BIOCONTROL POTENTIAL OF RHIZOSPHERE AND RHIZOPLANE FUNGI OF SELECTED GRASSES AGAINST CERTAIN FUNGAL DISEASES OF FOREST NURSERY SEEDLINGS

Thesis submitted to the University of Calicut in partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

in

BOTANY

by

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KSCSTE-Kerala Forest Research Institute

(An Institution under Kerala State Council for Science, Technology and Environment)

Peechi - 680 653, Thrissur, Kerala, India

CERTIFICATE

This is to certify that the thesis entitled "**Biocontrol potential of Rhizosphere and Rhizoplane fungi of selected grasses against certain fungal diseases of Forest Nursery seedlings**" submitted to the University of Calicut for the award of **Degree of Doctor of Philosophy in Botany,** by **Mr. Bharath Nair** is the result of bonafide research work carried out by him under my guidance in the Department of Forest Pathology, KSCSTE- Kerala Forest Research Institute, Peechi. Further, I certify that this or part thereof has not been the basis for the award of any other diploma or degree either in any institution or university.

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Certification of the Supervisor

This is to certify that the corrections/suggestions recommended by the adjudicators had been incorporated in the thesis entitled 'Biocontrol Potential of Rhizosphere and Rhizoplane Fungi of selected grasses against certain Fungal Diseases of Forest Nursery Seedlings' by Mr. Bharath Nair after the open defence. The contents in the thesis and the soft copy of the thesis are one and the same.

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DECLARATION

I, Bharath Nair, hereby declare that the thesis entitled "**Biocontrol potential of Rhizosphere and Rhizoplane fungi of selected grasses against certain fungal diseases of Forest Nursery seedlings",** embodies the results of bonafide research work done by me under the guidance of **Dr. G. E. Mallikarjuna Swamy,** Senior Scientist, Department of Forest Pathology, KSCSTE- Kerala Forest Research Institute, Peechi- 680653, Thrissur, Kerala. I further declare that this or part thereof has not been the basis for the award of any other diploma or degree either in any institution or university.

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Bharath Nair

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23.1. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

23.2. Teak seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

23.3. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

23.4. Teak seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

23.5. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

23.6. Teak seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

23.7. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

23.8. Teak seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 24

24.1. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

24.2. Teak seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

24.3. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

24.4. Teak seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

24.5. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

24.6. Teak seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration at 180 days

(pathogen: antagonist) in preventive treatment showing increased root and shoot length.

24.7. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

24.8. Teak seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 25

25.1. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

25.2. Teak seedlings amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

25.3. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

25.4. Teak seedlings amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

25.5. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

25.6. Teak seedlings amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

25.7. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

25.8. Teak seedlings amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 26

26.1. Mahogany seedlings grown in unamended potting medium.

26.2. Mahogany seedlings grown in unamended potting medium showing healthy root system.

26.3. Mahogany seedlings grown in potting medium amended with *Fusarium oxysporum* at 2 x 10^6 conidial suspensions showing wilt symptoms.

26.4. Mahogany seedlings grown in potting medium amended with *Fusarium oxysporum* at 2 x 10^6 conidial suspensions showing wilt symptoms.

26.5. Mahogany seedlings grown in potting medium amended with *Athelia rolfsii* showing wilt and rot symptoms.

26.6. Mahogany seedlings grown in potting medium amended with *Athelia rolfsii* showing wilt and rot symptoms.

26.7. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Clonostachys rosea* at 2 x 10^6 conidial suspensions.
26.8. Mahogany seedlings grown in potting medium amended with *Clonostachys rosea* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

PLATE - 27

27.1. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Penicillium multicolor* at 2 x 10^6 conidial suspensions.
27.2. Mahogany seedlings grown in potting medium amended with *Penicillium multicolor* at 2 x 10^6 conidial suspensions showing healthy shoot and root.
27.3. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Purpureocillium lilacinum* at 2 x 10^6 conidial suspensions.
27.4. Mahogany seedlings grown in potting medium amended with *Purpureocillium lilacinum* at 2 x 10^6 conidial suspensions showing healthy shoot and root.
27.5. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Trichoderma harzianum* at 2 x 10^6 conidial suspensions.
27.6. Mahogany seedlings grown in potting medium amended with *Trichoderma harzianum* at 2 x 10^6 conidial suspensions showing healthy shoot and root.
27.7. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Trichoderma koningii* at 2 x 10^6 conidial suspensions.
27.8. Mahogany seedlings grown in potting medium amended with *Trichoderma koningii* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

PLATE - 28

28.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.
28.2. Mahogany seedlings amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
28.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.
28.4. Mahogany seedlings amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.
28.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.
28.6. Mahogany seedlings amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
28.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

28.8. Mahogany seedlings amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 29

29.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

29.2. Mahogany seedlings amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

29.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

29.4. Mahogany seedlings amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

29.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

29.6. Mahogany seedlings amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

29.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

29.8. Mahogany seedlings amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 30

30.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

30.2. Mahogany seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

30.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

30.4. Mahogany seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

30.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.
30.6. Mahogany seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
30.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.
30.8. Mahogany seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 31

31.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.
31.2. Mahogany seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
31.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.
31.4. Mahogany seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.
31.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.
31.6. Mahogany seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
31.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.
31.8. Mahogany seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 32

32.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

32.2. Mahogany seedlings amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

32.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

32.4. Mahogany seedlings amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

32.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

32.6. Mahogany seedlings amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

32.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

32.8. Mahogany seedlings amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

ABBREVIATIONS

cfu g^{-1}	Colony forming units per gram
ISO	Isolates
GC-MS	Gas Chromatography Mass Spectrometry
et al.	And others (co-authors)
PGPF	Plant growth promoting fungi
BCA	Biological control agents
CWEDs	Cell Wall Degrading Enzymes
ISR	Induced Systemic Resistance
^oC	Degree Celsius
AFP	Antifungal Proteins
PAL	Phenylalanine Ammonia Lyase
PO	Peroxidase
PPO	Polyphenol Oxidase
%	Per cent
km^2	Square kilometer
Sp.	Species (singular)
ha	Hectare
cm	Centimeter
PDA	Potato dextrose Agar
mg L^{-1}	Milligram per litre
ml	Millilitre
h	Hour
min	Minute
dia.	Diameter
NSF	Non Sporulating Fungi
Sec	Seconds
ANOVA	Analysis of variance
DMRT	Duncan’s multiple range test
Mm	Millimeter
ml^{-1}	Per millilitre
g	Gram
CF	Colonization frequency

rpm	Rotation per minute
KOH	Potassium hydroxide
w/w	Weight per weight
µl	Microlitre
PI	Per cent Inhibition
CI	Colony Interaction
MI	Mycelial Interaction
IZ	Zone of Inhibition

Abstract

Efficient management of plant diseases is achieved better through environment friendly and sustainable approaches. Since there is a huge demand for healthy planting stock across the globe, application of chemicals is the norm to manage plant diseases though traditional physical and cultural methods are also practiced. Successful alternate approaches are on cards but dependability and sustainability are questionable. It is well known that soil and rhizosphere represent special ecological niches which are influenced by microbe-microbe and microbe-plant interactions in response to root secretions and root exudates. These ecological niches can be modified and transformed so as to manage plant diseases in an eco-friendly way. The present study was designed with the aim of identifying and evaluating the role of fungi isolated from the rhizosphere and rhizoplane of selected grasses as biocontrol agents and to assess their ability in promoting plant growth.

Fungi were isolated from the rhizosphere and rhizoplane regions of ten perennial grass species namely *Alloteropsis cimicina, Cynodon dactylon, Ischaemum indicum, Oplismenus compositus, Ottochloa nodosa, Panicum repens, Paspalidium flavidum, Paspalum conjugatum, Perotis indica* and *Setaria barbata.* Soil and root samples associated with the grass species were collected from selected sites in the Kerala parts of the Western Ghats located in northern, central, and southern Kerala during summer, rainy and winter seasons in 2016-2018. A total of 11915.58 fungal isolates from rhizoplane and 975.44 cfu g^{-1} of soil (10^4 dilution factor) from rhizosphere regions were isolated. Species

of *Absidia, Acremonium, Acrostalagmus, Alternaria, Arthobotrys, Aspergillus, Bipolaris, Cephalosporium, Chaetomium, Cladosporium, Clonostachys, Colletotrichum, Curvularia, Fusarium, Geotrichum, Helminthosporium, Mucor, Myrothecium, Nigrospora, Paecilomyces, Penicillium, Periconia, Pestalotiopsis, Phoma, Phomopsis, Purpureocillium, Rhizopus, Scopulariopsis, Talaromyces, Torula, Trichoderma* and *Verticillium* were found associated with the rhizoplane and rhizosphere of grasses. The per cent occurrence of fungi in rhizoplane and rhizosphere regions varied significantly between the grass species. Diversity of fungi was significantly high during the winter season followed by rainy and summer seasons irrespective of the grass species. A total of 134 fungal isolates, sixteen from *Alloteropsis cimicina*, 22 from *Cynodon dactylon*, 14 from *Ischaemum indicum*, 11 from *Oplismenus compositus*, 10 from *Ottochloa nodosa*, 15 from *Panicum repens*, 10 from *Paspalidium flavidum*, 19 from *Paspalum conjugatum*, 8 from *Perotis indica* and 9 from *Setaria barbata* were tested for antagonism on the basis of frequency of colonization in the rhizosphere and rhizoplane.

Disease survey across Central Forest Nurseries of Kerala was carried out to observe diseases of teak (*Tectona grandis* L.) and mahogany (*Swietenia macrophylla* King.) seedlings caused by wilt and rot symptoms and associated fungal pathogens. *Fusarium oxysporum* and *Athelia rolfsii* were found to be dominant pathogens causing root rot and wilt in both the seedlings and hence analyzed for *in vitro* antagonistic interactions against the fungal isolates mentioned above by dual culture assay. Bio-agents were also evaluated for antagonistic efficacy and disease causing abilities by leaf bioassay method on detached leaves of the host plants. The nine fungi selected namely,

Penicillium nigricans (ISO-11) from *Alloteropsis cimicina*, *Trichoderma harzianum* (ISO-33) and *Trichoderma koningii* (ISO-35) from *Cynodon dactylon*, *Aspergillus niger* (ISO-40) and *Purpureocillium lilacinum* (ISO-48) from *Ischaemum indicum*, *Penicillium multicolor* (ISO-58) from *Oplismenus compositus*, *Clonostachys rosea* (ISO-79) from *Panicum repens*, *Trichoderma* sp. (ISO-106) from *Paspalum conjugatum* and *Trichoderma pseudokoningii* (ISO-116) from *Paspalidium flavidum* were further tested for saprophytic competence and frequency of colonization as potential antagonistic agents.

The saprophytic competence and frequency of colonization was carried by Cambridge method using sterile paddy straw. The five fungal isolates namely, *Clonostachys rosea*, *Penicillium multicolor*, *Purpureocillium lilacinum*, *Trichoderma harzianum* and *Trichoderma koningii* were selected based on the saprophytic ability and colonization on paddy straw. These fungi were tested for root colonization ability in teak and mahogany seedlings using hydroponics. Conidial suspension of 2 x 10^6 at two concentrations (1 pathogen: 1 antagonist) and (1 pathogen: 5 antagonist) were tested. Antagonists at 1: 5 concentration (1 pathogen: 5 antagonist) was found to be highly effective in colonizing the roots and reducing the infection by *F. oxysporum* and *A. rolfsii*. *Clonostachys rosea, Trichoderma harzianum* and *Trichoderma koningii* colonized root tissues whereas *Penicillium multicolor* and *Purpureocillium lilacinum* were found attached to the root surface serving as a shield on the root against the pathogens.

All the five antagonists at a concentration of 1 pathogen: 5 antagonists were further analyzed for their field efficacy. Preventive (inoculation of seedling roots with

bio-agents followed by pathogens) and curative treatments (inoculation of pathogens followed by bio-agents) were the main experimental setup. Untreated plants served as controls. Besides these, plants treated with the pathogens and bio-agents were also maintained separately. All seedlings were observed for root and shoot length and fresh and dry weight. Seedlings of both the species exhibited variations in different parameters based on the antagonists used for inoculation.

Curative treatments were found to be more effective in improving plant growth. Analyses of soil myco-flora of seedlings under various treatments indicated a higher fungal density in seedlings inoculated with the pathogens compared to those inoculated with the bio-agents and preventive and curative treatments. The biochemical analyses of methanol extracts of plants under different treatments using GC-MS revealed that biochemical compounds produced specific to each treatment could play a decisive role in plant immunity. All the five antagonists were observed to be efficient in improving plant growth and hence curative treatment would serve as an alternate way to manage the disease.

Introduction

Life on earth is sustained by plants without which man and animals will be deprived of a major food source. Forests are also vital in sustaining life as they provide us - food, oxygen, shelter and recreation. They ensure good quality of life of humans being sources of over 5,000 commercially-traded products, ranging from pharmaceuticals to timber and clothing. The biodiversity of forests are the variety of genes, species and forest ecosystems underpins these goods and services, and is the basis for long-term forest health and stability.

Forest nurseries are meant for raising healthy seedlings which are often hindered mainly by diseases and pests. The availability of healthy and disease-free planting stock ensures their successful establishment and growth in planted forests (Bakshi, 1976; Bloomberg, 1985). Disease incidence in natural forests is also a concern but in most cases there happens a balancing act which control damage and spread.

A multitude of plant pathogens - fungi, bacteria, mollicutes, nematodes, viruses, viroids, algae and protozoa cause crop loss due to diseases and pests. Fungal pathogens account for a significant proportion of mortality and under-performance of nursery seedlings and planted forests worldwide. The impact affects not only national economy and food supplies but also cause mass migration of people and loss of major forest communities. *Phytophthora infestans* (Mont.) de Bary., a major pathogen of late blight of potato smacked Europe in 1840's resulting in the death of about a million of people due to starvation and migration of about a million in Ireland (Agrios, 2005). Ergotism is

another disease devastated France and spread across Europe in 1850's caused by sclerotia of *Claviceps purpurea* (Fr.) Tul., killing thousands of lives (Agrios, 2005). In 1870, the disease rust of coffee which affected Sri Lankan economy caused by fungal pathogen *Hemileia vastatrix* Berkeley & Broome (Agrios, 2005). From 1900, many worldwide catastrophes' caused by fungal pathogens have been reported namely, Chestnut disease caused by *Cryphonectria parasitica* (Murrill) M.E. Barr., Great Bengal famine by *Cochliobolus miyabeanus* (Ito & Kurib.) Drechsler ex Dastur., Dutch elm disease by *Ophiostoma ulmi* (Buisman) Melin & Nannf., Oak wilt and sudden death by *Ceratocystis fagacearum* (Bretz) J. Hunt., Butter-nut canker by *Sirocosus clavigignenti-juglandacearum* N.B. Nair, Kostichka & J.E. Kuntze., Cypress canker by three species of *Seiridium*, particularly *S. cardinal* (W.W. Wagener) B. Sutton & I.A.S. Gibson., Southern corn leaf blight caused *Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker. and Corn blight caused by *Cochliobolus heterostrophus* (Drechsler) Drechsler. (Agrios, 2005).

Forest nursery diseases such as collar rot, damping-off, root rot, foliar diseases, blights, powdery mildews and rusts are known to cause major loss of seedlings. Prevalence of the diseases decreased with the introduction of direct seedling of many species into the polythene container as well as in soil-less or soil free medium in root trainers. However, damping-off and root diseases persist still in seed bed nurseries and pose a major threat. Species of *Rhizoctonia, Fusarium, Sclerotium, Pythium, Phytophthora, Cylindrocladium* etc., are generally associated with these diseases either alone or in combinations. Disease control is mostly achieved by the application of chemicals.

Chemical protection of seedlings has been much relied upon in forest nurseries because of the guaranteed results. However, widespread and indiscriminate applications of chemical pesticides in forest nurseries have resulted in the emergence of pesticide resistance in pathogens, toxicity to non-target organisms and environmental contamination which have greatly reduced the desirability of chemical pesticides. This emphasized the need of novel ways of controlling plant pests and diseases to minimise over dependence on chemical agents. Biological management is thus being considered as a safe, eco-friendly and cost effective method to limit the use of chemicals in nurseries.

In this context, use of bio-pesticides serve as a novel method for pest and pathogen management and their demand is growing steadily over the past few decades. Their market share has shown a surge from a meagre 0.2% to 2.89% (2000 – 2005) and expected to exhibit an annual growth rate of 2.3% in years to come (Thakore, 2006).

It is well established that microbes colonize varied substrates and habitats and play pivotal roles in plant health and productivity (Wagg *et al*., 2014). The bulk of the microbial populations exquisitely colonize nutrient rich ecological niches like soil, rhizosphere, rhizoplane and phylloplane and are also the natural resources for microbial metabolites and other biotechnologically important products.

Rhizosphere soil compared to the surrounding bulk soil comprises a tremendous combination of soil microbes and diversified microbial activities and is considered as the largest ecosystem on earth with massive energy flux (Smalla *et al*., 2001; Barriuso *et al*., 2008). Most of these microorganisms generally make use of plant root-derived nutrients such as root exudates, mucilage, mucigel and secondary metabolites (Huang *et al*., 2014).

Plant roots derived chemicals probably alter physical and chemical characteristics of the soil and may also influence rhizosphere microbial diversity and composition of soil microbial community by selectively stimulating microorganisms with beneficial traits that are needed for both plant growth and health (Chaparro *et al*., 2014; Huang *et al*., 2014; Mukherjee *et al*., 2018). Interactions between microbes living on plant surfaces and those residing as endophytes in the host tissues represent a complex ecosystem and importance of these plant-microbe interactions on plant health is only beginning to understand.

Over the past few decades, a vast diversity of rhizosphere microorganisms and their relationships with roots namely associative, symbiotic, neutralistic or parasitic have been described and characterized (Haldar and Sengupta, 2015). In many cases, they are also being tested as bio-control candidates against soil-borne plant pathogens. Their applications as potential antagonists have been hindered by inconsistent field performance. The lack of rhizosphere competence and their survival in soil restricts large scale use of these microbes as potential bio-control agents. Rhizosphere competence and survivability are pre-requisites for effective biological management and knowledge of root-microbe interactions influenced by genetic and environmental determinants significantly contribute to the improvement and efficacy of these potential bio-control agents.

Biological agents interact directly or indirectly. Direct antagonism involves physical contact exhibited by competition and hyper-parasitism where no other organism would be required to exert a suppressive effect. In contrast, indirect antagonisms involve

stimulation of host plant defence pathways by non-pathogenic bio-control agents and involve no sensing or targeting a pathogen by the bio-control agents (Harman *et al*., 2004).

All these mechanisms may operate together or independently and their activities can result in the suppression of soil-borne plant pathogens. Due to the activity of bio-control agents, certain biochemical changes may occur in the host plant, which in turn is associated with plant defence mechanism.

Literature is abound on discussing the potentials of fungi as biological agents of managing plant and soil health, promoting plant growth and stimulating resistance and plant defence mechanisms. Fungal species namely *Trichoderma*, *Gliocladium*, *Chaetomium*, *Penicillium* etc., have been extensively used in biological management of diseases and plant growth promotion (Mathew and Gupta, 1998; Prasad *et al*., 1999; Pandey *et al*., 2005). Besides, non-pathogenic fungal strains namely *Pythium*, *Fusarium*, *Phialophora* etc., have also been described for their ability as potential antagonists (Li *et al*., 2005; Muslim *et al*., 2003; Nel *et al*., 2006; Yigit and Dikilitas, 2007; Mukhtar, 2008; Vinale *et al*., 2008; Contreras-Cornejo *et al*., 2009). Regardless of many fungal species characterised as potential biocontrol agents, only a few of them have been exploited and utilized on a commercial scale.

As already indicated, microbial populations in the rhizosphere regulate plant growth and development and exhibit antagonistic ability (Weller, 1988; Weller *et al*., 2002). These beneficial fungi include plant growth promoting (PGPF) and biological control agents (BCA). The detection and isolation of PGPF and BCA often involve

screening of thousands of isolates, only to find a few beneficial ones. Better information on the rhizosphere fungal communities can lead to a good understanding of their role in the soil ecosystem including the balancing of the pathogen population. Microbial interactions in the rhizosphere needed to be given importance as combined microbial activity which is proven to be significant also when applied individually (Singh and Singh, 2008).

The characteristic feature of an antagonist has to be saprophytic and capability to reside up to a depth of 5-15 cm in soil (Bardgett *et al*., 1997). The chances of colonization of the root system of grasses by saprophytes are high because grasses have a fibrous and shallow root system, which gets rejuvenated every growing season. The forest soil is rich in humus, undisturbed in nature and is largely free from pesticide contamination.

Rhizopshere and rhizoplane regions of grasses encompass a large number of microorganisms (Hyakumachi *et al*., 1992; 1993; Smith *et al*., 1999) and have been studied extensively for their biocontrol and plant growth promoting potentials in green house and field conditions (Shivanna *et al*., 1994; 1995; 1996a; 1996b; Hashiba *et al*., 2001; Shivanna *et al*., 2005). However, more systematic studies are mandatory to fully unravel soil-plant interactions and tap the potentials of micro fungi for beneficial purposes.

Aim and Objectives

Fungi constitute the most diverse, exceptional and widespread life form on earth and their diversity and distribution vary depending on different micro and macro climatic conditions and ecological niches across the globe. Literature survey evidences that numerous fungal species reside in the rhizosphere and rhizoplane regions of different plant species. However, only a few have been studied for their properties as bio control and plant growth promoting agents. Of these, only a finite number of species has been found to have any potential for exploitation. The present work was planned based on the hypothesis that there could be a fair number of fungal species with potential antagonistic properties. The objectives of the present study are:

1. To isolate and identify fungi in the rhizosphere and rhizoplane of selected grass species growing in the Kerala parts of the Western Ghats during different seasons.
2. To test the antagonistic ability of selected fungal isolates against certain plant pathogens causing diseases on seedling in forest nurseries.
3. To test the competitive saprophytic, root colonization, biological control and resistance inducing abilities of the selected fungi against the plant pathogens.

Review of Literature

Plants are the fundamental producers on which other members of an ecosystem confide. As plant pathogens are dependent on their hosts, they regulate various ecological and evolutionary processes in ecosystems (Augspurger, 1988; Barbosa, 1991; Burdon, 1991; Alexander, 1992; Dickman, 1992; Herms and Mattson, 1992). Plant diseases in natural ecosystems may present in epidemic or endemic levels and can alter forest community as a whole. On the other hand no microorganism is said to be disease causing unless being influenced by these three compulsory factors (i) a virulent pathogen, (ii) a susceptible host and (iii) a conducive environment (Agrios, 2005). However this model contrasts with certain regional disease outbreaks (Paillet, 2003; Rizzo *et al*., 2005; Shearer *et al*., 2007).

Plant disease causing organisms represent various branches on the tree of life including fungi, bacteria, protozoa, virus, nematodes, oomycetes etc. fungi being the most devastating. Fungi, their mode of infection contradicts to that of insects, where spore dispersal or mycelium spread disseminates to new hosts. Fungal pathogens could be specialists or generalists (Hersh *et al*., 2012). The management strategies depend upon the fungal groups.

Seedling health and viability influence forest reproduction. Forest seedlings, a major source of timber, pulp, firewood and other products are also affected by various diseases. Fungal diseases limit production of sufficient quantity and quality of plant stocks to satisfy a rapidly expanding and discerning world population (Hewitt, 1998). For

combating diseases, effective measures of chemical control have been developed over the years. Due to certainty in effective control, unchecked, repeated and comprehensive applications of toxic chemicals (fungicides) have been subjected at different stages in seedling production. Their broad-spectrum efficiency oftentimes resulted in the eradication of both beneficial and pathogenic organisms (Baker and Cook, 1974). Recently, chemicals have been designed specifically for effective against targeted organisms (Thomson, 1990). However, development of resistance by pathogens once introduced into field pose a great concern (Staub, 1991).

Though fungicides have played a major role in controlling diseases, excessive use of fungicides has resulted in a variety of harmful and undesirable effects not only on man and wildlife, but also on the ecosystem as a whole. This has led in the search of more acceptable approaches substituting chemicals with biological agents for disease management (Lawson and Dienelt, 1989). Biological control is present naturally in plant ecosystem which keeps pathogen population within limits (Baker, 1987). However in artificial systems such as agricultural fields or in nurseries specialized steps must be taken to promote biological balance of organisms so that suppression of plant disease and quality stock production can be achieved.

Biological control is defined as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists (Baker, 1987;

Campbell, 1989). Biological control involves in the reduction of number or ability of pathogens to cause disease thereby improving plant health (Baker and Cook, 1982).

Biological control usually has three objectives: 1) reduction of pathogen inoculum 2) reduction of host infection by the pathogen and 3) reduction of disease severity (Axelrood, 1991). Without biological control functioning in nature none of the wild plants susceptible to various pathogens would have survived (Baker and Cook, 1982). In natural ecosystems, innumerable microorganisms dwell in the soil or on plant surfaces which tend to safeguard plants. Therefore pathogens are not eliminated but their activities are repressed (Baker and Cook, 1982).

Biocontrol Mechanisms

Numerous microbial potentialities in suppressing diseases by various pathogens have been studied world-wide (Blakeman and Fokkema 1982; Andrews 1992; Tronsmo 1992; Blakeman 1993; Brasier 1998; Funck-Jensen and Lumsden 1999). A great amount of work has concentrated on diverse aspects such as rhizosphere microbial diversity and their impacts on plant growth and development (Avis *et al*., 2008; Compant *et al*., 2010). Research involving microbe-microbe and plant-microbe interactions has ignited curiosity among scientists to decipher such crosstalk.

Microorganisms interact in different pathways and mechanisms involved are vital in designing the optimum conditions for their effectiveness in a given patho-system (Pal and Gardener, 2006; Glick, 2012).

Antibiosis

Secondary metabolites produced by an organism interfere with the growth and metabolic activities of other microorganisms have been recognized (Whipps, 1997; Funck-Jensen and Lumsden, 1999). Numerous known antibiotics by different groups of microbes namely actinomycetes, bacteria and fungi have been thoroughly studied and their application against various pathogens in the management of diseases have been established world-wide (Delaney *et al*., 2001; Duffy *et al*., 2004; Krishnan *et al*., 2007; Kadir *et al*., 2008; Calderon *et al*., 2013).

Competition

The initiation and the extent of pathogen depend upon the availability of nutrients. In effect they are the initial colonizers but non pathogenic species later on compete for the same resource hence play a prime role in substrate colonization. The success of a pathogen in disease establishment depends upon host invasion and spore germination and reduction in the concentration of substrates limit the extent of the pathogen (Blakeman, 1993; Elad, 1995; Funck-Jensen and Lumsden, 1999).

Parasitism

The competitive interaction among the organisms where one overcomes the other and is most prominent in case of fungi (Elad, 1995). Hyperparasitism involve Cell Wall Degrading Enzymes (CWEDs) and in some cases secondary metabolites also disorganise hyphal cells and subsequently in the death of the hosts (Jeffries, 1995). Fungal bioagents, *Trichoderma*, *Gliocladium* and *Clonostachys* have been widely investigated for hyper-parasitic interactions. They develop specialized structures for attachment and

subsequently infect and kill the hosts via CWDEs or in association with secondary metabolites (Harman *et al*., 2004; Harman, 2006; Mukherjee *et al*., 2012; Karlsson *et al*., 2017; Nygren *et al*., 2018).

Induced Resistance (IR)

Induced resistance is plant's innate defence mechanism incite upon proper stimulation (van Loon *et al*., 1998). Enhancing plant resistance through triggering of plant defence pathways on the recognition of the pathogen is crucial in plant's vegetative phase. The resistance may be locally or systemic (Conrath *et al*., 2015). Induced Systemic Resistance (ISR) is attributed to a variety of microorganisms which help in plant's defence against various soil and foliar pathogens (Paulitz and Matta, 1999). Induced Systemic Resistance keeps a memory which in the presence of future stimuli results in faster and stronger response (Mauch-Mani *et al*., 2017).

A diverse array of microorganisms as biocontrol agents against various plant pathogens has been characterized and described in detail from different sources.

1. Soil mycoflora

Soil ecosystem is complex and is substantiated by the copious and multifaceted interactions among physical, chemical and biological components (Buscot, 2005). The heterogeneous microbial populations have a wide range functions on the soil such as decomposing organic material, in biogeochemical cycling of nutrients, maintaining soil fertility, mediating various metabolic processes etc. (Kirk, 2004; Wani *et al*., 2008; Khan *et al*., 2009b; Ahmed *et al*., 2009).

Pan and Ghosh (1997) analysed soils from different parts of West Bengal and tested isolated microbes efficacy against *Phytophthora colocasiae* the causal agent of leaf blight and corn rot of taro. *In vitro* dual culture resulted in 10 isolates, five of *Trichoderma viride*, three of *T. harzianum* and one each of *Gliocladium virens* and an unidentified sterile fungus out of 58 fungal isolates screened to be potential antagonists. Ghosh (2000) in his study found *Trichoderma viride*, *T. harzianum* and *G. virens* among numerous fungi isolated from soils of different parts of West Bengal to be effective in the *in vitro* management of leaf and foot rot pathogen *Phytophthora parasitica* var. *piperina* of *Piper betle*. *In vitro* mycoparasitic interactions noticed morphological changes like hyphal coiling, penetration and formation of haustoria like structure.

Integrated management of web blight of Ground nut caused by *Rhizoctonia solani* was determined using fungal species namely *Trichoderma viride*, *T. harzianum* and *Gliocladium virens* isolated from soil *in vitro*. Maximum seed germination and minimal disease incidence was exhibited by seed coating with combination of *G. virens* + thiram followed by *G. virens* alone (Dubey, 2000). Kumar and Dubey (2001) screened volatile and non-volatile metabolites of antagonists *Trichoderma viride*, *T. harzianum* and *Gliocladium virens* isolated from soil samples of Ranchi against *Fusarium solani* f. sp. *pisi* causing pea collar rot. *In vitro* studies revealed *T. harzianum* exhibited maximum seed germination and minimal disease incidence followed by *G. virens*.

Intana *et al*., (2003) evaluated 165 isolates of *T. harzianum* from 148 soil samples from different plantations in Thailand. Glass house experiments exhibited about 156 isolates guarded cucumber damping-off and 111 and 57 isolates elevated shoot and root

fresh weights, respectively. Repeated screening of isolates resulted in isolate 39, 35 and 22 among the 39 isolates tested to be effective in disease management and increasing shoot and root fresh biomass.

Bajwa *et al*., (2004) reported antagonistic potentiality of five *Trichoderma* sp. (*T. viride, T. harzianum, T. koningii, T. aureoviride* and *T. pseudokoningii*) and three *Aspergillus* sp. (*A. fumigatus, A. glaucus* and *A. oryzae*) against *Fusarium solani* causing wilt disease in *Dalbergia sisoo*. *In vitro* antagonistic activity noticed *Trichoderma harzianum* comparatively better antagonistic agent against all the fungal organisms studied exhibiting 52.4% pathogen colony inhibition. Potentiality of six biological agents namely *Trichoderma harzianum, T. viride, Gliocladium virens, Bacillus subtilis, B. cereus* and *Pseudomonas fluorescens* against three *Fusarium* sp. namely *Fusarium solani, F. moniliforme* and *Fusarium oxysporum* f.sp. *dalbergiae* in *in vitro* and *in vivo* studies resulted *G. virens* and *B. subtilis* to be highly effective (Kaushik *et al.,* 2005).

Fungal diversity of three sacred groves soil samples from Tiruchirapalli district was analysed. The peculiarity in the presence of *Aspergillus fumigatus*, *A. terreus*, *Cladosporium herbarum*, *Fusarium chlamydosporium*, *Mucor mucedo* and *Penicillium verrucosum* among the three sacred groves could be attributed to the dominant tree species like *Ficus religiosa, Diospyros ovalifolia, Albizzia amara, Terminalia chebula*, *Wrightia tinctoria* and *Cassine glauca* (Abubacker *et al*., 2005).

Adekunle (2006) evaluated the efficacy of *Trichoderma* sp. against *Macrophomina phaseolina in vitro* and *in vivo*. Cowpea seeds exhibited better plant

stands on treatment with *T. koningii* + starch (53.8% and 49.3% at 7 and 21 days respectively) followed by *T. harzianum* (55% and 45.8% at 7 and 21 days respectively).

Bastakotti *et al*., (2017) isolated five different *Trichoderma* sp. from 26 different soil samples from Himalayan region, Hilly region and Terai region of Nepal and determined for its efficacy against soil-borne phytopathogens namely *Fusarium oxysporum*, *Sclerotium rolfsii*, *Sclerotium sclerotiorum* and *Rhizoctonia solani*. *In vitro* dual culture exhibited 100% inhibitory activity against *S. rolfsii* and was found to be efficient compared with other pathogens, 62% against *R. solani,* 68% against *F. oxysporum* and 23% against *S. sclerotiorum*.

2. Rhizosphere and Rhizoplane mycoflora

Rhizosphere is a vital environment which comprises soil adjacent to the root system characterised by rich micro-flora. The composite microbial populations due to their specific metabolic activity have potential effect on soil functions (Ahmed *et al*., 2009). Rhizosphere has been classified into endo-rhizosphere, rhizoplane and ecto-rhizosphere zones (Clark, 1949; Lynch, 1987; Pinton *et al*., 2001) and microbial communities vary along these different zones (Assmus *et al*., 1995; Lemanceau *et al*., 1995; Bosse and Frenzel, 1997; Gilbert and Frenzel, 1998) as well as along the root axis (De Leij *et al*., 1993; Lemanceau *et al*., 1995; Gilbert and Fernzel, 1998), depending on the plant species (Grayston *et al*., 1998) and on soil type (Campbell *et al*., 1997).

Rhizosphere is the complex environment and is constituted in majority by bacteria among the pool of different microbial groups, fungi, archae, protozoans, nematodes,

algae and phytoplasmas (Hartel, 1999; van Elsas *et al*., 2007b). Standing and Killham (2007) attributed different parameters such as soil moisture, soil texture, temperature and pH that directly or indirectly influence metabolic activities and microbial composition.

Plants in due course of their growth and development release heterogeneous organic compounds (Curl and Truelove, 1986). This increases microbial population and enhance their activities in rhizosphere region compared to bulk soil (Grayston *et al*., 1996).

All these factors in combination with the variety of crop grown (Berg *et al*., 2006), plant developmental stage (Gomes *et al*., 2003) and soil characteristics (Nie *et al*., 2009) is referred as rhizopshere effect (Morgan and Whipps, 2001).

Rhizosphere responds to biogeochemical changes (Rougier, 1981) which correspond to the amount of soil carbon (Lynch and Whipps, 1990). Plants in various stages of their growth and development release divergent metabolites which also plays a decisive role in regulating microbial diversity (Bowen and Rovira, 1991; Bolton *et al*., 1992).

The plant roots derived chemical exudates influence neighbouring plants and rhizosphere (Flores *et al*., 1999). Divergent chemicals released namely phytoalexins, defence proteins and other unexplored chemicals play a pivotal role in defending plants from various pathogenic attacks. The densities of microbial inoculums associated to the plant roots and the distance of microbes from the roots at different stages of plant growth

is influenced by root exudates (Huisman, 1982) thus an apparent zone for novel bio-active compounds, including antimicrobials.

A great diversity of rhizosphere organisms have been described in detail possessing antagonistic potentiality against numerous soil borne plant pathogens (Pandey and Upadhyay, 2000; Lo and Lin, 2002; Grasso *et al.,* 2003; Montealegre *et al*., 2003; El-Mehalawy, 2004; Landa *et al.,* 2004; Nwaga *et al.,* 2007; Siddiqui and Akhtar, 2007).

Rhizosphere microbes alleviate plant health through various mechanisms (Srivastava, 2015). Various biotic, abiotic and edaphic factors govern the rhizosphere population. Chesters and Parkinson (1959) observed seasonal fungal succession dominated by Mucorales followed by *Fusarium* and other dematiaceous fungi in the rhizosphere of Oats. In 1960, Ivarson and Katznelson noticed different plant growth stages influence on the rhizosphere microbial population. Wajid Khan *et al*., (1975) studied rhizosphere and non-rhizosphere mycoflora of *Ricinus communis, Brassica oleracea* and *Brassica mutica* during different vegetative growth stages and noticed higher rhizosphere population during peak growth stage which gradually declined compared to non-rhizosphere regions which remained constant throughout. Besides, soil texture and physico-chemical properties also influence qualitative and quantitative rhizosphere micro-flora compared to non-rhizosphere (Taylor and Parkinson, 1964).

In 1967, Mishra compared rhizosphere and rhizoplane mycoflora of different plant species namely *Cynodon dactylon* L., *Trichodesma amplexicaule* Roth., *Abutilon indicum* G. Don and *Amaranthus spinosa* L. occupying common habitat and observed

fungal species specificity towards rhizoplane region than rhizosphere region. Rangarao and Mukerji (1971a) in their detailed study on rhizosphere and rhizoplane microflora of four cultivars of wheat observed comprehensive variant levels of microbial population in four wheat cultivars. Mishra (1979) isolated rhizoplane mycoflora of three fibre yielding plants and observed most of the fungi belonging to Deuteromycetes amongst which *Aspergillus*, *Fusarium* and *Penicillium* were common and some of the fungal species were plant specific.

Singh (1970) in his experiment on rhizopshere fungi of *Argemone mexicana* in natural and artificial conditions observed a variation in fungal population where the number of fungi was less in sterilized soil compared to others. Rhizosphere and rhizoplane mycoflora of *Vigna unguiculata* during different stages of plant growth resulted *Aspergillus, Fusarium, Penicillium* and *Trichoderma* to be frequent colonizers (Odunfa and Oso, 1979). Qualitative analysis of fungi of rhizosphere and non-rhizosphere regions in relation to age of eight varieties of *Abelmoschus esculentus* resulted in certain fungi to be specific with plant varieties as well as with the age (Srivastava and Dayal, 1980).

In 1981, Mendoza *et al*., compared grassland, forest and agricultural soil micro flora and observed *Aspergillus* and *Trichoderma* are the dominant genera. Abdel-Hafez (1982) documented rhizosphere and rhizoplane fungi from *Triticum vulgare* and *Zea mays* cultivated in Saudi Arabia. *Aspergillus* and *Penicillium* are dominated rhizoplane fungi where as *Fusarium* sp., *Dreschlera spicifera, Cephalosporium roseogriseum, Stemphylium botryosum, Acremonium strictum* and *R. solani* were predominant in

rhizopsphere regions. In 1988, Dubey and Dwivedi observed rhizoplane mycoflora in soybean both quantitatively and qualitatively differed with respect to growth and environmental factors. Oyeyiola and Hussain (1992) studied rhizosphere fungi associated with wheat plant and identified *Aspergillus* and *Penicillium* to be dominant genera.

Nautiyal (1997) evaluated 256 rhizosphere competent bacterial strains from chickpea for their biocontrol potentiality against *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia bataticola* and *Pythium* sp. *In vitro* studies resulted *Pseudomonas* sp. NBRI9926 and *Rhizobium* sp. NBRI9513 to be effective in managing all the three pathogenic species while greenhouse studies identified *Pseudomonas* sp. NBRI9926P3 to be better rhizosphere coloniser and biocontrol agent compared to *Rhizobium* sp. NBRI9513R7. Evaluation of fungal strains *Gliocladium roseum* and *Trichoderma virens* against chickpea seedling soft rot by *Botrytis cinerea* under controlled and in the field conditions noticed *G. roseum* more effective than *T. virens* against *B. cinerea* (Burgess and Keane, 1997). Patel and Anahosur (2001) reported *in vitro* efficacy of *Trichoderma harzianum* against soil borne pathogens of chick pea - *Fusarium oxysporum* f. sp. *ciceri*, *F. solani*, *Macrophoma phaseolina* and *Sclerotium rolfsii* by dual culture technique. *Trichoderma harzianum* exhibited myco-parasitic interactions against *M. phaseolina* and *S. rolfsii* whereas for *Fusarium* sp. initially exhibited antibiosis and later-on over-grew and inhibited the pathogen growth.

Kwasna *et al*., (1999) worked on wheat rhizosphere mycoflora - *Gliocladium roseum, Fusarium flocciferum* and *Verticillium psalliotae* against *Coemansia* sp. *Gliocladium roseum* and *F. flocciferum* exhibited myco-parasitic interaction involved

hyphal coiling and lysing. In 2003, Dal Bello *et al*., analysed biocontrol potentiality of wheat rhizosphere microflora against wheat seedling blight causal agent *Bipolaris sorokiniana*. *Bacillus subtilis* and *Gliocladium roseum* were effective *in vitro* but failed in managing the disease in field conditions. Kucuk and Kivanc (2004) studied three rhizopshere *Trichoderma harzianum* strains (T8, T11 and T15) interaction against wheat pathogens namely *Gaeumannomyces graminis* var. *tritici*, *Fusarium culmorum* and *F. moniliforme in vitro*. The *T. harzianum* strains T8, T11 and T15 inhibited pathogen growth by producing different metabolite in the medium such as β-1, 3 glucanase and chitinase.

Harman *et al*., (2004) reported *Trichoderma harzianum* T-22 strain effective in controlling *Pythium ultimum* and *Colletotrichum graminicola* pathogens in maize and roots were found colonised with T-22 strain whereby stimulated plants for innate defense mechanism. El-Mehalawy *et al*., (2004) screened maize rhizopshere microbes for antagonistic activity against late wilt disease caused by *Cephalosporium maydis in vitro*. A total of 85 actinomycetes and 40 yeast species were tested of which six actinomycetes and five yeast isolates were found to be effective.

Khan and Sinha (2005) analysed *Trichoderma* strains against *Rhizoctonia solani* causing sheath blight in *Oryzae sativa*. Five *Trichoderma* strains from rice leaves, *T. harzianum* and *T. hamatum* from rhizosphere of rice and *T. virens* from rice field soils resulted *T. harzianum* to be effective and prior treatment of soil with *T. harzianum* reduced disease incidence. Swain *et al*., (2018) isolated six isolates of *Trichoderma* sp., *Trichoderma harzianum, T. erinaceum* and four isolates of *T. atroviride* from different

tree barks from Odisha. All the isolates were tested against rice pathogens - *Rhizoctonia solani*, *Sclerotium rolfsii*, *Sclerotium oryzae* and *Helminthosporium oryzae*. *Trichoderma erinaceum* noticed outperformance compared to all the other isolates. Seed treatment with *T. erinaceum* resulted in high yield and effective disease management.

Motta *et al*. (2003) screened rhizosphere soil of sunflower and isolated 49 species of fungi comprising 159 strains. *Penicillium* and *Aspergillus* were found to be dominant genera associated. Out of 159 strains, 79 strains had the potentiality to hydrolyse inulin. In 1999, Prasad *et al*., tested fourteen isolates of *Trichoderma* and *Gliocladium* species against root or collar rot caused by *Sclerotium rolfsii* in sunflower. *In vitro* studies resulted in all the isolates effective in controlling the growth of the pathogen. Among the isolates *T. harzianum* PDBCTH2 showed maximum inhibition followed by PDBCTH8 and PDBCTH7 exhibiting 61.4%, 55.2% and 54.9% respectively. *Gliocladium virens* exhibited 39.9% mycelial inhibition. Biocontrol of *Macrophomina phaseolina* caused dry root rot of sunflower was tested using *Trichoderma* isolates in combination with organic amendments. Seed treatment with *T. viride* in combination with neem cake yielded better control compared to *T. harzianum* in combination with neem cake (Mani and Hepziba, 2003).

Biswas and Sen (2000) reported the control of stem rot of groundnut caused by *Sclerotium rolfsii*. Isolates of *Trichoderma harzianum* - T8, T10 and T2 were found effective in dual culture, among 11 isolates tested. In field trials both T8 and T10 isolates reduced stem rot incidence by 33 to 50% when applied as seed coating and upto 72 and 83% on direct soil application. Rao and Sitaramaiah (2000) studied the potentiality of

Trichoderma sp. against *Aspergillus niger* causing collar rot disease in groundnut. *In vitro* studies noticed growth of *A. niger* was effectively controlled by *T. koningii* followed by *T. harzianum* and *T. hamatum* and field trials also proved the ability of *Trichoderma* sp. in the reduction of disease incidence. Devi, (2005) isolated five *Trichoderma* sp. and one *Pseudomonas* sp. from rhizosphere regions of groundnut and screened for their activity against *Sclerotium rolfsii* causing root infection in groundnut. Among the agents *T. harzianum* and *T. viride* were found to be effective *in vitro* and in field conditions. Sreedevi *et al*., (2011) isolated *T. harzianum* from groundnut rhizosphere and tested for its ability against *Macrophomina phaseolina* causing root rot in groundnut. Defence enzymes such as peroxidase and polyphenol oxidase and defence compounds like total phenol and ortho-dihydric phenol were analysed and it was found that *T. harzianum* treated plants showed an increase in the levels of these biochemical compounds hence highlighted the ability of *T. harzianum* in the induction of systemic resistance in groundnut plants. Kaur *et al*., (2019) screened *Streptomyces* sp. MR14 isolated from mustard rhizosphere regions against various phytopathogens - *Alternaria alternata, Alternaria brassicicola, Alternaria mali, Alternaria solani, Cercospora beticola, Cladosporium herbarum, Colletotrichum acutatum, Colletotrichum gloeosporioides, Exserohilum* sp., *Fusarium oxysporum, Fusarium oxysporum* f.sp. *dianthi, Fusarium moniliforme,* and *Pyricularia oryzae*. The strain exhibited potent activity against all the pathogens, highest inhibitory activity was observed against *Pyricularia oryzae* (31mm). *Streptomyces* sp. MR14 cells/ supernatant/ solvent was also tested *in vivo* in the rhizosphere regions of tomato and was found to elicit plant innate defense mechanism as well as growth promotion ability.

Bunker and Mathur (2001) isolated five isolates of *Trichoderma* species isolated from rhizosphere of ginger and tested *in vitro* against *Rhizoctonia solani* caused dry root rot in chilly. *Trichoderma harzianum* effectively suppressed the growth of the pathogen and damaged the sclerotia formation of *R. solani*. Noveriza and Quimio (2004) reported antagonism of rhizosphere of black pepper mycoflora against *Phytophthora capsici in vitro*. A total of 149 isolates were isolated and of which eighteen isolates inhibited pathogen growth. In 2007, Ezziyyani *et al*., investigated biocontrol efficacy of *Trichoderma harzianum* and *Streptomyces rochei* against *Phytophthora* sp. causing root rot in pepper *in vitro*. Both bio-agents were effective where *T. harzianum* exhibited parasitic interaction over the pathogen, while antibiosis by *S. rochei* retarded pathogen growth. Berg *et al*., (2005) isolated rhizosphere and non rhizosphere fungi from oilseed rape and strawberry from different locations in Germany. A total of 4320 isolates were isolated and tested for their potentiality against *Verticillium dahlia* and resulted, 911 are potent antagonists. *Penicillium* and *Paeciliomyces* isolates were found to be dominant in rhizosphere regions of strawberry where as *Monographella* was abundant in rhizosphere regions of oilseed rape. In 2012, Abdel-Hafez *et al*., analysed rhizosphere and rhizoplane density and diversity of *Fusarium* and other fungal genera at different growth stages of lentil and sesame plants. A total of sixteen *Fusarium* species were isolated from rhizosphere and rhizoplane regions of both plants. *Fusarium oxysporum*, *F. solani* and *F. verticillioides* were reported from rhizopshere and rhizoplane regions of both plants, *F. solani* being isolated at all the growth stages of both plants. Among other genera,

Aspergillus and *Penicillium* were recorded from both plants rhizosphere and rhizoplane regions.

Sharma and Chandel (2003) isolated nine rhizosphere fungal isolates from gladiolus and screened for their antagonistic ability against *Fusarium oxysporum* f. sp. *gladioli*. *In vitro* analysis of nine isolates - *Trichoderma harzianum, T. viride* and *T. virens* were found effective against the pathogen in dual culture. Mass multiplication of biocontrol agents was also subjected on eleven different organic media, of which wheat bran promoted maximum mass production of *Trichoderma* sp. and temperature for mass culturing noticed 25ºC is the optimum. Grasso *et al*., (2003) isolated and screened for the activity of rhizosphere mycoflora of gerbera (*Fusarium* sp., *Trichoderma* sp., oomycetes and bacteria) against root rot caused by *Phytophthora cryptogea*. Isolates of *Fusarium* and *Trichoderma* significantly reduced the disease incidence. In 2003, Moreno *et al*., screened antifungal proteins (AFP) from *Aspergillus giganteus* and subjected against *Botrytis cinerea* causing gray mold in geranium plants. Application of AFP hindered mycelial growth as well as conidial germination. Microscopic analysis resulted in swollen hyphal tips and thereby reducing hyphal elongation. Jagathambigai *et al*., (2010) evaluated antagonistic effect of three *Trichoderma viride* and one *T. harzianum* isolate against *Sclerotium rolfsii* caused collar rot in ornamental plant *Zamioculcas zamiifolia* in Srilanka. All the isolates remarkably inhibited the pathogen *in vitro, Trichoderma viride* (TV-1) being the most prominent species controlled the mycelia growth. Field application of the isolate *T. viride* TV-1 significantly managed the incidence of collar rot by 75.54%. Two isolates each of three biocontrol microbes - *Pseudomonas fluorecens, Bacillus*

subtilis and *Trichoderma viride* were amended in the rooting media of *Anthurium* and were challenge inoculated against *Colletotrichum gloeosporioides* causing anthracnose or spadix rot disease. Peak activity of defence enzymes, Phenylalanine Ammonia Lyase (PAL), Peroxidase (PO), Polyphenol Oxidase (PPO), β-1-3-Glucanase and phenol accumulation was observed by Selvaraj and Ambalavanam (2013).

Abdulwahid *et al*. (1997) reported the rhizosphere, rhizoplane and non-rhizosphere fungal population of soils of tomato fields. Mycoflora abundance and species richness was high in rhizosphere region compared to non-rhizosphere and rhizoplane regions. Rhizoplane mycoflora from five varieties of tomato yielded - *Fusarium oxysporum, Ralstonia solanacearum, B. theobromae, T. koningii* and *A. niger*. Variety Shiny yielded highest fungal species in contrast to Marglobe and Ame varieties which yielded minimal (Osuinde and Ikediugwu, 2002). Saikia and Gandhi (2003) screened *Trichoderma viride, T. harzianum* and *Gliocladium virens* for their ability against *Rhizoctonia solani* causing stem rot in cauliflower. All the isolates were found to be effective against the pathogen *in vitro. Trichoderma viride* was the most potent bio-agent followed by *G. virens*. However, *T. harzianum* was least effective. *In vitro* assay revealed hyphal interactions by the antagonistic agents. In 2004, El-Mehalawy tested antagonistic activity of yeast isolated from rhizosphere of kidney bean against fungal pathogen *Fusarium oxysporum* causing wilt diasease. Isolated yeast species exhibited innate systemic resistance and plant growth promotion ability. Abeysinghe (2006) studied non-pathogenic *Fusarium oxysporum* isolates in contrary to the *Cucumis sativus* root and stem rot pathogen *F. oxysporum* f. sp. *radicis–cucumerinum*. Two of the three non-pathogenic

isolates on individual application reduced the disease incidence. However, application of the isolates in combination did not prove to be beneficial. Rini and Sulochana (2006) evaluated *Trichoderma* sp. and *Pseudomonas fluorescens* both individually and in combination against *Rhizoctonia* root rot under greenhouse and field conditions in chilly. *Trichoderma harzianum* (TR20) + *P. fluorescens* (P28) in combination proved to be efficient in managing the disease as well as improving plant yield. Efficacy of bioagents was studied against *Macrophomina phaseolina* causing root rot in egg plant. *Trichoderma harzianum* exhibited mycelial inhibition of 18.20 % against the pathogen *in vitro*. Talc-based field application noticed *T. harzianum, T. polysporum* or *T. viride* to be effective in managing the disease (Ramezani, 2008). Purwantisari *et al*., (2018) carried out soil application of *Trichoderma viride* suspensions against late blight disease caused by *Phytophthora infestans* in potato plants. The bio-agent suspension elevated glucanase and total phenol content besides enhancing plant innate resistance.

Sule and Oyeyiola (2012) studied the rhizosphere and rhizoplane mycoflora of Cassava cultivar TME-419. The various fungal species associated with Cassava are *Alternaria, Aspergillus, Acremonium, Brettanomyces, Botrytis, Byssochamys, Cladosporium, Doratomyces, Geotrichum, Humicola, Moniliella, Monascus, Neurospora, Oidiodendron, Penicillium, Pyricularia, Papulospora, Rhodotorula, Rhizopus, Saccharomyces, Sporothrix, Trichothecium* and *Trichoderma*. Of which *Byssochamys fulva* (23.1 %), *Geotrichum candidum* (10.9%) and *Papulospora coprophila* (10.2 %) were found to be dominant in rhizosphere where as *Papulospora coprophila* (16.1 %) and *Geotrichum candidum* (14.3%) were dominant in rhizoplane

regions. In 1997, Ulacio and colleagues studied the rhizosphere and rhizoplane mycoflora of tobacco plants. *Aspergillus* and *Fusarium* were observed to be dominant in both the rhizosphere and rhizoplane regions. Bioagents from ginger rhizosphere and rhizopalne regions were tested for their activity against rhizome rot caused by *F. solani* and *Pythium myriotylum*. Among the agents, *Trichoderma harzianum*, *T. aureoviride* and *G. virens* effectively controlled the pathogen growth *in vitro*. In fields, suspensions of *T. harzianum* reduced the density of rot pathogens and enhanced productivity (Ram *et al*., 2000). Zohair *et al*., (2018) screened for promising biocontrol agents from rhizosphere regions of medicinal plants. A total of 104 fungal isolates from different medicinal plants *Ocimum basilicum*, *Mentha piperita* and *Aloe vera* were isolated. Of which, 59 were screened against pathogenic fungi - *Fusarium solani*, *Rhizocotina solani*, *Sclerotium rolfsii* and *Verticillium dahlia*. *In vitro* antagonism was confirmed by confrontation method and scanning electron microscopy. *Aspergillus pseudocaelatus* and *Trichoderma gamsii* were identified as promising isolates which inhibited pathogen growth by 77.90% and 77.98% respectively and field evaluation resulted in improved seedling emergence and plant growth enhancement.

Dadwal *et al*. (1986) analysed rhizosphere microflora of teak plants of varying age. The prevalence of the microflora varied through different stages of the growth of the plant and seedling stage reported higher microflora density. A decline in the microflora abundance was observed with increase in the plant age. In 1995, Yasmeen and Jahan reported the frequency and the abundance of *Prosopis spicigera* rhizosphere fungi more in the vegetative stage as compared to the flowering stage. Yasmeen and Ajaz (1999)

isolated 23 fungal species belonging to 18 genera from rhizosphere and rhizoplane regions of *Albizia lebbek*. Rhizosphere region characterised with more number of fungi compared to rhizoplane and certain groups were specific to either rhizosphere or rhizoplane regions.

3. Phyllosphere and Phylloplane

Above ground plant parts, due to their intricate topographical features abode an impressive population of different microbial associates. The microbial communities comprise different genera of bacteria, fungi, yeasts, algae etc. and bacteria being the most predominant (Beattie and Lindow, 1995; Andrews and Harris, 2000). The phylloplane micro-flora derives utmost interest because of variant roles such as decomposition of senescent plant materials (Dickinson, *et al*., 1975; Osono, 2006), production of secondary metabolites (Buckley, *et al*., 1981; El-Said, 2001; McGinnis, *et al*., 2003; Thakur, *et al.*, 2014), possible biocontrol activity against various pathogens (Avis *et al*., 2001; Bakker, 2003; Evueh and Ogbebor, 2008; Halfeld-Vieira *et al*., 2008; Rivera *et al*., 2009; Evueh, 2010; Sowndhararajan *et al.*,2010) and in nutrient cycling (Lee *et al*., 2002; Bhat *et al*., 2015).

4. Endophytes

Endophyte is a microbe, that colonize inter or intracellular and asymptomatically within healthy plant tissues at least one phase of their life cycle (Azevedo *et al.*, 2000; Kaneko *et al*., 2010). Endophytes get nutrition and protection from the host plant and in return the host plants are benefitted owing to functional metabolites. Many endophytes

are known to control plant pathogenic fungi *in vitro* (Narisawa *et al*., 2004; Aneja *et al*., 2006; Kim *et al*., 2007; Mejia *et al*., 2008).

Market potential of biological control agents

Biopesticides, their eco-friendly approach in the management of plant pests have gained extensive significance worldwide in recent years (Mazid *et al*., 2011). Present farming practices with the recent trend of "Organic food production" primarily focuses on the application of biological based products in the management as well as enhancing the crop yield pave an alternate to chemical applicants (Chandrasekaran *et al*., 2012; Senthil-Nathan, 2013). World-wide research centres, considering chemical pesticide performance and environmental safety are in rigorous efforts to amplify qualitative application of bio-active products actively developing techniques for mass production, storage and shelf life with the ultimate aim of commercial application (Kumar, 2012; Senthil-Nathan, 2013).

Presently world-wide bio-pesticide demand is increasing and India has also shown an increase in the application of bio-active products over the time period. In India as on 2005, bio-pesticides represent 2.89% of the total pesticide market and are expected to increase in the coming years with an annual rise of 2.3% (Thakore, 2006). India due to its rich bio-diversity abode numerous biological organisms and plant derived products serves as clues for developing novel and effective bio-pesticides (Table 1).

Table 1. List of Biological pesticides used in horticulture and agriculture crops

Sl. No.	Biological agent (Trade name)	Target organisms	Crops
1	*Agrobacterium radiobacter* strain K 84. **(Galltrol)**	*Agrobacterium tumefaciens*	Ornamentals, fruits, Nuts
2	*Agrobacterium radiobacter* strain K 1026 **(Nogall)**	*A. tumefaciens* and *A. rhizogenes*	Ornamentals, fruits, Nuts
3	*Bacillus licheniformis* strain SB 0386**(Ecoguard, Novozymes Biofungicide Green Relief)**	Foliar pathogens and blights	Ornamental plants and ornamental turfs
4	*Bacillus pumilus* strain GB 34 **(GB34)**	*Rhizoctonia*, *Fusarium*	Soybean
5	*Bacillus subtilis* strain GB 03 **(Kodiak, Companion)**	*Rhizoctonia*, *Fusarium*, *Aspergillus* and others	Crop seeds including seeds of cotton, peanuts, soybeans, wheat, barley, peas and beans
6	*Bacillus subtilis* strain MBI 600 **(Subtilex, Histick N/T)**	*Rhizoctonia*, *Fusarium*, *Aspergillus*, *Alternaria*	Cotton, peanuts, soybeans, wheat, barley, corn, peas and beans
8	*Bacillus subtilis* var *amyloliquefaciens* strain FZB 24 **(Taegro)**	*Rhizoctonia*, *Fusarium*	Shade and forest tree seedlings, ornamental trees and shrubs
9	*Pseudomonas aureofaciens* strain Tx-1 **(Bio-ject, Spot-less)**	*Sclerotinia homeocarpa*, *Colletotrichum gaminicola*, *Pythium aphanidermatum*, *Microdochium nivale*	Golf course turf
10	*Pseudomonas chlororafis* strain 63-28 **(AtEze)**	*Pythium*, *Rhizoctonia solani*, *Fusarium oxysporum*	Vegetables and ornamentals in green house
11	*Pseudomonas fluorescens* strainA 506 **(Blight Ban A506, Frost Ban)**	Frost damage, Fire blight, bunch rot	Fruit crops, Almonds, Potatoes and Tomatoes
12	*Pseudozyma flocculosa* strain PF-A22UL **(Sporodex L)**	Powdery mildew	Roses and cucumbers in green house

Table 1 cont'd..

Sl. No.	Biological agent (Trade name)	Target organisms	Crops
13	*Pseudomonas syringae* starin ESC 10 **(Bio-save 10LP)**	Post-harvest diseases	Apples, pears, lemons, oranges and grapes
14	*Streptomyces griseoviridis* strain K 61 **(Mycostop)**	Soil borne pathogens	Food crops, ornamentals and tree seedlings
15	*Streptomyces lydicus* strain WYEC 108 **(Actinovate SP, Novozymes BioAg)**	*Pythium, Rhizoctonia, Fusarium, Phytophthora* Powdery Mildew, *Botrytis* blight and others	Greenhouse ornamentals, vegetables and herbs
16	*Ampelomyces quisqualis* strain M 10 **(AQ10 Bio-Fungicide)**	Powdery mildew	Fruits, vegetables and ornamental crops
17	*Aspergillus flavus* strain AF 36 **(*Aspergillus flavus* AF36)**	*Aspergillus flavus*	Cotton
18	*Aspergillus flavus* strain NRRL21,882 **(Afla-guard)**	*Aspergillus flavus*	Peanut
19	*Candida oleophila* starin I 182 **(Aspire)**	Post-harvest diseases	Fruits, vegetables, flowers, ornamentals and other plants
20	*Coniothyrium minitans* strain CON/M/91-08 **(Contans WG, Intercept)**	*Sclerotinia sclerotiorum* and *Sclerotinia minor*	Agricultural soil
21	*Gliocladium catenulatum* strain J 1446 **(Primastop)**	Soil borne pathogens	Vegetables, herbs, spices, ornamentals, tree and shrub seedlings
22	*Gliocladium virens* strain GL 21 **(Soilgard)**	Soil borne pathogens	Ornamental, vegetables and cotton
23	Killed *Myrothecium verrucaria* fermentation solids and soluble **(DiTera)**	Plant parasitic nematodes	Food, fiber and ornamental crops
24	*Trichoderma asperellum* strain ICC 012 plus *Trichoderma gamsii* strain ICC 080 **(BioTam 2.0)**	*Fusarium, Phytophthora, Pythium, Rhizoctonia, Thielaviopsis, Sclereotina*	Greenhouse ornamentals, vegetables and herbs

Table 1 cont'd..

Sl. No.	Biological agent (Trade name)	Target organisms	Crops
25	*Trichoderma asperellum* strain T34 **(Asperello T34)**	*Fusarium, Rhizoctonia, Pythium* and *Phytophthora*	Greenhouse ornamentals
26	*Trichoderma harzianum* ATCC 20,476 **(Binab-T)**	Tree wound pathogens	Ornamental, shade and forest trees
27	*Trichoderma harzianum* strain T 22 **(Root Shied, Plant Shield)**	Soil borne pathogens	Green houses, nurseries turf, home gardens and other outdoor soil
28	*Trichoderma harzianum* strain T 39 **(Trichodex)**	*Botrytis cinerea*	Most food crops
29	*Trichoderma harizanum* Rifai strain KRL-AG2 **(Root Shield)**	*Pythium, Rhizoctonia, Fusarium, Cylindrocladium, Thielaviopsis*	Greenhouse ornamentals, vegetables, and herbs.
30	*Ulocladium oudemansii* strain U 3 **(BotryStop)**	*Botrytis cinerea, Sclerotinia sclerotiorum*	Green house ornamentals, vegetables

Source: L. Pundt, Extension Educator, UConn Extension. 2015. Updated July 2018

Forest cover in Kerala is distributed in 11521.813 km^2 of the total geographical area of the state. Of the total forest cover, plantations constitute about 1556.897 km^2 i.e. 13.513% as per Kerala Forest statistics, 2018. A number of plant species being cultivated which serves as sources for various forest and non-wood forest products and contribute towards the state economy. Fore-seeing the limitation in the availability of the land and hike in the demand for various forest and non-wood forest products forced for the sufficient supply of resources to replenish the planting stocks.

Health of a plant depends upon planting stocks adjudged by numerous factors of which diseases are major. Proper nursery practices and periodic monitoring minimize causalities and if at all diseases do occur, can be managed by the application of

chemicals. Again disease management relies on the proper identification of symptoms and subsequently their cause.

In Kerala, forest health monitoring initiated in 1970's with the outbreak of diseases in Eucalypt plantations. *Cylindrocladium quinqueseptatum* severely affected eucalypt seedlings and resulted in heavy mortality hindering healthy planting stock production in the nurseries and their subsequent out-plantings. In due time, an epidemic caused by *Corticium salmonicolor* resulting pink diseases in *E. tereticornis* became prominent. The disease severely affected almost 55-95 % eucalypt plantations at low and mid altitudes (Gibson and Armitage, 1979).

Initial efforts on *Cylindrocladium* leaf blight and pink disease by Bakshi (1972), Sehgal *et al.*, (1978) and Seth *et al.*, (1978) and water blister in teak by Bakshi and Boyce (1958) and later in 1985, Sharma *et al.*, comprehensively described various nursery and plantation diseases associated with different plant species and their causal agents. Maintenance of seedlings under tropical climate is one of the major tasks for the nursery people as they have to confront with disease problems which if not timely intervened may end up in great devastation.

Teak

Teak (*Tectona grandis* L.) is a large deciduous tree of family Verbenaceae typically known for its high timber quality and desirable wood properties. Teak is indigenous to Southeast Asia and is also grown as one of the major plantation species in different parts of the world (Hedegart, 1976; Keogh, 1979). Tropical Asia accounts for 94% of total teak plantations of which India contributes a major share (44%) followed by

Indonesia (31%) and then by Thailand and Myanmar (7% and 6%), respectively (Ball *et al*., 2000).

In India, first teak plantation was established in Nilambur, Kerala in 1846 initiated by Conolly in 1842 and since 1960's large scale commercial cultivation of teak is practised. Subramanian *et al*., (2000) reported that about 1.5 million ha of teak plantations exist in India under Government Forest Departments and other forest development corporations. Teak plantation in Kerala is spread across 77237.981 ha, which constitutes about 49.61% of total plantation area as per Kerala Forest statistics, 2018. Different planting sources such as seedlings, stumps and tissue culture raised plants are widely in use in nurseries but quality planting stocks are limited, diseases being the major hindrances (Table 2).

Table 2. Diseases of teak seedlings across various forest nurseries and plantations in Kerala

Sl. No.	Diseases in teak seedlings	Pathogens associated	Plant part infected
1	Bacterial collar rot	*Pseudomonas solanacearum*	Collar
2	Bacterial wilt	*Pseudomonas solanacearum*	Root
3	Powdery Mildew	*Uncinula tectonae*	Leaf
4	Leaf Rust	*Olivea tectonae*	Leaf
5	*Phomopsis* Leaf spot	*Phomopsis variosporum*	Leaf
6	*Pseudoepicoccum* Zonate Leaf spot	*Pseudoepicoccum tectonae*	Leaf
7	*Colletotrichum* Leaf spot	*Colletotrichum* state *Glomerella cingulata*	Leaf
8	*Sclerotium* Leaf spot	*Sclerotium rolfsii*	Leaf
9	Pink disease	*Corticium salmonicolor*	Stem
10	Die-back	*Phialophora richardsiae*	Tree
11	Collar rot	*Rhizoctonia solani*	Collar
12	Die-back (Insect)	*Cossus cadambae*	Tree
13	Stem Rot	*Marasmiellus ignobilis*	Stem
14	Mistletoe (Phanerogamic Parasite)	*Dendrophthoe falcata*	Branches

Sources: Sharma *et al.*, 1985, Mohanan, 2001

Mahogany

Mahogany (*Swietenia macrophylla* King.) an important timber species of family Meliaceae is native to Central America and is widely distributed in various parts of the world namely South Eastern Mexico, Central and South America, Philippines, Sri Lanka, Solomon Island, Fiji, Martinique and Western Somoa (Gullison *et al.*, 1996). In India, Mahogany, an exotic tree species was introduced in 1795 in Royal Botanical Garden, Calcutta from West Indies (Troup, 1921).

In Kerala, the species was first introduced in South Malabar in 1872 and later in 1893 a small Mahogany plantation was established in Edacode, North Forest Division. Due to adaptability and remarkable wood qualities, Mahogany gained importance among tree growers and was widely established in different parts of Kerala. According to Kerala Forest Statistics, 2018, Mahogany plantations occupy about 518.152 ha constituting 0.33% of total plantation area. Planting stocks are majorly propagated through seeds. As tropical climate limit seed viability, seedling diseases hinder their quality propagation (Table 3).

Table 3. Diseases of Mahogany seedlings across various forest nurseries and plantations in Kerala

Sl. No.	Diseases in mahogany seedlings	Pathogens associated	Plant part infected
1	Seedling wilt	*Sclerotium rolfsii*	Root
2	Damping off	*Rhizoctonia solani* *Fusarium oxysporum*	Seedling

Source: Mohanan, 2001

With advent in science and technology in the past few years and modified nursery practices (Sharma *et al.*, 1985; Sharma and Mohanan, 1992; Mohanan, 2007) implied significance to the phyto-sanitation strategies thus giving importance to the maintenance of seedling health.

Materials and Methods

Objective – I

Isolation and characterization of rhizosphere and rhizoplane fungi of certain grasses of Kerala parts of the Western Ghats during different seasons

a) Study area

The study sites were randomly selected in 1) Northern (Paithalamala (Kannur), Brahmagiri (Wayanad) and Chembra Peak (Wayanad)), 2) Central (Kole wetlands (Thrissur), Pandarawara (Palakkad) and Karimala (Palakkad)) and 3) Southern (Gandhi SmritiVanam (Alapuzha), Vagamon (Idukki) and Ponmudi (Thiruvananthapuram)) Kerala parts of the Western Ghats based on the type of vegetation and altitude.

In each location, three study sites were identified and in each study site, three quadrats (10x10m) representing three replicates were established. Field visits were made to the study areas at least once in summer (March-April), rainy (July-August) and winter (November-December) seasons during 2016-2018.

b) Selection of perennial grass species

Ten perennial grass species were collected and identified on the basis of morphological and floral characteristics and were compared with those described in standard manuals and flora (Sreekumar and Nair, 1991). Grass species selected were *Alloteropsis cimicina*, *Cynodon dactylon*, *Ischaemum indicum*, *Oplismenus compositus*, *Ottochloa nodosa*, *Panicum repens*, *Paspalidium flavidum*, *Paspalum conjugatum*, *Perotis indica* and *Setaria barbata*.

c) Isolation and characterization of fungal species from rhizosphere and rhizoplane regions of grass species

1) Collection of samples

Ten grasses of each species were identified. The zone of soil immediately adjacent to roots of each plant up to 5-15 cm depth was removed carefully without damaging root using a trowel. Samples consisting of soil as well as the root system were collected in sterile polypropylene bags and labeled.

2) Isolation and identification of fungal species from rhizosphere and rhizoplane regions of grasses

The root system of different grass species were carefully removed from soil and gently shaken to remove excess soil. The soil particles closely adhering to the root system were collected by gentle scraping using a sterile spatula and brushing with a camel hair brush. The collected soil formed the rhizosphere sample. The root system without soil particles was considered as the rhizoplane sample.

Rhizosphere soil samples were subjected to dilution plating on potato dextrose agar (PDA, Himedia) amended with streptomycin (30 mg L^{-1}) (Dhingra and Sinclair, 1993). One gram of rhizosphere soil sample taken in a sterile test tube containing 9 ml sterile distilled water was thoroughly mixed. The soil samples were serially diluted and the process was repeated until the desired dilution was obtained. 0.1 millilitre of aliquot of the desired dilutions (10^{-3} and 10^{-4}) was aseptically pipetted out into sterilized petridishes containing 12-15 ml of molten PDA. Petridishes containing the aliquots were

spread evenly using a sterile spreader. The spread plates were incubated in an incubation chamber at 12/12 h regimes of light and darkness at 25±2ºC for five to seven days.

To isolate rhizoplane fungi, ten root samples of each grass species were washed in slow running tap water for 20-30 min and then washed in 1% sodium hypochlorite solution followed by sterile distilled water twice and excess water was blotted out using sterile blotter papers. The root along the axis was divided in to root base, middle and root tip regions and each region was fragmented into 1 cm long segments. Root segments were placed on 9cm dia. petridishes containing PDA medium amended with streptomycin (30 mg L^{-1}) and incubated as described earlier.

Individual hyphal tips of the fungal colonies growing on PDA were picked from the growing margin with a fine tipped sterile needle and inoculated individually on PDA plate and incubated for five to seven days at 25±2ºC to obtain the pure culture. The fungal species were identified based on colony characteristics and morphology of the fruiting bodies and conidia/spores using standard identification manuals (Ellis, 1971; Barnett and Hunter, 1972; Domsch and Gams, 1972; 1980; Ellis, 1976; Sutton, 1980; Arx, 1981; Subramanian, 1983; Ramarao and Manoharachary; 1990; Ellis and Ellis, 2001).

The fungal isolates that failed to produce reproductive propagules on PDA after 14 days of incubation were considered as non-sporulating fungal isolates (NSF). Further, the NSF isolates were observed for their morphological characteristics.

Statistical analysis

The number of colonies formed on dilution plates were counted, averaged and multiplied by dilution factor (10^4) to determine the number of colony forming units (cfu g^{-1}) in soil sample. The colonization frequency (%) of rhizoplane fungi was calculated as the number of root segments colonized by a specific fungus divided by the total number of segments plated x100 (Fisher and Petrini, 1987). The dominantly occurring fungi were calculated as the colony frequency of a particular species divided by sum of colonization frequency x100 (Kumaresan and Suryanarayanan, 2002). The frequency of occurrence of the rhizosphere and rhizoplane fungi in different samples was determined by the Simpson diversity *(D')* and Shannon diversity *(H')* indices by following the procedures of Magurran (1988).

$$\text{Simpson diversity index } D' = 1 - \sum ni(ni-1)/N(N-1)$$

$$\text{Shannon diversity index } H' = -\sum ni/N \ln ni/N$$

Where,

ni – Number of individuals
N – Total number of individuals

Objective - II

Test the antagonistic ability of rhizosphere and rhizoplane fungal isolates against selected fungal pathogens causing diseases in forest nursery seedlings

a) Testing of rhizosphere and rhizoplane fungal isolates for their antagonistic properties *in vitro*

One hundred and thirty four fungal isolates (both sporulating and non-sporulating ones) isolated from the rhizosphere and rhizoplane regions of 10 grass species were selected on the basis of frequency of colonization in the rhizosphere/rhizoplane.

b) Isolation and identification of fungal pathogens causing root rot and wilt diseases of Teak and Mahogany seedlings

Central Forest Nurseries of Kerala at Kannavam (Kannur), Nilambur (Malappuram), Chettikulam (Thrissur) and Kulathupuzha (Kollam) were surveyed for diseases of teak (*Tectona grandis* L.) and Mahogany (*Swietenia macrophylla* King.) seedlings. Attention was paid to examine roots for any infection. Infected root samples were collected and processed following standard procedures under ambient laboratory conditions. Samples were surface disinfected using 1% sodium hypochlorite solution for 30-60 sec followed by sterile distilled water twice as described previously and incubated on moistened sterile blotter discs contained in Petridishes under darkness for 5-7 days. The infected samples were also incubated by Agar Plate method. Infected root samples were disinfected as previously described and 0.5-1cm long root segments were placed on 9 cm dia. Petridishes containing PDA amended with streptomycin (30 mg L^{-1}) and incubated as described earlier. Isolated fungal colonies were identified using standard

manuals and individual colonies were sub-cultured to obtain pure cultures for further experiments.

c) *In vitro* antagonism by dual culture technique

Fusarium oxysporum (Schlecht. emend. Snyder and Hansen) and *Athelia rolfsii* ((Curzi) C.C. Tu and Kimbr.) causes root rot and wilt diseases in teak and mahogany seedlings, respectively, were isolated from infected seedlings. Selected fungi isolated from the rhizosphere and rhizoplane of the 10 grass species were cultured and maintained on their respective medium under 12/12h light and darkness cycle at 25±2ºC for five days. Five millimetre diameter discs of the selected fungi of grass species and the test pathogens were obtained from the growing margin of five-day-old colony culture using a sterile cork borer. Culture discs, one from each of the pathogens and one test antagonistic fungus (*Fusarium oxysporum + Trichoderma harzianum*, *F. oxysporum + Aspergillus niger*; *Athelia rolfsii + Trichoderma harzianum, A. rolfsii + Aspergillus niger* etc.) were placed at a distance of four centimetres from each other on the culture media contained in Petridishes and three replicates were maintained. Plates were incubated in an incubation chamber as described above. The control plates contained either the disc of the pathogen or test fungus placed in the centre. Inhibition of the pathogen by the test fungus was calculated in comparison to the control (Kucuk and Kivanc, 2004).

$$I = C\text{-}T / C \times 100$$

Where,

I= Inhibition in mycelia growth (%)
C= Growth of mycelium in control (mm)
T= Growth of mycelium in treatment (mm)

Statistical analysis

Experiments were conducted in the factorial design. Triplicate data were subjected to analysis of variance (ANOVA). Once 'F' values were significant, means were separated by Duncan's multiple range test (DMRT, $P_{0.05}$).

d) Evaluation of Antagonism

The evaluation of antagonistic activity between the antagonists and the test pathogen was scored 1-5 (Bell *et al*., 1982). This method was typically followed for the antagonists exhibiting mycelia interaction. The cultures were observed after ten days of incubation. The given isolate was considered to be antagonist if the score was ≤ 2 and not highly antagonist if the score was ≥ 3 (Table 4).

Table 4. Evaluation of Antagonism of rhizosphere and rhizoplane fungal isolates of selected grasses

Colony Interaction	Scale of Antagonism
Complete overgrowth of the antagonist over the pathogen	1
75% overgrowth of the antagonist over the pathogen	2
Both the antagonist and the pathogen grow 50% and neither organism dominate	3
75% overgrowth of the pathogen and withstand antagonism	4
Complete overgrowth of the pathogen	5

Source Bell *et al*., 1982

e) *In vitro* leaf bioassay of selected antagonistic rhizosphere and rhizoplane fungi of selected grass species

The young hyphal tips of the five-day-old colony culture of the candidate fungal isolate were cultured aseptically on PDA medium. Ten mycelial discs of size 5mm dia. were incubated in 1% sucrose solution at 25±2º C for 10 days. After 10 days, the spore

suspension was filtered using sterile muslin cloth and the number of spores was counted using a haemocytometer. The spore count was adjusted to 2×10^6 spores ml^{-1}.

In the case of non-sporulating fungal isolates, the mycelial mat was separated from the sucrose solution using sterile muslin cloth, washed in sterile distilled water and blotted out. The mycelial mat was weighed and macerated (1 g of mycelial mat/5 ml of sterile water) using pestle and mortar to obtain the mycelial suspension (Meera *et al*., 1994).

Healthy leaves of teak and mahogany seedlings were collected and washed with sterile distilled water followed by 70% ethanol and were placed in Petridishes containing two layers of sterilized moistened blotter discs. Abaxial leaf surface was gently brushed with spore suspension of each fungal species and incubated at 25±2º C for 4-5 days. For practical bioassay, the results were recorded on the basis of "symptom" and "symptom-free" only.

Objective – III

To test the competitive saprophytic, root colonization, biological control and resistance inducing abilities of selected antagonistic fungal isolates against selected pathogens

a) Selection of candidate fungal isolates

Based on the previous experiments, nine fungal isolates *Penicillium nigricans* (ISO-11) from *Alloteropsis cimicina*, *Trichoderma harzianum* (ISO-33) and *Trichoderma koningii* (ISO-35) from *Cynodon dactylon*, *Aspergillus niger* (ISO-40) and *Purpureocillium lilacinum* (ISO-48) from *Ischaemum indicum*, *Penicillium multicolor* (ISO-58) from *Oplismenus compositus*, *Clonostachys rosea* (ISO-79) from *Panicum repens*, *Trichoderma* sp. (ISO-106) from *Paspalum conjugatum* and *Trichoderma pseudokoningii* (ISO-116) from *Paspalidium flavidum* were selected and subjected for further antagonistic efficacies. These isolates were tested for their saprophytic and root colonization abilities.

b) Mass production of the inocula of candidate fungal isolates

Selected fungal isolates were cultured on PDA medium. Five mm mycelia discs were obtained from actively growing margins of seven day old cultures by using a cork borer inoculated in Potato Dextrose Broth and were incubated for 14 days at 25±2ºC. Mycelia mats were then separated by filtering through Whatsman No.1 filter paper, dried, grounded using mortar and pestle and centrifuged at 10000 rpm for 15 min to remove hyphal debris to obtain spore suspension. The number of spores was counted using a haemocytometer and was adjusted to 2×10^6 spores ml^{-1}.

In case of non-sporulating fungal isolates, the mycelial mat was separated from the Potato Dextrose broth using sterile muslin cloth, washed in sterile distilled water and blotted out. The mycelia mat was weighed and macerated (1 g of mycelial mat/5 ml of sterile water) using pestle and mortar to obtain the mycelial suspension (Meera *et al*., 1994).

c) Testing of saprophytic ability of candidate fungal isolates

Saprophytic ability of nine selected antagonists was tested by the Cambridge method (Garrett 1970). Freshly harvested paddy straws were cut in to 1-cm long segments and autoclaved. Sterile plastic cups of 7 cm were perforated at the bottom and plugged with sterile cotton pads. These cups were filled with 200 g of autoclaved potting medium up to 7-cm height of the cup and placed with paddy straw segments at 1cm apart from the bottom of the cup. Eighteen autoclaved paddy straw pieces were placed in a radial fashion and sterile potting medium was over laid up to 8-cm height of the cup. 40 ml of conidial suspensions were poured over the potting medium separately and each set was placed in individual plastic trays containing sterile distilled water. The cups were not watered from the top but the potting medium in the cup was allowed to imbibe water only through capillary action from the holes at the bottom of the cup. Colonization of paddy straw segments was determined at 21 days of incubation.

d) Colonization frequency

Paddy straw segments removed after regular intervals of incubation were washed in slow running tap water, then twice in sterile distilled water and incubated on antibiotic amended PDA medium at 25±2^{o}C for 7 days. The fungal colonies growing from these

segments were identified and compared with the characteristics of the original culture. Per cent colonization by the nine selected antagonists at different depth levels at given time was determined using the following formula.

$$\text{Colonization frequency (CF)} = \frac{\text{Number of fungus isolated in each bits}}{\text{Total number of bits observed}} \text{ X } 100$$

e) Testing for root colonization ability of antagonistic fungi and root pathogens

Selected fungal antagonists and the root pathogens namely, *F. oxysporum* and *A. rolfsii* were studied for their interactions with roots of teak and mahogany. Mycelial discs were incubated in 1% sucrose solution for 14 days at 25±2^{o}C and were separated out by filtering through Whatsman No.1 filter paper and centrifuged at 10000 rpm for 15 min to remove hyphal debris. Conidial suspensions obtained were then suspended in sterile distilled water and the concentration was adjusted to 2 x 10^6 using haemocytometer. Fifteen day old teak and mahogany seedlings roots dipped with different concentrations volume/volume (1pathogen: 1 antagonists and 1 pathogen: 5 antagonists) and a separate set for control was also maintained. Seedlings were incubated for 21 days and were observed for possible interactions by root clearing method. Root samples were treated with 10% KOH solution for 1 hour in a hot water bath at 60^{o}C and were washed with distilled water and treated with 2% HCl solution. Samples were stained with 0.05% Trypan blue in lactic acid and kept in a hot water bath for 10-15 min. Samples were de-stained with lactic acid and were observed under the microscope to observe mycelial interactions. Microscopic slides were observed under Leica DM2000 LED microscope

and photo-micrographs were taken using attached Leica DMC2900 camera on the microscope.

f) Biological control and resistance inducing abilities of selected antagonistic Fungi

1) Field experiments

In field conditions, teak and mahogany seedlings planted in grow bags were tested for bio-control abilities by antagonistic fungi during June to December 2019. The sterilized grow bags were filled with solarized soil. Seedlings were grown individually and were allowed to stand for one month. Apparently healthy seedlings without disease symptoms were selected for biological control and resistance inducing abilities. The grow bags with individual seedlings were drenched with spore suspensions of 1 pathogen: 5 antagonists (w/w) (containing 2 x 10^6 spores) mixed with talc powder. Bio-agents and the pathogens treatments were carried out in preventive and curative manner, respectively.

i) Preventive treatment

In preventive treatment, spore suspensions of bio-agents were drenched prior to that of pathogens.

ii) Curative treatment

In curative treatment, spore suspensions of pathogens were drenched prior to that of bio-agents.

Experimental design

Experiment was carried for 6- month period (June to December).

June: One month old seedlings were transferred to polybags

July: Two month old apparently healthy seedlings were ready for treatment

August: Preventive and curative treatments were carried out

September: kept for one month

October: Preventive and curative treatments given as 2nd dose

November: kept for one month

December: Biomass of seedlings were evaluated

2) Biomass evaluation

Plants were uprooted carefully after the treatment period, washed thoroughly, dry and fresh weights were determined. For dry weights, plant samples were dried in oven at 60°C for 72 hrs. Root and shoot lengths of plants were determined and the data were subjected to statistical analysis.

Statistical analysis

The data were subjected to analysis of variance (ANOVA). Once 'F' values were significant, means were separated by Duncan's multiple range tests (DMRT, $P_{0.05}$).

3) Microbial frequency

Soil samples from the rhizosphere region of teak and mahogany seedlings from the above experiment were analysed for microbial colonisation to evaluate the effect of inoculum density applied. Soil dilution method (10^{-3} dilution) was used for each

individual treatment. The number of colonies formed on dilution plates were counted, averaged and multiplied by dilution factor (10^3) to determine the number of colony forming units (cfu g^{-1}) in soil sample.

4) Phyto-chemical analysis

Plant samples of control and different treatments were subjected for phyto-chemical evaluation by Gas Chromatography and Mass Spectroscopy (GC-MS). Methanol extracts of plants (80%) were prepared and analysed for biochemical compounds.

GC-MS analysis

GC-MS analysis was carried out using QP2010S-Shimadzu GC-MS instrument (30m x 0.25mm x 0.25µm, Rxi-5Sil MS). 1µl of the chloroform extract was injected into the GC-MS instrument. Initially the column temperature was maintained at 80^{o}C for 2 minutes, followed by a temperature gradient from 80^{o}C to 260^{o}C and held constant for 10 minutes and finally raised temperature to 280^{o}C and held constant for 6 minutes. The instrument operated in a split mode and NIST 11 and WILEY 8 libraries were used for compound analysis.

Results and Discussions

Objective - 1

Isolation and characterization of rhizosphere and rhizoplane fungi of certain grasses of Kerala parts of the Western Ghats during different seasons

Grass species were collected from (1) Northern (Paithalamala (Kannur), Brahmagiri (Wayanad) and Chembra Peak (Wayanad)) (2) Central (Kole wetlands (Thrissur), Pandarawara (Palakkad) and Karimala (Palakkad)) and (3) Southern (Gandhi Smriti Vanam (Alapuzha), Vagamon (Idukki) and Ponmudi (Thiruvananthapuram)) Kerala parts of the Western Ghats and were identified on the basis of vegetative and floral characteristics by using standard manuals and flora (Plate 1-3).

Details of the subfamily of 10 species of grasses selected for the study are given in the Table 5. These species of grasses were found to occur in all the three seasons - summer (March-April), rainy (July-August) and winter (November-December) seasons of the study period 2016-2018.

Table 5. Details of the subfamily of grass species selected for the isolation of rhizosphere and rhizoplane mycoflora

Sl. No.	Grass species (Common name)	Sub family
1	*Alloteropsis cimicina* (L.) Stapf. (Summer grass)	Panicoideae
2	*Cynodon dactylon* (L.) Pers. (Bermuda grass)	Chloridoideae
3	*Ischaemum indicum* (Houtt.) Merr. (Indian muriana grass)	Panicoideae
4	*Oplismenus compositus* (L.) P. Beauv. (Running mountain grass)	Panicoideae
5	*Ottochloa nodosa* (Kunth) Dandy. (Slender panic grass)	Panicoideae
6	*Panicum repens* (L.) (Torpedo grass)	Panicoideae
7	*Paspalidium flavidum* (Retz.) A. Camus. (Shot grass)	Panicoideae
8	*Paspalum conjugatum* P.J.Bergius. (Hilo grass)	Panicoideae
9	*Perotis indica* (L.) Kuntze. (Indian comet grass)	Chloridoideae
10	*Setaria barbata* (Lam.) Kunth. (Bristly fox tail grass)	Panicoideae

PLATE - 1

1.1. Habit of *Alloteropsis cimicina* (L.) Stapf.

1.2. Habit of *Cynodon dactylon* (L.) Pers.

1.3. Habit of *Ischaemum indicum* (Houtt.) Merr.

PLATE - 1

1.1

1.2

1.3

PLATE - 2

2.1. Habit of *Oplismenus compositus* (L.) P. Beauv.

2.2. Habit of *Ottochloa nodosa* (Kunth) Dandy.

2.3. Habit of *Panicum repens* (L.).

2.4. Habit of *Paspalum conjugatum* P.J.Bergius.

PLATE - 2


2.1


2.2


2.3


2.4

PLATE - 3

3.1. Habit of *Paspalidium flavidum* (Retz.) A. Camus.

3.2. Habit of *Setaria barbata* (Lam.) Kunth.

3.3. Habit of *Perotis indica* (L.) Kuntze.

PLATE - 3


3.1


3.2


3.3

Rhizosphere and rhizoplane regions of the selected grass species yielded a total of 94 species of fungi belonging to 32 genera (Table 6). Fungal colonies were grouped into five true divisions namely Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota and Basidiomycota. The majority of the fungal genera isolated from the rhizosphere and rhizoplane regions of grass species belonged to the division Ascomycota followed by Zygomycota (Table 7 and 8). This observation agrees with the findings of Vargas-Gastelum *et al*. (2015) and Suleiman *et al*., (2019) who studied the rhizosphere flora of *Vachellia pachyceras* in Valle de Las Palmas, Mexico. Studies for the fungal communities in rhizosphere soil under conservation tillage at Yanglin, Shaanxi, China resulted in Ascomycota (average 68.7%) as dominant fungal phyla followed by Zygomycota (average 13.3%) and Basidiomycota (average 4.1%) (Wang *et al*., 2017). In another study by Zhang *et al*., (2019) for rhizosphere fungi of *Ferula sinkiangensis* from Yining city, Xinjiang, China observed higher abundance as well as species richness for Ascomycota among the other phyla. Similarly, Fuentes *et al*., (2020) reported the abundance for Ascomycota with least representation of Glomeromycota and Chytridiomycota in the rhizosphere regions of *Baccharis scandens* and *Solanum chilense* from the Atacama Desert, Chile. The dominance of Ascomycota members may be attributed to the primary decomposers that fall in this order and their competence over the carbon source released by the roots (Hannula *et al.,* 2012; Ma *et al.,* 2013). No fungal species belonging to divisions Chytridiomycota, Glomeromycota and Basidiomycota were isolated.

Table 6. Percentage frequencies of occurrence of fungal isolates from the Rhizosphere and Rhizoplane regions of ten selected grass species

Fungal Species	Occurrence of fungal isolates from the Rhizosphere and Rhizoplane regions of ten selected grass species (%)																			
	Alloterpsis cimicina		***Cynodon dactylon***		***Ischaemum indicum***		***Oplismenus compositus***		***Ottochloa nodosa***		***Panicum repens***		***Paspalum conjugatum***		***Paspalidium flavidum***		***Perotis indica***		***Setaria barbata***	
	RZ	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**
Absidia van Tieghem.	0.00	0.00	0.00	1.11	0.11	0.00	0.00	0.00	0.17	0.00	0.23	0.43	0.31	0.00	0.37	0.51	0.00	0.00	0.32	0.75
Absidia glauca Hagem.	0.00	0.00	0.00	1.11	0.11	0.00	0.00	0.00	0.17	0.00	0.23	0.43	0.31	0.00	0.37	0.51	0.00	0.00	0.32	0.75
Acremonium Link.	0.00	0.00	0.00	0.33	0.00	0.17	0.37	0.00	0.00	0.41	0.26	0.36	0.00	0.00	0.37	0.68	0.00	0.00	0.00	0.00
Acremonium sp.	0.00	0.00	0.00	0.33	0.00	0.17	0.37	0.00	0.00	0.41	0.26	0.36	0.00	0.00	0.37	0.68	0.00	0.00	0.00	0.00
Alternaria Nees	0.34	0.88	0.00	0.54	0.82	0.74	0.49	0.79	0.37	0.63	0.44	0.29	0.25	0.74	0.23	0.6	0.23	0.61	0.37	0.99
Alternaria alternata (Fr.) Keissl.,	0.34	0.88	0.00	0.54	0.34	0.00	0.00	0.79	0.37	0.00	0.00	0.00	0.00	0.00	0.23	0.60	0.23	0.34	0.37	0.68
A. tenuissima (Kunze) Wiltshire.	0.00	0.00	0.00	0.00	0.48	0.74	0.49	0.00	0.00	0.63	0.44	0.29	0.25	0.74	0.00	0.00	0.00	0.27	0.00	0.31
Arthrobotrys corda.	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.20	0.26	0.75	0.31	0.31	0.00	0.32	0.31	0.00	0.00
Arthrobotrys sp.	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.20	0.26	0.75	0.31	0.31	0.00	0.32	0.31	0.00	0.00
Aspergillus Micheli	21.48	28.5	31.9	34.01	27.72	29.00	33.70	32.37	38.68	35.92	32.98	31.56	32.64	36.04	32.81	32.46	30.55	29.01	26.66	27.24
*A. niger*Tiegh.,	7.40	6.56	5.85	7.09	6.52	7.67	8.20	11.10	7.53	8.33	6.12	4.76	2.48	2.94	3.70	2.87	7.97	7.92	7.33	6.56
*A. nidulans*Eidam,	3.98	4.76	3.63	4.67	1.61	2.18	2.84	3.14	6.73	7.01	8.39	9.79	6.43	6.81	2.15	2.10	2.36	2.11	0.00	0.00
A. flavus (Sakag. & K. Yamada)	1.65	4.24	5.38	3.94	1.78	1.86	3.47	4.00	6.16	6.98	2.79	1.90	2.70	2.16	7.79	7.26	2.82	2.45	1.92	2.06
A. glaucus (L.) Link.	3.08	5.14	4.05	4.93	4.71	6.45	2.98	2.90	3.15	2.00	2.21	1.11	2.73	2.58	2.00	2.15	2.59	2.49	1.60	2.20
A. ustus(Bainier) Thom & Church,	0.00	0.00	2.27	2.38	0.00	0.74	0.00	0.00	5.00	5.85	6.36	6.28	0.00	0.00	0.00	0.00	0.00	0.00	3.15	2.66
*A. terreus*Thom,	0.00	1.63	1.16	1.84	1.52	0.00	1.81	0.00	0.00	0.00	0.00	0.87	3.12	3.98	1.89	2.10	1.66	1.77	0.00	0.00
A. ochraceous Wilhelm.	0.70	0.00	3.16	3.42	0.00	0.00	0.83	0.00	0.00	0.00	1.60	0.00	3.01	3.39	2.15	1.47	6.20	5.84	0.00	0.00
A. wentii Wehmer	1.21	1.87	1.86	1.84	2.23	2.18	0.00	0.00	1.62	1.95	1.25	0.00	2.70	3.70	1.61	1.66	0.00	0.00	1.35	1.48

Table 6 Cont'd..

Fungal Species	Occurrence of fungal isolates from the Rhizosphere and Rhizoplane regions of ten selected grass species (%)																			
	Alloterpsis cimicina		***Cynodon dactylon***		***Ischaemum indicum***		***Oplismenus compositus***		***Ottochloa nodosa***		***Panicum repens***		***Paspalum conjugatum***		***Paspalidium flavidum***		***Perotis indica***		***Setaria barbata***	
	RZ	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**
A. ruber Thom & Church,	0.00	0.00	1.64	0.00	2.17	1.70	1.18	0.00	1.73	0.00	0.75	1.47	0.00	0.00	0.00	0.00	0.76	0.00	5.04	5.11
A. penicillioides Speg.	1.51	0.59	0.00	1.34	0.59	0.00	5.56	6.93	1.79	0.00	0.00	0.00	0.58	0.00	5.56	6.17	0.00	0.00	0.00	0.00
A. tamari Kita	0.00	0.00	0.00	0.00	2.03	0.65	0.00	0.00	0.00	0.00	1.04	1.13	0.00	0.00	0.00	0.00	1.95	1.91	0.00	0.00
A. repens (Corda) Sacc	0.00	0.00	1.55	0.00	0.00	0.00	3.47	2.11	1.65	1.37	0.75	1.71	4.42	5.43	0.00	0.00	0.00	0.00	0.60	1.23
A. versicolor (Vuillemin)Tiraboschi.	1.93	3.70	1.36	2.57	4.55	5.57	3.36	2.18	3.32	2.43	1.71	2.55	4.48	5.05	5.96	6.68	4.25	4.53	5.67	5.95
Bipolaris Shoemaker	0.00	0.76	0.00	0.40	0.40	0.00	0.00	0.00	0.34	1.08	0.46	0.31	0.31	0.00	0.59	0.41	0.00	0.00	0.49	0.80
B. cynodontis Wallwork et al.,	0.00	0.43	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.59	0.41	0.00	0.00	0.00	0.00
B. oryzae (Breda de Haan) Shoemaker,	0.00	0.33	0.00	0.40	0.00	0.00	0.34	0.00	0.34	1.08	0.46	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.80
Botryodiplodia (Sacc.) Sacc.,	0.34	0.00	0.39	0.40	0.00	0.19	0.00	0.00	0.60	0.65	0.23	0.31	0.25	0.43	0.00	0.19	0.35	0.58	0.00	0.00
B. theobromae Pat.,	0.34	0.00	0.00	0.40	0.00	0.19	0.00	0.00	0.60	0.65	0.00	0.00	0.25	0.43	0.00	0.19	0.35	0.58	0.00	0.00
B. oncidii (Henn.) Petr. & Syd.,	0.00	0.00	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cephalosporium	0.06	0.00	0.22	0.00	0.54	0.38	2.49	2.45	0.88	0.55	0.32	0.00	3.14	1.99	0.00	0.00	0.00	0.44	0.37	0.75
Cephalosporium sp.	0.06	0.00	0.22	0.00	0.54	0.38	2.49	2.45	0.88	0.55	0.32	0.00	3.14	1.99	0.00	0.00	0.00	0.44	0.37	0.75
Chaetomium Kunze,	0.08	0.00	0.17	0.05	0.23	0.36	0.00	0.26	0.00	0.00	0.78	0.75	0.00	0.00	0.11	0.00	0.00	0.00	0.26	0.51
C. globosum Kunze,	0.08	0.00	0.00	0.00	0.23	0.36	0.00	0.00	0.00	0.00	0.78	0.75	0.00	0.00	0.11	0.00	0.00	0.00	0.26	0.51
C. spirale Zopf,	0.00	0.00	0.17	0.05	0.00	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cladosporium Link	3.9	6.59	7.37	5.59	4.83	5.24	5.59	3.84	4.83	4.62	3.80	4.91	3.03	3.01	4.21	3.38	3.49	5.09	3.64	4.26
C. fulvum Cooke,	0.00	0.00	1.47	0.40	0.31	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. herbarum (Pers.) Link,	1.91	3.10	2.66	2.76	2.03	1.94	2.78	1.89	2.24	2.09	2.18	2.96	1.17	1.33	2.20	1.71	2.15	2.74	1.60	1.91

Table 6 Cont'd..

Fungal Species	Occurrence of fungal isolates from the Rhizosphere and Rhizoplane regions of ten selected grass species (%)																			
	Alloterpsis cimicina		***Cynodon dactylon***		***Ischaemum indicum***		***Oplismenus compositus***		***Ottochloa nodosa***		***Panicum repens***		***Paspalum conjugatum***		***Paspalidium flavidum***		***Perotis indica***		***Setaria barbata***	
	RZ	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**
C. cladosporioides (Fresen.) G.A. de Vries,	1.99	3.48	3.24	2.43	2.48	2.80	2.81	1.94	2.58	2.53	1.63	1.95	1.86	1.68	2.00	1.66	1.34	2.35	2.03	2.35
Colletotrichum Corda	0.48	1.99	0.64	1.18	1.07	0.45	0.00	0.62	3.18	2.24	1.22	1.78	1.47	1.61	1.69	2.07	1.48	1.89	1.00	1.04
C. acutatum J.H. Simmonds.	0.00	0.81	0.19	0.14	0.37	0.00	0.00	0.00	0.00	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. capsici (Syd.) E.J. Butler & Bisby	0.00	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.88	0.89	0.00	0.00	0.47	0.00	0.59	0.84	0.00	0.00	0.23	0.00
C. gloeosporioides (Penz.) Penz. & Sacc.,	0.48	0.73	0.44	0.85	0.31	0.00	0.00	0.00	1.93	1.35	0.70	0.99	1.00	1.61	1.10	1.23	0.96	1.38	0.77	1.04
C. dematium (Pers.) Grove.	0.00	0.00	0.00	0.19	0.40	0.45	0.00	0.62	0.37	0.00	0.00	0.79	0.00	0.00	0.00	0.00	0.52	0.51	0.00	0.00
Clonostachys Corda	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.40	2.09	0.22	0.26	0.00	0.00	0.00	0.00	0.00	0.00
C. rosea (Link) Schroers, Samuels, Seifert & W. Gams,	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.40	2.09	0.22	0.26	0.00	0.00	0.00	0.00	0.00	0.00
Curvularia Boedijn,	2.83	5.05	7.34	5.42	4.38	5.64	5.33	6.21	5.20	6.5	3.43	5.32	2.53	3.49	4.69	5.31	5.50	5.33	5.18	4.55
C. clavata B.L. Jain,	0.00	0.69	1.03	1.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.00	0.82	1.04	0.00	0.00	0.00	0.00
C. lunata (Wakker) Boedijn,	1.63	2.11	2.72	2.43	1.86	2.13	2.47	2.30	1.93	2.09	1.25	2.19	0.64	1.11	2.06	1.88	1.77	1.62	2.49	2.20
C. maculans (C.K. Bancr.) Boedijn,	1.21	2.25	2.47	1.98	2.51	2.84	2.32	2.37	2.75	3.30	1.54	2.14	1.00	1.49	1.81	2.39	3.03	2.83	2.69	2.35
C. pallescens Boedijn,	0.00	0.00	1.14	0.00	0.00	0.67	0.54	1.53	0.51	1.11	0.64	0.99	0.53	0.88	0.00	0.00	0.70	0.87	0.00	0.00
Fusarium Link	12.34	10.83	5.99	11.01	7.20	10.42	8.09	11.94	7.53	7.03	4.93	6.09	6.40	6.02	7.34	6.97	8.41	6.95	8.48	8.3
F. acutatum Nirenberg & O'Donnell,	0.00	0.00	0.00	0.92	0.31	0.00	0.37	0.00	0.51	0.58	0.00	0.00	0.39	0.38	0.00	0.00	0.00	0.00	0.60	0.00
F. culmorum (Wm.G.Sm.) Sacc.	2.55	1.68	0.14	1.01	1.19	1.63	0.40	0.74	0.88	0.43	0.00	0.00	0.00	0.00	1.10	1.47	0.52	0.00	0.00	0.00
F. equiseti (Corda) Sacc.	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.11	0.00	0.00	0.00	0.00	0.00

Table 6 Cont'd..

Fungal Species	Occurrence of fungal isolates from the Rhizosphere and Rhizoplane regions of ten selected grass species (%)																			
	Alloterpsis cimicina		***Cynodon dactylon***		***Ischaemum indicum***		***Oplismenus compositus***		***Ottochloa nodosa***		***Panicum repens***		***Paspalum conjugatum***		***Paspalidium flavidum***		***Perotis indica***		***Setaria barbata***	
	RZ	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**
C. lunata (Wakker) Boedijn,	1.63	2.11	2.72	2.43	1.86	2.13	2.47	2.30	1.93	2.09	1.25	2.19	0.64	1.11	2.06	1.88	1.77	1.62	2.49	2.20
C. maculans (C.K. Bancr.) Boedijn,	1.21	2.25	2.47	1.98	2.51	2.84	2.32	2.37	2.75	3.30	1.54	2.14	1.00	1.49	1.81	2.39	3.03	2.83	2.69	2.35
C. pallescens Boedijn,	0.00	0.00	1.14	0.00	0.00	0.67	0.54	1.53	0.51	1.11	0.64	0.99	0.53	0.88	0.00	0.00	0.70	0.87	0.00	0.00
Fusarium Link	12.34	10.83	5.99	11.01	7.20	10.42	8.09	11.94	7.53	7.03	4.93	6.09	6.40	6.02	7.34	6.97	8.41	6.95	8.48	8.3
F. acutatum Nirenberg & O'Donnell,	0.00	0.00	0.00	0.92	0.31	0.00	0.37	0.00	0.51	0.58	0.00	0.00	0.39	0.38	0.00	0.00	0.00	0.00	0.60	0.00
F. culmorum (Wm.G.Sm.) Sacc.	2.55	1.68	0.14	1.01	1.19	1.63	0.40	0.74	0.88	0.43	0.00	0.00	0.00	0.00	1.10	1.47	0.52	0.00	0.00	0.00
F. equiseti (Corda) Sacc.	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.11	0.00	0.00	0.00	0.00	0.00
F. roseum Link,	2.22	1.87	0.17	0.14	0.88	1.17	1.35	1.46	0.00	0.00	1.04	1.23	0.00	0.00	0.00	0.00	0.79	0.75	1.29	1.14
F. oxysporum Schltdl.	3.08	3.48	2.27	3.61	1.95	3.04	2.47	2.42	2.44	2.02	2.18	2.38	2.53	2.09	2.74	2.48	2.94	2.35	2.78	2.83
F. verticillioides (Sacc.) Nirenberg	2.50	2.11	1.86	1.91	1.58	2.18	0.00	1.99	0.88	0.89	0.52	0.75	1.45	1.64	1.67	1.64	1.86	1.89	2.46	2.20
F. semitectum Berk. & Ravenel,	0.00	0.00	0.00	1.84	0.00	0.00	2.49	3.21	1.22	1.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F. solani (Mart.) Sacc.	1.99	1.68	1.55	1.58	1.30	2.41	0.77	2.11	1.59	1.64	1.19	1.73	1.86	1.92	1.72	1.37	2.30	1.96	1.35	2.13
Geotrichum Link.	0.62	0.52	0.00	0.00	1.33	0.50	0.49	0.74	0.88	0.99	2.93	2.21	0.19	0.40	0.00	0.00	3.46	2.62	2.61	2.23
Geotrichum sp.	0.08	0.07	0.00	0.00	0.88	0.50	0.00	0.00	0.00	0.00	1.97	1.71	0.00	0.00	0.00	0.00	2.56	1.96	0.00	0.31
G. candidum Link.	0.53	0.45	0.00	0.00	0.45	0.00	0.49	0.74	0.88	0.99	0.96	0.51	0.19	0.40	0.00	0.00	0.90	0.65	2.61	1.91
Helminthosporium Link.	0.00	0.00	0.3	0.35	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.43	0.00	0.00	0.00	0.00
Helminthosporium sp.	0.00	0.00	0.3	0.35	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.43	0.00	0.00	0.00	0.00
Humicola Traaen.	0.00	0.00	0.36	0.00	0.31	0.43	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Humicola sp.	0.00	0.00	0.36	0.00	0.31	0.43	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 6 Cont'd..

Fungal Species	Occurrence of fungal isolates from the Rhizosphere and Rhizoplane regions of ten selected grass species (%)																			
	Alloterpsis cimicina		***Cynodon dactylon***		***Ischaemum indicum***		***Oplismenus compositus***		***Ottochloa nodosa***		***Panicum repens***		***Paspalum conjugatum***		***Paspalidium flavidum***		***Perotis indica***		***Setaria barbata***	
	RZ	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**
Macrophomina Petr	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.39	0.17	0.00	0.00	0.00	0.11	0.00	0.23	0.34	0.00	0.00
Macrophomina phaseolina (Tassi) Goid.,	0.00	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.39	0.17	0.00	0.00	0.00	0.11	0.00	0.23	0.34	0.00	0.00
Mucor P. Micheli ex L.,	1.99	1.68	1.94	2.69	1.58	2.34	1.35	2.45	0.00	2.41	1.10	2.55	2.31	2.89	2.23	2.82	3.90	3.68	3.61	4.28
M. racemosus Fresen.	1.99	1.68	1.27	1.81	1.58	1.51	1.35	2.04	1.31	2.41	0.00	1.08	1.31	1.68	1.21	1.23	2.53	2.45	1.95	1.91
M. irregularis Stchigel, Cano, Guarro & Ed.Alvarez	0.00	0.00	0.67	0.87	0.00	0.84	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.21	1.02	1.28	1.37	1.24	1.32	1.86
M. delicatus L.S. Loh & Kuthub.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.94	0.00	1.10	1.47	0.00	0.00	0.00	0.31	0.00	0.00	0.34	0.51
Myrothecium Tode.	0.39	0.33	0.83	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.00	0.58	0.5	0.20	0.00	0.00	0.00	0.54	0.56
M. cinctum(Corda) Sacc.,	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M.roridum Tode.	0.39	0.33	0.58	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.50	0.20	0.00	0.00	0.00	0.54	0.56
Nigrospora Zinun.	0.50	0.43	0.00	0.00	0.51	0.93	0.00	0.29	0.23	0.43	0.32	0.00	0.53	0.74	0.25	0.22	0.23	0.00	0.00	0.31
N. sphaerica Mason.	0.00	0.00	0.00	0.00	0.51	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N. oryzae (Berk. & Br.) Petch.	0.50	0.43	0.00	0.00	0.00	0.31	0.00	0.29	0.23	0.43	0.32	0.00	0.53	0.74	0.25	0.22	0.23	0.00	0.00	0.31
Paecilomyces Samson.	0.00	0.00	1.69	1.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.30	2.10	0.00	0.00	0.00	0.00
P. variotii Bainier,	0.00	0.00	1.69	1.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.30	2.10	0.00	0.00	0.00	0.00
Penicillium Link.	35.84	22.34	24.33	19.09	28.23	26.97	27.04	25.96	23.86	23.66	28.36	30.12	24.99	23.14	27.87	28.38	30.11	28.89	35.74	32.01
P. chrysogenum Thom,	10.24	6.61	4.96	6.34	2.85	2.18	4.04	2.92	4.74	5.56	4.09	3.27	5.93	6.26	3.56	3.54	7.16	7.68	5.10	3.53
P. oxalicum Currie & Thom,	6.93	3.84	1.55	0.00	0.00	0.00	0.00	1.65	2.75	1.47	0.00	0.00	3.92	4.43	3.36	3.18	3.23	2.59	0.00	0.00
N. oryzae (Berk. & Br.) Petch.	0.50	0.43	0.00	0.00	0.00	0.31	0.00	0.29	0.23	0.43	0.32	0.00	0.53	0.74	0.25	0.22	0.23	0.00	0.00	0.31

Table 6 Cont'd..

Fungal Species	Occurrence of fungal isolates from the Rhizosphere and Rhizoplane regions of ten selected grass species (%)																			
	Alloterpsis cimicina		***Cynodon dactylon***		***Ischaemum indicum***		***Oplismenus compositus***		***Ottochloa nodosa***		***Panicum repens***		***Paspalum conjugatum***		***Paspalidium flavidum***		***Perotis indica***		***Setaria barbata***	
	RZ	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**
P. digitatum (Pers.) Sacc.,	0.00	0.00	3.99	3.51	6.13	7.20	7.08	7.84	0.00	0.00	0.00	0.00	3.20	2.61	0.00	0.00	0.00	0.00	7.76	7.77
P. decumbens Thom,	4.63	3.13	3.71	2.29	1.10	0.41	2.49	2.11	1.22	0.00	6.18	7.72	0.00	0.00	1.58	1.35	0.00	0.00	1.35	1.89
P. multicolor J.F.H. Beyma,	0.00	0.00	2.27	0.00	1.72	0.00	6.28	6.62	0.00	0.00	2.73	3.56	0.00	0.00	1.30	1.47	1.22	0.00	0.00	0.00
P. nigricans Bainier,	2.55	2.16	2.00	2.43	5.00	5.19	1.03	1.10	0.80	1.37	1.65	1.03	0.89	0.00	2.12	2.10	2.53	2.93	5.70	5.88
P. citrinum Thom,	3.67	2.32	2.19	1.84	1.07	1.63	0.00	0.00	1.90	1.71	0.00	0.00	3.01	3.94	5.87	6.10	2.30	2.28	2.55	1.89
P. javanicum J.F.H. Beyma,	1.93	0.00	0.00	0.00	4.55	6.00	1.84	0.00	0.00	0.00	4.85	5.87	0.00	0.00	0.00	0.00	6.14	5.50	6.19	6.46
P. albicans G. Bainier.	1.99	1.68	0.00	0.00	0.00	0.00	2.15	2.18	6.67	7.63	0.00	0.00	2.87	2.11	1.89	2.19	0.00	0.00	0.77	0.00
P. frequentans Westling,	0.00	0.00	1.30	1.34	1.10	0.00	0.00	0.00	2.47	2.43	1.04	0.00	1.81	1.40	5.96	5.64	0.00	0.00	0.95	0.00
P. implicatum P. Biourge.	2.72	1.61	1.08	1.08	0.59	0.00	2.12	0.55	0.00	0.00	3.60	4.16	2.59	2.40	0.00	0.00	1.77	1.91	0.00	0.00
P. pallidum G. Sm.,	1.18	1.00	1.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.92	1.91
P. roqueforti Thom.	0.00	0.00	0.00	0.26	4.12	4.37	0.00	0.98	3.29	3.49	4.21	4.50	0.78	0.00	2.23	2.80	5.76	6.01	3.47	2.69
Periconia Tode.	0.00	0.00	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P. abyssa Kohlm.	0.00	0.00	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pestalotiopsis Steyaert.	3.2	3.46	3.66	2.92	2.80	2.77	2.04	3.26	1.11	1.37	3.08	2.19	2.81	2.68	1.95	2.65	3.67	3.9	2.89	3.9
Pestalotipsis sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P. maculans (Corda) Nag Raj.	1.29	1.59	0.67	1.13	0.00	0.00	0.60	1.10	0.45	0.75	0.35	0.29	0.64	0.00	1.21	1.50	1.72	1.79	0.60	1.23
P. microspora (Speg.) G.C. Zhao & N. Li,	1.15	1.87	1.86	1.79	1.64	1.65	0.95	1.27	0.34	0.63	0.00	0.00	0.86	1.23	0.73	1.16	1.95	1.72	0.00	0.00
P. palustris Nagraj.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.21	1.90	0.00	0.00	0.00	0.00	0.00	0.00	1.89	2.20
P. versicolor Speg.	0.76	0.00	1.14	0.00	1.16	1.12	0.49	0.89	0.31	0.00	0.00	0.00	1.31	1.45	0.00	0.00	0.00	0.39	0.40	0.46

Table 6 Cont'd..

Fungal Species	Occurrence of fungal isolates from the Rhizosphere and Rhizoplane regions of ten selected grass species (%)																			
	Alloterpsis cimicina		***Cynodon dactylon***		***Ischaemum indicum***		***Oplismenus compositus***		***Ottochloa nodosa***		***Panicum repens***		***Paspalum conjugatum***		***Paspalidium flavidum***		***Perotis indica***		***Setaria barbata***	
	RZ	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**
P. roqueforti Thom.	0.00	0.00	0.00	0.26	4.12	4.37	0.00	0.98	3.29	3.49	4.21	4.50	0.78	0.00	2.23	2.80	5.76	6.01	3.47	2.69
Phoma Saccardo	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	1.92	1.71	0.54	0.75	0.00	0.00	0.00	0.00
Phoma sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.14	0.00	0.00	0.00	0.00	1.92	1.71	0.54	0.75	0.00	0.00	0.00	0.00
Phomopsis Sacc. & Roum.	0.00	0.00	0.00	0.00	0.45	0.00	1.49	0.89	0.54	0.58	2.12	1.64	2.09	1.68	0.00	0.00	0.00	0.00	0.00	0.00
Phomopsis sp.	0.00	0.00	0.00	0.00	0.45	0.00	1.49	0.89	0.54	0.58	2.12	1.64	2.09	1.68	0.00	0.00	0.00	0.00	0.00	0.00
Purpureocillium Luangsa-ard, Hywel-Jones, Houbraken & Samson	0.00	0.00	0.00	0.00	3.36	2.13	0.00	0.00	0.00	0.00	0.00	0.00	3.37	1.80	0.00	0.00	0.00	0.00	0.00	0.00
P. lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson	0.00	0.00	0.00	0.00	3.36	2.13	0.00	0.00	0.00	0.00	0.00	0.00	3.37	1.80	0.00	0.00	0.00	0.00	0.00	0.00
Rhizopus Ehrenb.	3.28	3.27	2.19	3.16	1.69	1.89	1.69	1.82	2.50	2.72	1.42	1.76	0.75	0.64	1.36	1.71	1.08	1.5	0.92	1.55
*R. arrhizus*var. tonkinensis (Vuill.) R.Y. Zheng & X.Y. Liu,	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.36	0.00	0.00	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R. microsporus var. microsporus Tiegh.,	0.95	0.81	0.30	0.64	0.88	0.00	0.00	0.00	0.91	0.99	0.46	0.77	0.53	0.64	0.00	0.00	0.00	0.00	0.23	0.51
R. stolonifer (Ehrenb.) Vuill.	2.33	2.46	1.88	2.52	0.82	1.89	1.20	1.46	1.59	1.73	0.52	0.99	0.22	0.00	1.36	1.71	1.08	1.50	0.69	1.04
Scopulariopsis Bainier	1.32	0.00	1.19	0.66	0.00	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S. brevicaulis (Sacc.) Bainier	1.32	0.00	1.19	0.66	0.00	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sordaria Ces. & De Not.	0.31	0.26	1.22	1.08	0.00	0.00	0.00	0.00	0.65	0.00	0.52	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00
S. fimicola(Roberge ex Desm.) Ces. & De Not.,	0.31	0.26	0.36	0.45	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00

Table 6 Cont'd..

Fungal Species	Occurrence of fungal isolates from the Rhizosphere and Rhizoplane regions of ten selected grass species (%)																			
	Alloterpsis cimicina		***Cynodon dactylon***		***Ischaemum indicum***		***Oplismenus compositus***		***Ottochloa nodosa***		***Panicum repens***		***Paspalum conjugatum***		***Paspalidium flavidum***		***Perotis indica***		***Setaria barbata***	
	RZ	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**	**RZ**	**RP**
Talaromyces C.R.Benj.	5.24	4.67	1.58	1.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.70	2.44	0.71	0.27	0.41	0.65	0.00	0.31
T. flavus (Klocker) Stolk and Samson.	5.24	4.67	1.58	1.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.70	2.44	0.71	0.27	0.41	0.65	0.00	0.31
Trichoderma Pers.	3.70	6.35	3.69	4.64	8.75	7.03	4.82	4.68	5.99	7.13	4.96	4.02	5.98	5.93	6.66	6.63	4.77	5.94	5.24	4.53
T. harzianum Rifai,	3.39	6.09	3.69	4.64	5.05	4.71	4.82	4.68	4.09	4.77	4.96	4.02	4.48	4.08	4.49	4.29	4.77	5.50	4.78	4.26
T. koningii Oudem.,	0.31	0.26	0.00	0.00	3.70	2.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.46	0.27
T. pseudokoningii Rifai.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.90	2.36	0.00	0.00	0.00	0.00	2.17	2.34	0.00	0.00	0.00	0.00
Trichoderma sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.50	1.85	0.00	0.00	0.00	0.00	0.00	0.00
Torula Pers.,	0.00	0.00	0.39	0.64	0.00	0.00	0.49	0.55	0.00	0.00	0.00	0.46	0.00	0.00	0.48	0.00	0.17	0.00	0.00	0.00
T. herbarum(Pers.) Link	0.00	0.00	0.39	0.64	0.00	0.00	0.49	0.55	0.00	0.00	0.00	0.46	0.00	0.00	0.48	0.00	0.17	0.00	0.00	0.00
Verticillium Nees.	1.77	2.11	2.02	1.51	3.05	2.25	3.36	0.74	0.23	0.39	1.83	0.60	0.47	1.54	0.73	1.47	1.63	2.28	1.69	1.14
V. dahliae Kleb.	1.04	1.09	1.14	1.51	2.23	1.46	1.35	0.00	0.00	0.00	0.75	0.00	0.47	1.00	0.00	0.00	0.79	1.24	0.46	0.00
V. albo-atrum Reinke and Berthold	0.73	0.62	0.89	0.00	0.82	0.79	0.95	0.74	0.23	0.39	0.52	0.00	0.00	0.55	0.42	0.87	0.00	0.00	1.23	1.14
V. longisporum (C.Stark) Karapapa, Bainbr. and Heale.	0.00	0.40	0.00	0.00	0.00	0.00	1.06	0.00	0.00	0.00	0.55	0.60	0.00	0.00	0.31	0.60	0.84	1.04	0.00	0.00
No: of Genera	21	18	24	26	23	21	19	19	19	20	26	21	25	24	25	20	17	16	18	20
No: of Species	48	48	59	58	57	48	49	46	48	46	57	47	56	48	51	46	43	42	47	47

RZ- Rhizosphere, RP- Rhizoplane

Table 7. Fungi belonging to different groups in the rhizoplane regions of ten grass species on PDA in three different seasons

Grass Species and Fungal Divisions	Seasonal occurrence of fungal species in different seasons (%)		
	Rainy	**Winter**	**Summer**
Alloteropsis cimicina			
Chytridiomycota	0.00 (0.00)[1]	0.00 (0.00)	0.00 (0.00)
Zygomycota	13.33 (2.79)	16.13 (2.96)	7.77 (2.44)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	370 (77.38)	387.77 (71.15)	233.33 (73.17)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	94.85 (19.84)	141.12 (25.89)	77.78 (24.39)
TOTAL	**478.18**	**545.02**	**318.88**
Cynodon dactylon			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	16.67 (3.04)	42.21 (7.58)	13.33 (3.51)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	387.76 (70.65)	392.22 (70.46)	190.01 (50.01)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	144.44 (26.32)	122.23 (21.96)	176.67 (46.49)
TOTAL	**548.87**	**556.66**	**380.01**
Ischaemum indicum			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	24.44 (6.18)	30 (7.48)	20.78 (6.18)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	212.34 (53.68)	222.22 (55.40)	236.67 (70.37)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	158.88 (40.17)	148.89 (37.12)	78.89 (23.46)
TOTAL	**395.56**	**401.11**	**336.34**
Oplismenus compositus			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	54.44 (14.71)	34.61 (6.98)	12.22 (3.57)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	210.00 (56.76)	333.34 (67.24)	166.67 (48.70)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	105.56 (28.53)	127.78 (25.78)	163.34 (47.73)
TOTAL	**370.00**	**495.73**	**342.23**
Ottochloa nodosa			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	2.22 (0.69)	8.76 (1.97)	5.43 (1.75)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	272.23 (84.48)	297.67 (66.87)	201.45 (64.73)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	47.78 (14.83)	138.74 (31.17)	104.36 (33.53)
TOTAL	**322.23**	**445.17**	**311.24**

Table 7 Cont'd..

Grass Species and Fungal Divisions	Seasonal occurrence of fungal species in different seasons (%)		
	Rainy	**Winter**	**Summer**
Panicum repens			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	17.78 (4.34)	4.44 (0.97)	16.67 (4.49)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	176.67 (43.09)	394.44 (85.86)	237.78 (64.07)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	215.56 (52.57)	60.00 (13.07)	116.66 (31.44)
TOTAL	**410.01**	**458.88**	**371.11**
Paspalidium flavidum			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	2.47 (0.94)	5.55 (1.04)	1.17 (0.64)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	218.89 (83.40)	390.00 (72.78)	102.23 (55.74)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	41.11 (15.66)	140.34 (26.19)	80.00 (43.62)
TOTAL	**262.47**	**535.89**	**183.40**
Paspalum conjugatum			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	23.33 (4.50)	21.11 (3.49)	5.55 (1.12)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	331.12 (63.81)	445.55 (73.58)	277.77 (55.80)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	164.46 (31.69)	138.90 (22.94)	214.44 (43.08)
TOTAL	**518.91**	**605.56**	**497.76**
Perotis indica			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	6.78 (2.21)	8.96 (2.02)	7.64 (2.80)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	198.67 (64.74)	289.21 (65.07)	165.78 (60.68)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	101.43 (33.05)	146.32 (32.92)	99.78 (36.52)
TOTAL	**306.88**	**444.49**	**273.20**
Setaria barbata			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	2.46 (0.86)	2.22 (0.59)	3.33 (2.14)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	183.46 (63.79)	261.11 (69.12)	75.55 (48.57)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	101.66 (35.35)	114.45 (30.29)	76.66 (49.29)
TOTAL	**287.58**	**377.78**	**155.54**

[1]Number in parenthesis indicate per cent incidence in comparison to the total number of fungi in each grass species.

Table 8. Fungi belonging to different groups in the rhizosphere regions of ten grass species on PDA in three different seasons

Grass Species and Fungal Divisions	Seasonal occurrence of fungal species in different seasons (cfu g^{-1})		
	Rainy	**Winter**	**Summer**
Alloteropsis cimicina			
Chytridiomycota	0.00 (0.00)[1]	0.00 (0.00)	0.00 (0.00)
Zygomycota	1.01 (3.04)	1.11 (3.05)	0.89 (2.93)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	29.91 (90.01)	33.27 (91.50)	27.71 (91.18)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	2.31 (6.95)	1.98 (5.45)	1.79 (5.89)
TOTAL	**33.23**	**36.36**	**30.39**
Cynodon dactylon			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	1.83 (4.81)	2.01 (4.23)	1.45 (4.11)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	33.18 (87.27)	39.31 (82.76)	31.79 (90.01)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	3.01 (7.92)	6.18 (13.01)	2.08 (5.89)
TOTAL	**38.02**	**47.50**	**35.32**
Ischaemum indicum			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	1.96 (5.14)	1.38 (3.02)	0.91 (2.77)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	33.46 (87.79)	39.69 (86.75)	29.63 (90.07)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	2.69 (7.06)	4.68 (10.23)	2.36 (7.17)
TOTAL	**38.11**	**45.75**	**32.90**
Oplismenus compositus			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	0.54 (1.84)	1.04 (3.11)	0.67 (2.37)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	27.45 (93.24)	30.34 (90.78)	26.18 (92.64)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	1.45 (4.93)	2.04 (6.11)	1.41 (4.99)
TOTAL	**29.44**	**33.42**	**28.26**

Table 8 Cont'd..

Grass Species and Fungal Divisions	Seasonal occurrence of fungal species in different seasons (cfu g^{-1})		
	Rainy	**Winter**	**Summer**
Ottochloa nodosa			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	0.43 (1.50)	1.01 (3.07)	0.78 (2.77)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	26.59 (92.65)	29.78 (90.35)	25.57 (90.93)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	1.68 (5.85)	2.17 (6.58)	1.77 (6.29)
TOTAL	**28.70**	**32.96**	**28.12**
Panicum repens			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	1.64 (5.95)	1.77 (5.13)	0.76 (3.27)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	23.46 (85.12)	29.96 (86.87)	21.10 (90.79)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	2.46 (8.93)	2.76 (8.01)	1.38 (5.94)
TOTAL	**27.56**	**34.49**	**23.24**
Paspalidium flavidum			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	1.18 (3.69)	1.78 (5.16)	0.98 (4.24)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	28.76 (90.10)	31.13 (90.28)	21.07 (91.17)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	1.98 (6.20)	1.57 (4.55)	1.06 (4.59)
TOTAL	**31.92**	**34.48**	**23.11**
Paspalum conjugatum			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	1.34 (3.52)	1.76 (3.99)	0.75 (3.11)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	31.89 (83.57)	35.78 (81.30)	28.36 (88.29)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	4.93 (12.92)	6.47 (14.70)	3.01 (9.37)
TOTAL	**38.16**	**44.01**	**32.12**
Perotis indica			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	1.43 (4.48)	1.54 (4.33)	1.37 (4.39)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	28.58 (89.59)	31.38 (88.17)	27.98 (89.62)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	1.89 (5.93)	2.67 (7.50)	1.87 (5.99)
TOTAL	**31.90**	**35.59**	**31.22**

Table 8 Cont'd..

Grass Species and Fungal Divisions	Seasonal occurrence of fungal species in different seasons (cfu g^{-1})		
	Rainy	**Winter**	**Summer**
Setaria barbata			
Chytridiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Zygomycota	0.97 (4.49)	1.21 (4.35)	0.96 (4.88)
Glomeromycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Ascomycota	19.65 (90.85)	25.56 (91.78)	17.69 (89.89)
Basidiomycota	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Non Sporulating Fungi Isolates	1.01 (4.67)	1.08 (3.88)	1.03 (5.23)
TOTAL	**21.63**	**27.85**	**19.68**

[1]Number in parenthesis indicate per cent incidence in comparison to the total number of fungi in each grass species.

Zygomycota - Species of *Absidia, Mucor* and *Rhizopus* and Ascomycota - Species of *Acremonium, Alternaria, Arthrobotrys, Aspergillus, Bipolaris, Botryodiplodia, Cephalosporium, Chaetomium, Cladosporium, Clonostachys, Colletotrichum, Curvularia, Fusarium, Geotrichum, Helminthosporium, Humicola, Macrophomina, Myrothecium, Nigrospora, Paecilomyces, Penicillium, Periconia, Pestalotiopsis, Phoma, Phomopsis, Scopulariopsis, Sordaria, Torula, Trichoderma* and *Verticillium.*

In addition, varying percentages of non-sporulating fungi was also observed in the rhizosphere and rhizoplane regions of different grass species. Those fungal colonies which failed to sporulate in the PDA medium were categorized as non-sporulating fungi (NSF) and the present study resulted in the isolation of 26 non-sporulating fungi (Table 9).

In the present study, the rhizoplane was dominated by *Aspergillus, Cladosporium, Curvularia, Fusarium, Penicillium* and *Trichoderma*. *Aspergillus niger, C. maculans, F. oxysporum, F. verticillioides* (*F. moniliforme*), *F. solani, P. chrysogenum* and *T. harzianum* occurred as major fungal species in the rhizoplane. Root colonization of fungal species in grass species varied among the upper, middle and basal portions of the root. The maximum colonization was observed in the middle region followed by upper and basal portions of the root.

Table 9. List of non-sporulating fungal isolates (NSF) from rhizosphere and rhizoplane regions of selected grass species

Sl. No.	Grass species	Non-sporulating fungal isolate	Colony morphology	Hyphal septation
1	*Alloteropsis cimicina*	NSF AC-1	Upper surface: White colour, cottony mycelium, olive-green pigmentation, margins irregular Lower surface: Orange colour, striations with irregular margin	Septate
		NSF AC-2	Upper surface: White colour followed by orange, floccose, margins irregular Lower surface: Cream colour, irregular margin	Septate
2	*Cynodon dactylon*	NSF CD-1	Upper surface: White colour, floccose, margins irregular hairy Lower surface: White colour, irregular margin	Aseptate
		NSF CD-2	Upper surface: Cream colour followed by orange, cottony mycelium, cream pigmentation, margins irregular Lower surface: Yellow colour, striations with irregular margin	Septate
		NSF CD-3	Upper surface: Grey colour, velvety, margins entire Lower surface: Cream colour, irregular margin	Septate
3	*Ischaemum indicum*	NSF II-1	Upper surface: Red colour followed by white, floccose, margins wavy Lower surface: Cream colour, striations with wavy margin	Septate
		NSF II-2	Upper surface: Black colour, velvety, margins entire Lower surface: Black colour and entire margin	Septate
4	*Oplismenus compositus*	NSF OC-1	Upper surface: Red colour, cottony mycelium, margins irregular Lower surface: Peach colour, striations with irregular margin	Aseptate
		NSF OC-2	Upper surface: White colour, aerial raised, margins irregular Lower surface: Off-white and irregular margin	Septate

Table 9 Cont'd..

Sl. No.	Grass species	Non-sporulating fungal isolate	Colony morphology	Hyphal septation
		NSF OC-3	Upper surface: White colour, cottony mycelium with irregular margins Lower surface: Cream colour, striations with irregular margin	Aseptate
		NSF OC-4	Upper surface: Black colour, aerial raised and margins irregular Lower surface: Peach colour, striations with irregular margin	Septate
5	*Ottochloa nodosa*	NSF ON-1	Upper surface: White colour followed by red, floccose, margin irregular hairy Lower surface: red colour later turns to cream, striations with irregular margin	Aseptate
		NSF ON-2	Upper surface: White colour, aerial raised, red pigmentation, margin peltate Lower surface: Cream colour later turns to red, striations with peltate margin	Septate
6	*Panicum repens*	NSF PR-1	Upper surface: White colour followed by peach, cottony, margins peltate hairy Lower surface: Off-white colour later turns to red, striations with peltate margin	Aseptate
7	*Paspalidium flavidum*	NSF PF-1	Upper surface: White-orange colour, floccose, margin peltate Lower surface: Cream colour, striations, hairy margin	Septate
		NSF PF-2	Upper surface: Yellow colour followed by white, floccose, margin undulate Lower surface: Cream colour later turns to brown, sulcations, undulate margin	Septate
8	*Paspalum conjugatum*	NSF PC-1	Upper surface: Black colour, cottony, margin entire Lower surface: Grey colour, striations with sulcations, entire margin	Aseptate
		NSF PC-2	Upper surface: Cream colour, floccose, margin irregular Lower surface: Peach colour and irregular margin	Septate
		NSF PC-3	Upper surface: Yellow colour, velvety, margin entire, yellow pigmentation Lower surface: off-white colour, stellate sulcations, entire margin	Septate

Table 9 Cont'd..

Sl. No.	Grass species	Non-sporulating fungal isolate	Colony morphology	Hyphal septation
		NSF PC-4	Upper surface: Brown colour, cottony mycelium, margin irregular Lower surface: Brown colour and irregular margin	Septate
9	*Perotis indica*	NSF PI-1	Upper surface: White colour followed by violet, cottony, margin irregular Lower surface: White colour later turns to peach and irregular margin	Septate
		NSF PI-2	Upper surface: Brown colour, floccose and irregular margin Lower surface: Brown colour with concentric black rings and margin irregular	Septate
10	*Setaria barbata*	NSF SB-1	Upper surface: Grey colour, velvety and margin entire Lower surface: Cream colour later turns to pale pink and margin entire	Septate
		NSF SB-2	Upper surface: White colour, cottony mycelium, irregular margin and producing cream pigmentation Lower surface: Cream colour and irregular margin	Septate
		NSF SB-3	Upper surface: Red colour, cottony mycelium, irregular margin and producing cream pigmentation Lower surface: Cream colour, striation and irregular margin	Aseptate
		NSF SB-4	Upper surface: Brown colour, floccose and irregular margin Lower surface: Brown colour later turns to black, irregular margin	Septate

In agreement with the results of this study, Bolton *et al*. (1993) reported that fungal colonies were not distributed uniformly throughout the root length. They opined that the maximum fungal colonization at the root tip and nearby regions may be due to root exudations which promote fungal colonization. Naim (1965), who studied the rhizoplane of *Aristida coerulescens* found a fewer fungal species at the base compared

with the root tip. Contrary to this, Ali (1997) observed that certain species of fungi like *Rhizoctonia* and *Fusarium* colonized basal portions of the root in large numbers. Yang and Crowley (2000) suggested that the reformed soil chemistry in response to root exudation judiciously influenced the microbial population. Besides, microbial density and structure also confide upon the nutritional prominence and the type of the soil (Mahafee and Kloepper, 1997; Lupawayi *et al*., 1998; Griffiths *et al*., 1999).

Rhizosphere regions of grass speices were found to be dominated by species of the genera *Aspergillus, Cladosporium, Curvularia, Fusarium, Mucor, Paecilomyces, Penicillium* and *Trichoderma*.

These fungi are predominantly saprophytic in nature and have been found associated with various plant species (Sharma and Chandel, 2003; Khan and Sinha, 2005; Oyeyiola, 2009). Of these, *A. niger, C. lunata, C. maculans, F. oxysporum, P. chrysogenum* and *T. harzianum* occurred as major fungal species.

Higher percentage colonization of fungi in the rhizosphere was observed for *Cynodon dactylon* (Chloridoideae subfamily) and *Ischaemum indicum* followed by *Paspalum conjugatum* (Panicoideae subfamily).Whereas in the grass rhizoplane regions, higher fungal percentage colonization was recorded from *Cynodon dactylon* (Chloridoideae subfamily) and *Paspalum conjugatum* (Panicoideae subfamily).

Fungal isolates were analyzed quantitatively to estimate the richness of fungal species and was denoted by Shannon diversity index (H') and the occurrence of dominant fungi was denoted by Simpson diversity index (D') from rhizosphere and rhizoplane regions of grass species. The diversity was analyzed separately for three different seasons and also for all seasons combined. In the case of rhizoplane regions, for convenience all the three root regions (tip, middle and basal) were combined and averaged for all the three seasons.

Diversity indices for rhizoplane mycoflora of ten grass species during three seasons exhibited a significantly higher diversity (D') for the winter season followed by rainy and summer seasons. Diversity of fungal isolates varied irrespective of the grass species. During winter season among the grass species a higher diversity was observed for *Cynodon dactylon*, *Ischaemum indicum*, *Ottochloa nodosa*, *Paspalidium flavidium* and *Paspalum conjugatum*. In rainy season *Alloteropsis cimicina*, *Cynodon dactylon*, *Paspalidium flavidum*, *Perotis indica* and *Setaria barbata* exhibited a higher diversity of fungal isolates while *Alloteropsis cimicina*, *Ischaemum indicum*, *Ottochloa nodosa* and *Paspalidium flavidium* recorded a higher diversity of fungal isolates during summer season. When fungal species richness (H') for rhizoplane regions of grasses during different seasons were analyzed, *Ottochloa nodosa* (winter), *Paspalidium flavidium* (rainy) and *Cynodon dactylon* (summer) recorded a significantly higher species richness (Table 10).

Table 10. Species diversity of fungal communities in the rhizoplane region of grass species during three seasons

Sl. No.	Grass species	Diversity Index[1]					
		Simpson Diversity Index (D')			Shannon Diversity Index (H')		
		Summer	Winter	Rainy	Summer	Winter	Rainy
1	*Alloteropsis cimicina*	0.88[2]	0.85	0.87	2.35	2.05	2.17
2	*Cynodon dactylon*	0.76	0.88	0.88	2.99	2.22	2.17
3	*Ischaemum indicum*	0.87	0.88	0.86	2.13	2.20	2.02
4	*Oplismenus compositus*	0.82	0.85	0.85	1.92	1.97	2.02
5	*Ottochloa nodosa*	0.88	0.89	0.82	2.23	2.43	1.83
6	*Panicum repens*	0.74	0.86	0.85	1.75	1.96	2.14
7	*Paspalidium flavidum*	0.87	0.88	0.89	2.18	2.19	2.23
8	*Paspalum conjugatum*	0.80	0.88	0.83	1.82	2.22	1.93
9	*Perotis indica*	0.81	0.83	0.87	2.21	1.98	2.15
10	*Setaria barbata*	0.71	0.84	0.87	1.53	1.93	2.10

[1]Experiment was conducted in different seasons (2016-2018), [2]Data is an average of three replicates

When all the seasons were combined and analyzed for diversity indices, *Alloteropsis* cimicina, *Ischaemum indicum*, *Paspalidium flavidum* and *Perotis indica* were found to abide a higher diversity fungal species while higher fungal species richness were found to be associated with *Alloteropsis cimicina*, *Cynodon dactylon*, *Ischaemum indicum*, *Paspalidium flavidum* and *Perotis indica* (Table 11).

Table 11. Species diversity of fungal communities in the rhizoplane region of grass species in all the seasons

Sl. No.	Grass species	Diversity index[1]	
		Simpson Diversity Index (D')	Shannon Diversity Index (H')
1	*Alloteropsis cimicina*	0.87[2]	2.21
2	*Cynodon dactylon*	0.84	2.49
3	*Ischaemum indicum*	0.87	2.12
4	*Oplismenus compositus*	0.84	1.97
5	*Ottochloa nodosa*	0.84	1.98
6	*Panicum repens*	0.81	1.95
7	*Paspalidium flavidum*	0.88	2.21
8	*Paspalum conjugatum*	0.83	1.98
9	*Perotis indica*	0.88	2.15
10	*Setaria barbata*	0.82	1.94

[1]Experiment was conducted in different seasons (2016-2018), [2]Data is an average of three replicates

Likewise rhizosphere regions also exhibited a higher diversity of fungal species during winter season. When Simpson diversity was observed separately for three seasons, *Cynodon dactylon*, *Oplismenus compositus* and *Setaria barbata* recorded a higher diversity during winter season whereas during summer and rainy seasons a higher diversity for fungal species was noted for *Oplismenus compositus* and *Setaria barbata*. Shannon diversity index for rhizosphere fungi during winter season noted significantly higher fungal species richness for *Cynodon dactylon*, *Oplismenus compositus* and *Setaria barbata* while summer and rainy seasons recorded a higher rhizosphere fungal species for *Cynodon dactylon* and *Setaria barbata* grasses (Table 12).

Table 12. Species diversity of fungal communities in the rhizosphere region of grass species during three seasons

Sl. No.	Grass species	Diversity Index[1]					
		Simpson Diversity Index (D')			Shannon Diversity Index (H')		
		Summer	Winter	Rainy	Summer	Winter	Rainy
1	*Alloteropsis cimicina*	0.71[2]	0.79	0.72	1.49	1.67	1.33
2	*Cynodon dactylon*	0.82	0.89	0.82	1.89	2.08	1.87
3	*Ischaemum indicum*	0.81	0.80	0.83	1.68	1.75	1.75
4	*Oplismenus compositus*	0.89	0.88	0.86	1.82	1.98	1.62
5	*Ottochloa nodosa*	0.63	0.79	0.65	1.29	1.68	1.42
6	*Panicum repens*	0.64	0.68	0.66	1.27	1.41	1.31
7	*Paspalidium flavidum*	0.69	0.72	0.70	1.15	1.49	1.30
8	*Paspalum conjugatum*	0.76	0.79	0.82	1.75	1.78	1.75
9	*Perotis indica*	0.63	0.63	0.63	1.29	1.29	1.29
10	*Setaria barbata*	0.91	0.90	0.88	1.96	2.17	2.08

[1]Experiment was conducted in different seasons (2016-2018), [2]Data is an average of three replicates

When all the seasons were combined and analyzed for rhizosphere fungal diversity indices, *Setaria barbata* noticed a higher value for Simpson index indicating a higher diversity of fungal species while Shannon index for species richness associated

with the rhizosphere regions recorded a higher value for *Cynodon dactylon* and *Setaria barbata* compared with the other selected grass species (Table 13).

Table 13. Species diversity of fungal communities in the rhizosphere region of grass species in all the seasons

Sl. No.	Grass species	Diversity Index[1]	
		Simpson Diversity Index (D')	Shannon Diversity Index (H')
1	*Alloteropsis cimicina*	0.75[2]	1.51
2	*Cynodon dactylon*	0.84	1.92
3	*Ischaemum indicum*	0.80	1.72
4	*Oplismenus compositus*	0.87	1.78
5	*Ottochloa nodosa*	0.65	1.33
6	*Panicum repens*	0.66	1.35
7	*Paspalidium flavidum*	0.71	1.28
8	*Paspalum conjugatum*	0.81	1.77
9	*Perotis indica*	0.63	1.29
10	*Setaria barbata*	0.89	2.13

[1]Experiment was conducted in different seasons (2016-2018), [2]Data is an average of three replicates

Qureshi *et al*. (2004) recorded 16 and 10 fungal species associated with the rhizosphere and rhizoplane regions of *Cynodon dactylon,* respectively from Pakistan. Vasanthakumari and Shivanna (2011) in Badra Wildlife sanctuary, Karnataka observed a higher diversity of fungi in the rhizopshere and rhizoplane of *Ischaemum ciliare* in the winter season compared to rainy and summer seasons. They noted that species of *Aspergillus*, *Chaetomium*, *Penicillium* and *Trichoderma* are frequently associated with the grass rhizosphere. A similar study on the rhizosphere and rhizopane fungal communities of grasses belonging to subfamily Chloridoideae indicated higher diversity of fungi during winter season and also among the grass species studied, *Cynodon dactylon* had a greater diversity compared to the other grass species (Shivanna and Vasanthakumari, 2011).

This study showed that the number of fungal colonies in the rhizosphere and rhizoplane regions was higher during the winter season followed by rainy and summer seasons. It is reported that soil characteristics such as availability of nutrients, moisture, P^H etc. enhance microbial association and the lack of favorable conditions during dry seasons limit microbial activity and in turn effective colonization (Mahaffee and Kloepper, 1997). Potts (1994) illustrated water scarcity which results in dehydration of cells and death of roots will affect colonization of root by microbial communities. Also, it is well established that soil moisture and temperature are the major determinants influencing microbial activities in soil (Kaiser *et al*., 2010b; 2011).

Dominant rhizosphere fungi such as species of *Aspergillus*, *Chaetomium*, *Gliocladium*, *Penicillium* and *Trichoderma* are known for their antagonistic and biological control properties (Mathew and Gupta, 1998; Ghini *et al*., 2000; Sheroze *et al*., 2003; Aggarwal *et al*., 2004; Poddar *et al*., 2004; Sabuquillo *et al*., 2010). The present study which evaluated the potential of selected rhizosphere fungi of grasses for potential of biocontrol against certain root pathogens of forestry species was prompted by these studies.

Rhizosphere and rhizoplane fungi vary in species diversity and abundance depending on the host species, soil microclimate, type of exudates released by the roots and microbial interactions within regions. Studies are abound which illustrate the potentials of ability of grass rhizosphere fungi in plant growth promotion and biocontrol of plant diseases (Hyakumachi *et al*. 1992; Shivanna *et al*., 1994; 1996).

Objective - 2

To test the antagonistic ability of fungal isolates against the selected fungal pathogens causing diseases in forest nursery seedlings

Fungal isolates from the rhizosphere and rhizoplane of grass species were tested for antagonistic activity against the two fungal pathogens *Fusarium oxysporum* and *Athelia rolfsii* causing root diseases in teak and mahogany seedlings (Table 14). The selection of isolates was made based on frequency of colonization in the rhizosphere and rhizoplane regions.

Table 14. Rhizosphere and rhizoplane mycoflora of selected grass species selected for *in vitro* antagonistic efficacy

Sl. No.	Grass species	Fungal isolates selected for *in vitro* antagonistic activity
1	*Alloteropsis cimicina*	*Aspergillus niger, A. penicillioides, A. ruber* *Curvularia maculans* *Fusarium* sp. *Paecilomyces variotii* *Penicillium chrysogenum,P. citrinum, P. nigricans* *Phomopsis* sp. *Talaromyces flavus* *Trichoderma harzianum,* 1NSF (Non-sporulating species)
2	*Cynodon dactylon*	*Aspergillus glaucus, A. niger, A. ustus, A. repens, A. tamari, A. versicolor* *Geotrichum* sp. *Phomopsis* sp. *Penicillium albicans, P. citrinum, P. javanicum, P. oxalicum, P. pallidum,* *Pestalotiopsis palustris* *Trichoderma harzianum, T. koningii* *Verticillium vertcillioides* 2 NSF

Table 14 Cont'd..

Sl. No.	Grass species	Fungal isolates selected for *in vitro* antagonistic activity
3	*Ischaemum indicum*	*Aspergillus glaucus, A. niger, A. versicolor* *Curvularia maculans* *Penicillium digitatum, P. javanicum, P. roquefortii* *Purpureocillium lilacinum* *Trichoderma harzianum, T. koningii,* 2 NSF
4	*Oplismenus compositus*	*Aspergillus niger, A. penicillioides* *Cephalosporium* sp. *Fusarium* sp. *Penicillium digitatum, P. multicolor* *Trichoderma harzianum* 3 NSF
5	*Ottochloa nodosa*	*Aspergillus flavus, A. nidulans, A. niger, A. ustus* *Curvularia maculans* *Penicillium albicans, P. crysogenum,* *P. roquefortii* *Trichoderma harzianum, T. pseudokoningii*
6	*Panicum repens*	*Aspregillus nidulans, A. niger, A. ustus* *Clonostachys rosea* *Geotrichum* sp. *Penicillium decumbens, P. implicatum,* *P. javanicum, P. multicolor, P. roqueforti* *Phomopsis* sp. *Pestalotiopsis palustris* *Trichoderma harzianum*
7	*Paspalum conjugatum*	*Aspergillus nidulans, A. ochraceous, A. repens, A. terreus, A. versicolor, A. wentii* *Cephalosporium* sp. *Penicillium crysogenum, P. citrinum, P. oxalicum* *Phoma* sp. *Phomopsis* sp. *Purpureocillium lilacinum* *Talaromyces flavus* *Trichoderma harzianum, Trichoderma* sp. NSF

Table 14 Cont'd..

Sl. No.	Grass species	Fungal isolates selected for *in vitro* antagonistic activity
8	*Paspalidium flavidum*	*Aspergillus flavus, A. penicillioioides,* *A. versicolor* *Curvularia maculans* *Paecilomyces variotii* *Penicillium citrinum, P. frequentens* *Trichoderma harzianum, T. pseudokoningii* NSF
9	*Perotis indica*	*Aspergillus niger, A. ochraceus* *Curvilaria maculans* *Geotrichum* sp. *Penicillium crysogenum, P. javanicum, P. roquefortii* *Trichoderma harzianum*
10	*Setaria barbata*	*Aspergillus niger, A. ruber, A. versicolor* *Geotrichum* sp. *Penicillium digitatum, P. javanicum, P. nigricans* *Pestalotiopsis palustris* *Trichoderma harzianum*

Selection of fungal pathogens

The selection of fungal pathogens is discussed in Materials and methods. The infected root samples of teak and mahogany seedlings yielded *Fusarium* spp., *Athelia rolfsii* (*Sclerotium rolfsii*) and *Rhizoctonia solani* in culture (Table 15 and 16) (Plate 4 and 5). The pathogens were evaluated for pathogenicity and the dominant pathogens against both the seedlings were subjected for *in vitro* interactions against the grass fungal isolates.

Table 15. Nursery diseases of teak seedlings and pathogens associated

Sl. No.	Nursery diseases	Pathogens associated
1	Root wilt, rot	*Fusarium oxysporum*
2	Root rot, collar rot	*F. solani*, *F. Verticillioides* (*F. moniliforme*)
3	Collar rot, damping off	*Athelia rolfsii* (*Sclerotium rolfsii*), *Rhizoctonia solani*

PLATE - 4

4.1. Teak seedlings showing root rot and wilt disease symptoms in Kulathupuzha Central Nursery.

4.2. Mahogany seedlings showing root rot disease symptoms in Chettikulam Central Nursery.

PLATE - 4

PLATE - 5

5.1. Pure culture of fungal pathogen *Fusarium oxysporum* in PDA medium.	5.4. Pure culture of fungal pathogen *Athelia rolfsii* in PDA medium.
5.2. Microscopic view of hyphae showing conidiophores and micro-conida of *Fusarium oxysporum*.	5.5. Microscopic view of hyphae of *Athelia rolfsii* hyphae.
5.3. Microscopic view of hyphae showing conidiophores and macro-conida of *Fusarium oxysporum*.	5.6. Formation of sclerotia of *Athelia rolfsii*.

PLATE - 5

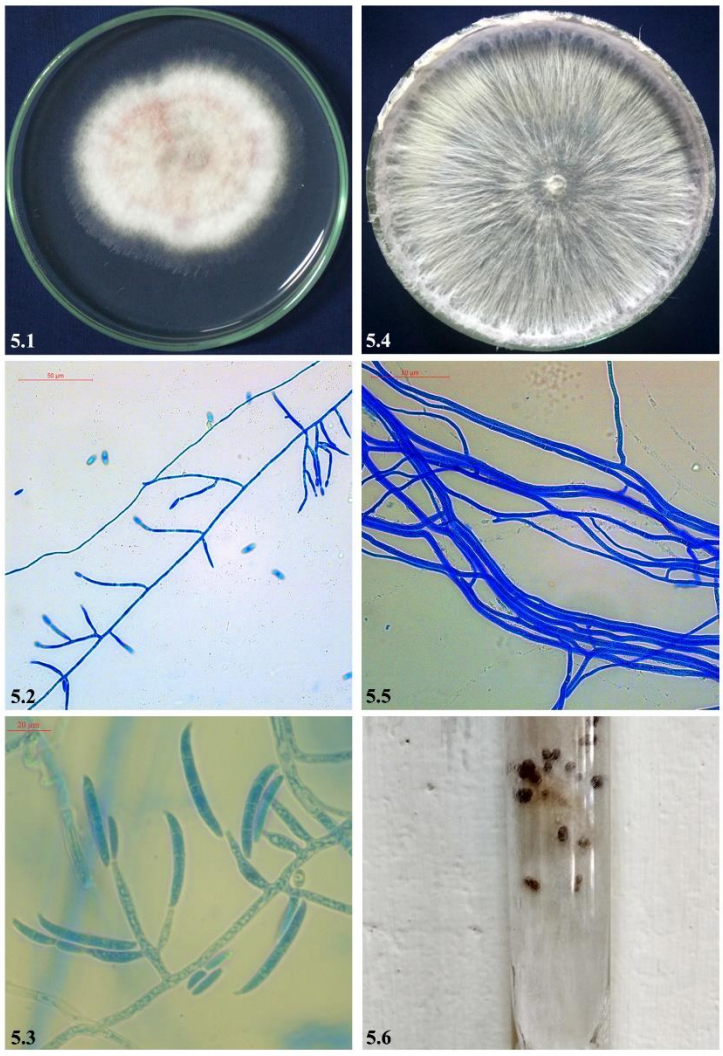


Table 16. Nursery diseases of mahogany seedlings and pathogens associated

Sl. No.	Nursery diseases	Pathogens associated
1	Root wilt, Rot	*Fusarium oxysporum*
2	Root rot, Collar rot	*F. solani*, *F. Verticillioides* (*F. moniliforme*)
3	Collar rot, Damping off	*Athelia rolfsii* (*Sclerotium rolfsii*)

a) *In vitro* antagonistic activity by dual culture

Among the pathogens isolated, *Fusarium oxysporum* from teak and *Athelia rolfsii* (*Sclerotium rolfsii*) from mahogany were found to be pathogenic for both the plant species. Selected pathogens were tested for their *in vitro* antagonistic activity against the selected 134 rhizosphere and rhizoplane fungal isolates.

Fungal isolates exhibited mycelial interaction (MI) as well as inhibition zone (IZ) formation against the selected pathogens. The *in vitro* study revealed varied antagonistic activity between isolates and among the two pathogens.

In the case of *Fusarium oxysporum*, of the 134 isolates tested, 16% (22 isolates) exhibited mycelial interactions of 60% and above, whereas 56% (75 isolates) exhibited mycelial interactions in the range 12 to 59%. Also, 7% (9 isolates) exhibited an inhibition zone and the remaining 21% (28 isolates) exhibited no interaction at all against the pathogen. In the case of *Athelia rolfsii*, 7% (9 isolates) exhibited mycelial interactions of 60% and above, whereas 37% (49 isolates) exhibited mycelial interactions in the range 7 to 59%. Also, 6% (8 isolates) produced an inhibition zone and the remaining 50% (68 isolates) exhibited no interaction against the pathogen.

Trichoderma harzianum (ISO-14) isolated from the grass species *Alloteropsis cimicina* showed the maximum antagonistic activity against both the pathogens. In the case of *Fusarium oxysporum, Penicillium nigricans* (ISO-11) was close behind followed by *Paecilomyces variotii* (ISO- 07) and *Talaromyces flavus* (ISO-13). Except for

Penicillium chrysogenum (ISO-08) and *P. citrinum* (ISO-10) which formed an inhibition zone rest of the isolates exhibited moderate inhibitory activity against *F. oxysporum*. *Curvularia maculans*, *Fusarium* sp. and *Phomopsis* sp. exhibited no antagonistic activity at all. Whereas for *Athelia rolfsii* except for *Trichoderma harzianum* (ISO-14) rest of the isolates caused moderate inhibitory activity and nine isolates noticed no inhibitory activity. However, *Penicillium citrinum* (ISO-10) formed a zone of inhibition (Table 17).

Table 17. Fungal species isolated from rhizosphere and rhizoplane regions of *Alloteropsis cimicina* exhibiting antagonism against the selected pathogens

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		***Athelia rolfsii***	
		PI	**CI**	**PI**	**CI**
1	*Aspergillus niger* ISO-01	42.86±2.86[1 d2]	M I	48.15±1.28[d]	M I
2	*Aspergillus niger* ISO-02	37.14±2.85[c]	M I	-	-
3	*Aspergillus penicillioides* ISO-03	33.33±1.65[c]	M I	14.07±1.29[a]	M I
4	*Aspergillus ruber* ISO-04	16.19±3.29[b]	M I	-	-
5	*Curvularia maculans* ISO-05	-	-	-	-
6	*Fusarium* sp. ISO-06	-	-	-	-
7	*Paecilomyces variotii* ISO-07	63.81±1.65[ef]	M I	42.96±1.28[c]	M I
8	*Penicillium chrysogenum* ISO-08	-	IZ	-	-
9	*Penicillium chrysogenum* ISO-09	12.38±1.65[a]	M I	-	-
10	*Penicillium citrinum* ISO-10	-	IZ	-	IZ
11	*Penicillium nigricans* ISO-11	66.67±3.29[f]	M I	56.29±2.56[e]	M I
12	*Phomopsis* sp. ISO-12	-	-	-	-
13	*Talaromyces flavus* ISO-13	60.00±2.86[e]	M I	41.48±1.28[c]	M I
14	*Trichoderma harzianum* ISO-14	70.48±1.65[g]	M I	60.74±1.28[f]	M I
15	*Trichoderma harzianum* ISO-15	46.66±1.65[d]	M I	34.81±1.28[b]	M I
16	NSF-1 ISO-16	-	-	-	-

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ - Inhibition zone, '-'- Absent

Of the 22 fungal isolates selected from *Cynodon dactylon*, *Trichoderma harzianum* (ISO-33) exhibited prominent mycelial inhibitory activity against *Fusarium oxysporum*. *Penicillium oxalicum* (ISO-28) and *P. citrinum* (ISO-30) formed inhibition zones. The remaining isolates exhibited moderate activity with three isolates exhibiting

no activity at all. For *Athelia rolfsii*, *T. harzianum* (ISO-33) and *T. koningii* (ISO-35) isolates exhibited significant mycelial inhibitory activity compared to other isolates. *Penicillium oxalicum* (ISO-28) and *P. citrinum* (ISO-30) produced zone of inhibition by restricting the mycelial growth of the pathogen (Table 18). The remaining isolates failed to produce activity against the pathogen.

Table 18. Fungal species isolated from rhizosphere and rhizoplane regions of *Cynodon dactylon* exhibiting antagonism against the selected pathogens

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		*Athelia rolfsii*	
		PI	CI	PI	CI
1	*Aspergillus niger* ISO-17	34.28±2.85[1 g2]	M I	14.07±1.28[b]	M I
2	*Aspergillus niger* ISO-18	26.67±1.65[e]	M I	12.59±1.28[a]	M I
3	*Aspergillus tamari* ISO-19	21.91±1.65[bcd]	M I	-	-
4	*Aspergillus glaucus* ISO-20	47.62±1.65[h]	M I	41.48±1.28[e]	M I
5	*Aspergillus glaucus* ISO-21	19.05±1.65[b]	M I	22.96±1.28[c]	M I
6	*Aspergillus ustus* ISO-22	24.76±1.65[de]	M I	-	-
7	*Aspergillus repens* ISO-23	31.43±2.86[f]	M I	-	-
8	*Aspergillus versicolor* ISO-24	29.52±1.65[f]	M I	-	-
9	*Geotrichum* sp. ISO-25	12.36±1.66[a]	M I	-	-
10	*Phomopsis* sp. ISO-26	13.34±1.65[a]	M I	-	-
11	*Penicillium javanicum* ISO-27	20.95±1.65[bc]	M I	-	-
12	*Penicillium oxalicum* ISO-28	-	IZ	-	IZ
13	*Penicillium pallidum* ISO-29	15.24±1.65[a]	M I	-	-
14	*Penicillium citrinum* ISO-30	-	IZ	-	IZ
15	*Penicillium albicans* ISO-31	36.19±1.65[g]	M I	-	-
16	*Pestalotiopsis palustris* ISO-32	-	-	-	-
17	*Trichoderma harzianum* ISO-33	73.34±1.65[k]	M I	65.93±1.28[g]	M I
18	*Trichoderma harzianum* ISO-34	63.81±1.65[i]	M I	37.78±2.22[d]	M I
19	*Trichoderma koningii* ISO-35	67.62±1.65[j]	M I	52.59±1.28[f]	M I
20	*Verticillium vertcillioides* ISO-36	22.86±21.55[cd]	M I	-	-
21	NSF-2 ISO-37	-	-	-	-
22	NSF-3 ISO-38	-	-	-	-

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ – Inhibition zone, '-'- Absent

In the case of *Ischaemum indicum*, of the 14 isolates, except for *Penicillium digitatum* (ISO-45) and *Trichoderma harzianum* (ISO-49) noticed maximum activity and

rest of the isolates showed only a moderate inhibitory activity against *F. oxysporum* and remaining isolates showed no activity. Similarly, in the case of *Athelia rolfsii*, maximum mycelial inhibition was shown by *Trichoderma harzianum* (ISO-49) whereas other isolates showed only moderate activity. Six out of 14 isolates showed no activity. The isolate *Purpureocillium lilacinum* (ISO-48) restricted the growth of the pathogen by forming an inhibition zone around the pathogen (Table 19).

Table 19. Fungal species isolated from rhizosphere and rhizoplane regions of *Ischaemum indicum* exhibiting antagonism against the selected pathogens

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		*Athelia rolfsii*	
		PI	CI	PI	CI
1	*Aspergillus niger* ISO-39	18.09±1.65[1a2]	M I	-	M I
2	*Aspergillus niger* ISO-40	51.43±4.95[d]	M I	40.74±1.28[c]	M I
3	*Aspergillus glaucus* ISO-41	40.95±1.65[c]	M I	45.18±1.28[d]	M I
4	*Aspergillus versicolor* ISO-42	-	-	-	-
5	*Curvularia maculans* ISO-43	-	-	-	-
6	*Penicillium Chrysogenum* ISO-44	15.24±1.65[a]	M I	-	-
7	*Penicillium digitatum* ISO-45	60.95±3.30[f]	M I	40.74±2.57[c]	M I
8	*Penicillium javanicum* ISO-46	26.66±1.65[b]	M I	12.59±1.28[b]	M I
9	*Penicillium roquefortii* ISO-47	21.91±1.65[b]	M I	-	-
10	*Purpureocillium lilacinum* ISO-48	47.62±1.65[c]	M I	-	IZ
11	*Trichoderma harzianum* ISO-49	61.91±2.86[fg]	M I	53.33±1.28[f]	M I
12	*Trichoderma koningii* ISO-50	57.14±0.00[e]	M I	48.15±0.00[e]	M I
13	NSF-4 ISO-51	-	-	-	M I
14	NSF-5 ISO-52	15.24±1.65[a]	M I	13.33±2.23[a]	M I

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ – Inhibition zone, '-'- Absent

The grass *Oplismenus composites* yielded 11 fungal isolates, out of that *Trichoderma harzianum* (ISO-60) and *Penicillium multicolor* (ISO-58) exhibited a significant mycelial inhibitory activity against both the pathogens compared to other isolates. Except for three isolates i.e. *Cephalosporium* sp., *Fusarium* sp. and NSF-6 rest

of the isolates exhibited moderate inhibitory activity against the pathogens studied. *Penicillium digitatum* (ISO-59) inhibited mycelial growth and elongation of both the pathogens by forming an inhibition zone (Table 20).

Table 20. Fungal species isolated from rhizosphere and rhizoplane regions of *Oplismenus compositus* exhibiting antagonism against the selected pathogens

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		*Athelia rolfsii*	
		PI	CI	PI	CI
1	*Aspergillus niger* ISO-53	52.38±1.65[1e2]	M I	41.48±1.28[b]	M I
2	*Aspergillus niger* ISO-54	39.05±1.65[d]	M I	-	-
3	*Aspergillus penicillioides* ISO-55	20.95±1.65[c]	M I	-	-
4	*Cephalosporium* sp. ISO-56	-	-	-	-
5	*Fusarium* sp. ISO-57	-	-	-	-
6	*Penicillium multicolor* ISO-58	64.76±1.65[f]	M I	49.63±1.28[c]	M I
7	*Penicillium digitatum* ISO-59	-	IZ	-	IZ
8	*Trichoderma harzianum* ISO-60	66.66±1.65[f]	M I	54.82±1.29[d]	-
9	NSF-6 ISO-61	-	-	-	-
10	NSF-7 ISO-62	15.24±0.00[b]	M I	10.37±1.28[a]	M I
11	NSF-8 ISO-63	9.52±1.65[a]	M I	-	-

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ – Inhibition zone, '-'- Absent

Antagonism tests using isolates from *Ottochloa nodosa* showed that *Trichoderma harzianum* (ISO-72) and *T. pseudokoningii* (ISO-73) noticed significantly higher mycelial inhibitory activity against *F. oxysporum.* The other isolates showed only moderate inhibitory activity on the pathogen culture. C*urvularia maculans* and *Penicillium albicans* did not inhibit growth. In the case of *Athelia rolfsii*, all except *Trichoderma harzianum* (ISO-72) exhibited a low to moderate inhibition against the pathogen. None of the isolates exhibited a zone of inhibition against cultures of both the pathogens (Table 21).

Table 21. Fungal species isolated from rhizosphere and rhizoplane regions of *Ottochloa nodosa* exhibiting antagonism against the selected pathogens

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		*Athelia rolfsii*	
		PI	CI	PI	CI
1	*Aspergillus niger* ISO-64	48.57±2.86[1e2]	M I	40.74±3.39[b]	M I
2	*Aspergillus flavus* ISO-65	38.09±1.65[d]	M I	-	-
3	*Apsergillus nidulans* ISO-66	30.47±1.65[c]	M I	-	-
4	*Aspergillus ustus* ISO-67	20.95±1.65[b]	M I	9.63±1.28[a]	M I
5	*Curvularia maculans* ISO-68	-	-	-	-
6	*Penicillium chrysogenum* ISO-69	55.24±1.65[f]	M I	50.37±1.28[c]	M I
7	*Penicillium albicans* ISO-70	-	-	-	-
8	*Penicillium roquefortii* ISO-71	15.24±1.65[a]	M I	9.63±1.28[a]	M I
9	*Trichoderma harzianum* ISO-72	67.62±1.65[h]	M I	62.96±1.28[d]	M I
10	*Trichoderma pseudokoningii* ISO-73	60.00±2.86[g]	M I	51.85±1.28[c]	M I

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ – Inhibition zone, '-'- Absent

Fifteen fungal isolates from *Panicum repens* tested, out of that *Clonostachys rosea* (*Gliocladium roseum*) (ISO-79) and *Trichoderma harzianum* (ISO-88) inhibited the growth of *F. oxysporum* mycelium significantly compared to other fungal isolates which exhibited only a moderate activity. *Geotrichum* sp. and *Pestalotiopsis palustris* exhibited no activity against the pathogen. For *Athelia rolfsii*, *Clonostachys rosea* (*Gliocladium roseum*) and *Trichoderma harzianum* exerted the maximum inhibitory activity over the pathogen with other isolates exhibiting a low to moderate inhibitory activity. *Clonostachys rosea* exhibited parasitic interactions against both the pathogens. Microscopic observations revealed hyphal penetration and coiling resulting in cytoplasmic disintegrations (Table 22). The ability of this fungus to curb hyphal elongation of the pathogen by parasitizing and subsequently deforming hyphal structures and also the ability to produce chemicals such as gliotoxin has been demonstrated by various researchers (Mathew and Gupta, 1998; Kwasna *et al*., 1999; Pandey and Upadhyay, 2000).

Table 22. Fungal species isolated from rhizosphere and rhizoplane regions of *Panicum repens* exhibiting antagonism against the selected pathogens

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		*Athelia rolfsii*	
		PI	CI	PI	CI
1	*Aspergillus niger* ISO-74	55.24±1.65[1g2]	M I	41.48±2.56[d]	M I
2	*Aspergillus niger* ISO-75	43.81±1.65[e]	M I	30.37±3.39[c]	M I
3	*Aspregillus ustus* ISO-76	-	-	-	-
4	*Aspergillus nidulans* ISO-77	36.19±1.64[d]	M I	-	-
5	*Aspergillus nidulans* ISO-78	33.34±1.64[c]	M I	-	-
6	*Clonostachys rosea*ISO-79	70.47±1.65[h]	M I	65.19±2.57[f]	M I
7	*Geotrichum* sp. ISO-80	-	-	-	-
8	*Penicillium javanicum* ISO-81	18.09±1.65[a]	M I	-	-
9	*Penicillium decumbens* ISO-82	21.91±1.65[b]	M I	12.59±1.28[a]	M I
10	*Penicillium implicatum* ISO-83	17.14±1.65[a]	M I	-	-
11	*Penicillium multicolor* ISO-84	47.68±1.64[f]	M I	45.28±1.29[e]	M I
12	*Penicillium roquefortii* ISO-85	41.91±1.65[e]	M I	16.30±1.28[b]	M I
13	*Phomopsis* sp. ISO-86	23.80±1.65[b]	M I	-	-
14	*Pestalotiopsis palustris* ISO-87	-	-	-	-
15	*Trichoderna harzianum* ISO-88	71.43±2.86[i]	M I	65.93±2.57[f]	M I

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ – Inhibition zone, '-'- Absent

Among the fungal isolates isolated from *Paspalum conjugatum*, *Trichoderma harzianum* isolates (ISO-104, 105) and *Trichoderma* sp. (ISO-106) exerted significantly higher mycelial inhibitory activity against *F. oxysporum*. *Penicillium oxalicum* (ISO-98) and *P. citrinum* (ISO-99) restricted the pathogen growth by forming a zone of inhibition while the rest of the isolates exhibited a low to moderate inhibitory activity. In the case of *Athelia rolfsii*, except for *Trichoderma harzianum* (ISO-104, 105), the rest of the isolates exhibited only a moderate inhibitory activity. *Penicillium citrinum* (ISO-99) and *Purpureocillium lilacinum* (ISO-102) formed an inhibition zone around the pathogen thereby restricting the mycelial growth (Table 23).

Table 23. Fungal species isolated from rhizosphere and rhizoplane regions of *Paspalum conjugatum* exhibiting antagonism against the selected pathogen

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		*Athelia rolfsii*	
		PI	CI	PI	CI
1	*Aspergillus repens* ISO-89	26.66±1.65[1d2]	M I	-	M I
2	*Aspergillus ochraceus* ISO-90	29.52±1.65[e]	M I	-	-
3	*Aspergillus terreus* ISO-91	23.81±1.64[c]	M I	08.15±1.28[a]	M I
4	*Aspergillus wentii* ISO-92	17.14±1.65[b]	M I	-	-
5	*Aspergillus versicolor* ISO-93	-	-	-	-
6	*Aspergillus nidulans* ISO-94	13.34±1.65[a]	M I	-	-
7	*Cephalosporium* sp. ISO-95	-	-	-	-
8	*Penicillium chrysogenum* ISO-96	49.52±0.00[h]	M I	40.74±2.56[b]	M I
9	*Penicillium chrysogenum* ISO-97	35.24±1.64[f]	M I	-	-
10	*Penicllium oxalicum* ISO-98	-	IZ	-	-
11	*Penicillium citrinum* ISO-99	-	IZ	-	IZ
12	*Phoma* sp. ISO-100	-	-	-	-
13	*Phomopsis* sp. ISO-101	-	-	-	-
14	*Purpureocillium lilacinum* ISO-102	42.86±2.86[g]	M I	-	IZ
15	*Talaromyces flavus* ISO-103	48.57±1.65[h]	M I	42.96±1.28[c]	M I
16	*Trichoderma harzianum* ISO-104	65.71±1.64[k]	M I	62.96±0.00[f]	M I
17	*Trichoderma harzianum* ISO-105	60.95±1.65[j]	M I	54.82±1.29[e]	M I
18	*Trichoderma* sp. ISO-106	61.91±1.65[j]	M I	50.37±1.29[d]	M I
19	NSF-9 ISO-107	53.33±3.30[i]	M I	43.70±1.28[c]	M I

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ – Inhibition zone, '-'- Absent

Among the 10 isolates from *Paspalidium flavidum*, *Trichoderma harzianum* (ISO-115) and *T. pseudokoningii* (ISO-116) exhibited maximum inhibitory activity against *F. oxysporum*. *Penicillium citrinum* (ISO-113) and *P. frequentens* (ISO-114) restricted the pathogen growth by forming a zone of inhibition. Rest of the isolates exerted a low to moderate activity against the pathogen. Against *Athelia rolfsii*, *Trichoderma harzianum* (ISO-115) and *T. pseudokoningii* (ISO-116) showed the maximum growth inhibition. *Penicillium citrinum* (ISO-113) formed a zone of inhibition around the pathogen and the

remaining isolates exerting a moderate inhibitory activity. Four out of ten isolates showed no activity against the test pathogen (Table 24).

Table 24. Fungal species isolated from rhizosphere and rhizoplane regions of *Paspalidium flavidum* exhibiting antagonism against the selected pathogens

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		*Athelia rolfsii*	
		PI	CI	PI	CI
1	*Aspergillus penicillioioides* ISO-108	18.09±1.65[1a2]	M I	-	-
2	*Aspergillus flavus* ISO-109	29.52±2.85[b]	M I	12.59±1.28[a]	-
3	*Aspergillus versicolor* ISO-110	-	-	-	-
4	*Curvularia maculans* ISO-111	-	-	-	-
5	*Paecilomyces variotii* ISO-112	47.62±1.64[c]	M I	41.48±1.26[b]	M I
6	*Penicillium citrinum* ISO-113	-	IZ	-	IZ
7	*Penicillium frequentens* ISO-114	-	IZ	-	-
8	*Trichoderma harzianum* ISO-115	64.76±0.08[e]	M I	60.74±1.18[d]	M I
9	*Trichoderma pseudokoningii* ISO-116	65.71±1.6[e]	M I	62.96±1.28[e]	M I
10	NSF-10 ISO-117	52.38±0.00[d]	M I	45.19±2.56[c]	M I

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ – Inhibition zone, '-'- Absent

Of the eight selected fungal isolates which occur in the rhizosphere/rhizoplane of *Perotis indica*, *Trichoderma harzianum* (ISO-125) showed significantly higher mycelial inhibition activity against *F. oxysporum* while the other species exhibit only a moderate activity. Three isolates failed to show any activity against the pathogen. In the case of *Athelia rolfsii*, none of the isolates were found to be significantly antagonistic against the pathogen. Five out of eight isolates exhibited no potential activity (Table 25).

Among the isolates from *Setaria barabata*, *Trichoderma harzianum* (ISO-134) showed significantly high inhibitory activity against both the pathogens. None of the isolates were found to exhibit any visual zone of inhibition against both the pathogens (Table 26).

Table 25. Fungal species isolated from rhizosphere and rhizoplane regions of *Perotis indica* exhibiting antagonism against selected pathogen

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		*Athelia rolfsii*	
		PI	CI	PI	CI
1	*Aspergillus niger* ISO-118	46.67±1.64[1d2]	M I	41.48±2.56[b]	M I
2	*Aspergillus ochraceus* ISO-119	40.95±1.65[c]	M I	-	-
3	*Curvularia maculans* ISO-120	-	-	-	-
4	*Geotrichum* sp. ISO-121	-	-	-	-
5	*Penicillium chrysogenum* ISO-122	29.52±1.64[b]	M I	18.52±1.28[a]	M I
6	*Penicillium javanicum* ISO-123	24.76±1.65[a]	M I	-	-
7	*Penicillium roquefortii* ISO-124	-	-	-	-
8	*Trichoderma harzianum* ISO-125	68.57±0.00[e]	M I	55.56±2.25[c]	M I

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ – Inhibition zone, '-'- Absent

Table 26. Fungal species isolated from rhizosphere and rhizoplane regions of *Setaria barbata* exhibiting antagonism against the selected pathogens

Sl. No.	Fungal antagonists	Percent inhibition (PI) and colony interaction (CI) of fungal isolates against selected fungal pathogens*			
		Fusarium oxysporum		*Athelia rolfsii*	
		PI	CI	PI	CI
1	*Aspergillus niger* ISO-126	49.52±1.64[1d2]	M I	40.74±2.25[b]	M I
2	*Aspergillus ruber* ISO-127	24.76±2.85[c]	M I	-	-
3	*Aspergillus versicolor* ISO-128	21.91±1.18[b]	M I	14.07±2.56[a]	M I
4	*Geotrichum* sp. ISO-129	-	-	-	-
5	*Penicillium javanicum* ISO-130	16.19±0.08[a]	M I	-	-
6	*Penicillium nigricans* ISO-131	50.48±1.16[d]	M I	40.74±0.00[b]	M I
7	*Penicillium digitatum* ISO-132	49.52±1.65[d]	M I	42.96±1.28[b]	M I
8	*Pestalotiopsis palustris* ISO-133	-	-	-	-
9	*Trichoderma harzianum* ISO-134	68.57±1.64[e]	M I	64.43±0.00[c]	M I

*Data is an average of three replicates, [1]Standard deviation, [2]DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference, MI- Mycelial interaction, IZ – Inhibition zone, '-'- Absent

Dual culture studies displayed mycelial interactions and inhibition zone formation (Plate 6 and 7). The mode of interactions of any particular isolate varied between pathogens studied. Among the isolates, *T. harzianum* (isolate 33 and 88) exhibited a

PLATE - 6

6.1. *Clonostachys rosea* showing mycelial interaction against *Athelia rolfsii*.	6.2. *Clonostachys rosea* showing mycelial interaction against *Fusarium oxysporum*.
6.3. *Trichoderma harzianum* showing mycelial interaction against *Athelia rolfsii*.	6.4. *Trichoderma harzianum* showing mycelial interaction against *Fusarium oxysporum*.
6.5. *Purpureocillium lilacinum* showing antibiosis against *Athelia rolfsii*.	6.6. *Purpureocillium lilacinum* showing antibiosis against *Fusarium oxysporum*.
6.7. *Paecilomyces variotii* showing no interaction against *Athelia rolfsii*.	6.8. *Paecilomyces variotii* showing antibiosis against *Fusarium oxysporum*.

PLATE - 6

PLATE - 7

7.1. *Penicillium multicolor* showing mycelial interaction against *Athelia rolfsii*.	7.2. *Penicillium multicolor* showing mycelial interaction against *Fusarium oxysporum*.
7.3. *Penicillium javanicum* showing no interaction against *Athelia rolfsii*.	7.4. *Penicillium javanicum* showing mycelial interaction against *Fusarium oxysporum*.
7.5. *Aspergillus glaucus* showing no interaction against *Athelia rolfsii*.	7.6. *Aspergillus glaucus* showing mycelial interaction against *Fusarium oxysporum*.
7.7. *Aspergillus ochraceus* showing antibiosis against *Athelia rolfsii*.	7.8. *Aspergillus ochraceus* showing mycelial interaction against *Fusarium oxysporum*.

PLATE - 7

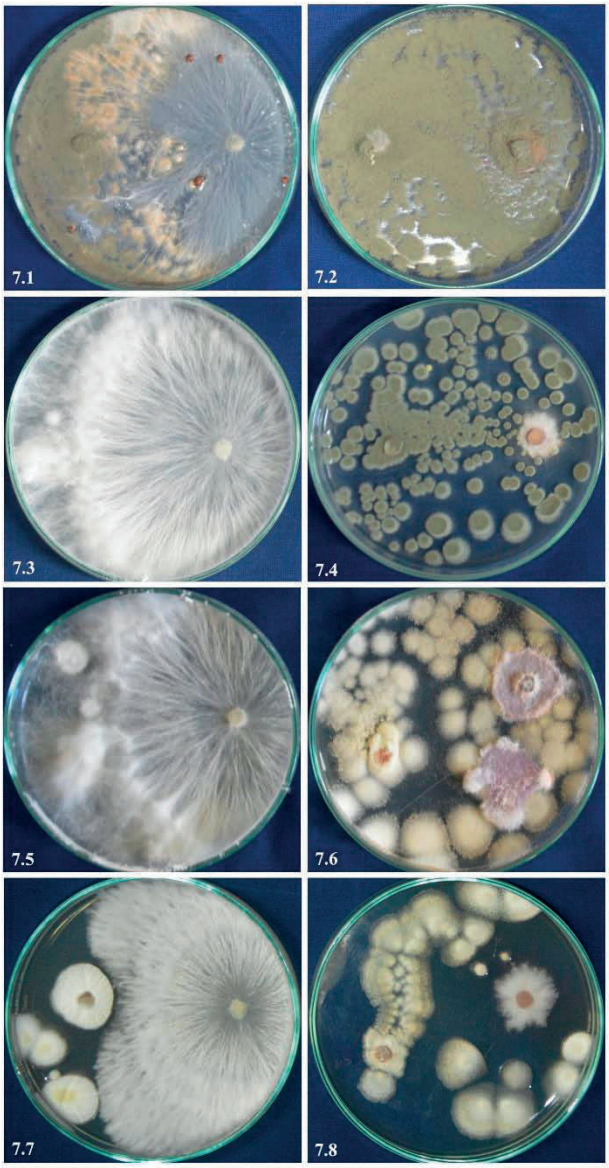
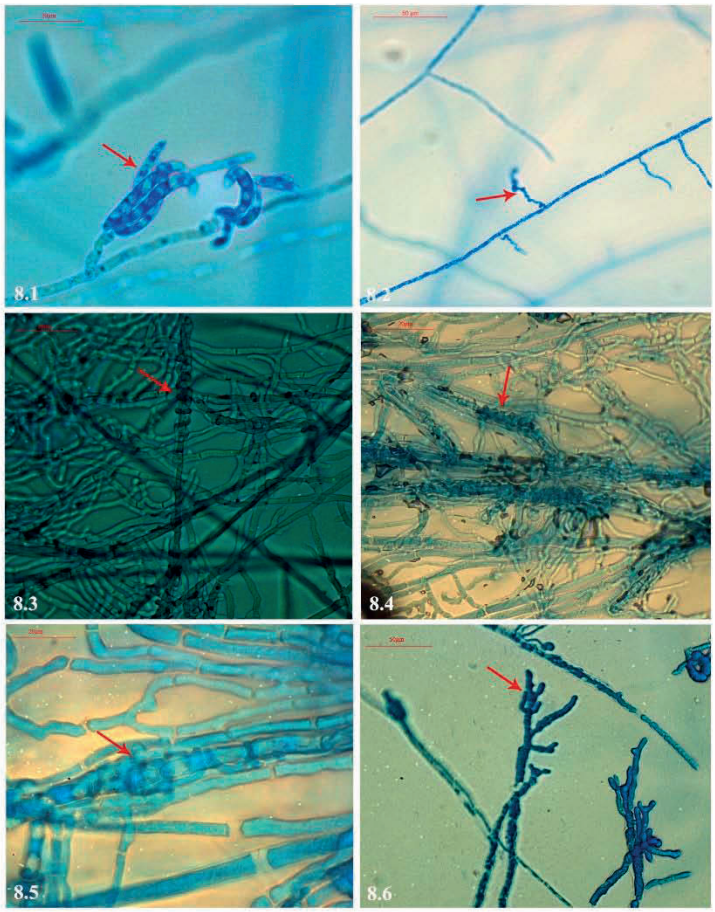


PLATE - 8

8.1. Microscopic view of *Clonostachys rosea* showing conidia and conidiophore deformation of *Fusarium oxysporum*.	8.2. Microscopic view of *Trichoderma harzianum* showing conidiophore deformation of *Fusarium oxysporum*.
8.3. Microscopic view of *Trichoderma harzianum* showing hyphal coiling of *Athelia rolfsii*.	8.4. Microscopic view of *Trichoderma harzianum* showing hyphal coiling of *Athelia rolfsii*.
8.5. Microscopic view of *Trichoderma harzianum* showing hyphal coiling of *Athelia rolfsii*.	8.6. Microscopic view of hyphal deformation of *Fusarium oxysporum* due to antagonist activity of *Purpureocillium lilacinum* hyphae.

PLATE - 8



significantly high mycelial interaction compared to other *T. harzianum* isolates. Similarly, *T. koningii* (isolate 35) and *T. pseudokoningii* (isolate 116) exhibited comparatively higher activity among the isolates studied. Microscopic observations revealed that *Trichoderma* species penetrated in to the hyphae of *F. oxysporum* and caused deformation of conidia and conidiophores. *Trichoderma* caused extensive hyphal coiling and penetration in *Athelia rolfsii* (Plate 8). A number of studies on vegetable crops involving the influence of the species of *Gliocladium*, *Trichoderma* and *Penicillium* as antagonistic agents against different plant pathogen have been highlighted by various research workers (Mathew and Gupta, 1998; Prasad *et al*., 1999; Pandey and Upadhyay, 2000).

Mukherjee *et al*., (1995b) evaluated the efficacy of *Gliocladium virens* and *Trichoderma harzianum* isolated from soil samples, Pantnagar, India against *Rhizoctonia solani* and *Sclerotium rolfsii* infecting chick pea. They observed both *G. virens* and *T. harzianum* were equally effective in inhibiting the mycelial growth of the test pathogens in dual culture assay and observed by SEM the mycelial of the antagonists parasitizing sclerotia and penetrating the hyphae thereby inhibiting mycelial growth of the pathogens. Similarly, Rudresh *et al*., (2005) studied nine isolates of *Trichoderma* spp. against chick pea fungal pathogens *Rhizoctonia solani*, S*clerotium rolfsii* and *Fusarium oxysporum* f. sp. *ciceri in vitro* and *in vivo*. Dual culture assay namely inverted plate technique and poisoned food technique revealed *T. harzianum*-PDBCTH 10 to be more effective in inhibiting mycelial growth of *R. solani* and *S. rolfsii* followed by *T. viride*-PDBCTV 32 and *T. virens*-PDBCTVs 12, whereas *T. virens*-PDBCTVs 12 was found to inhibit

Fusarium oxysporum f. sp. *ciceri* mycelial growth to a greater extent than other isolates. Greenhouse studies imparted *T. harzianum*-PDBCTH 10 to be an effective biological control agent against *Rhizoctonia* root rot and *Sclerotium* collar rot whereas *T. virens*-PDBCTVs 12 was found effective against *Fusarium* wilt. In an another study *Trichoderma viride* was evaluated against soya bean pathogens *Fusarium oxysporum* f. sp. *adzuki* and *Pythium arrhenomanes* from Ontario, Canada *in vitro* and found effective against both the pathogens via myco-parasitism (John *et al*., 2010). *Fusarium* wilt of faba bean (*Vicia faba*) caused by *Fusarium oxysporum* from different localities of Assiut governorate, Egypt was evaluated against biocontrol agents *Trichoderma harzianum*, *T. viride*, *Gliocladium roseum*, *G. catenulatum* and *Saccharomyces cerevisiae*. Fourteen isolates of *F. oxysporum* from diseased faba bean plants were evaluated for growth abilities *in vitro* by dual culture and tested antagonistic fungi were able to inhibit the growth of *F. oxysporum* significantly. Green house experiments showed that faba bean seed coatings with the antagonistic agents strongly suppressed *Fusarium* wilt symptoms (Mahmoud, 2016). Recently in 2020, Rivera-Mendez and colleagues studied efficacy of three native Costa Rican *Trichoderma asperellum* (BCC-1, BCF-2 and BCF-7) strains against white rot causal agent *Sclerotium cepivorum* in onion (A*llium cepa*) by dual culture (mycelial interactions) and cellophane membrane tests (hydrolytic enzyme/metabolic activity). They observed *T. asperellum* BCC-1 strain was highly effective in inhibiting mycelial growth of the pathogen by 81 and 90% in dual culture and cellophane membrane tests. *Trichoderma* species mycelial parasitism and also antibiotic and lytic enzymes production have been highlighted by various researchers (Kucuk and Kivanc, 2004; Grosch *et al.*, 2007; Zafari *et al*., 2008; Hajieghrari *et al*., 2008).

Among other isolates studied, species of *Aspergillus*, *Paecilomyces*, *Penicillium*, *Purpureocillium* and *Talaromyces* also exhibited a significant degree of antagonism against both the pathogens. The current study also revealed the differential activity of the isolates against the two pathogens while a few exhibited antagonism against *F. oxysporum* the same had no antagonistic activity against *A. rolfsii* and vice versa. Based on the results of the *in vitro* studies, further studies were conducted to confirm the efficacy of the isolates against the two pathogens.

b) Evaluation of antagonistic abilities of fungal isolates selected from *in vitro* trials

`The present study revealed different antagonistic abilities (especially hyphal interaction) of isolates of *Trichoderma* against the pathogens. This observation for *Trichoderma* is shared by various workers (Bell *et al*., 1982; Maity and Sen, 1984; Mishra, 2010). The variation in activity could be attributed to the involvement of several genes in both the antagonist and the pathogen in modulating the antagonism. The current study identified 19 fungal isolates *Paecilomyces variotii* (ISO-07), *Penicillium nigricans* (ISO-11), *Talaromyces flavus* (ISO-13), *Aspergillus glaucus* (ISO-20), *Penicillium oxalicum* (ISO-28), *Penicillium citrinum* (ISO-30), *Trichoderma harzianum* (ISO-33), *Trichoderma koningii* (ISO-35) *Aspergillus niger* (ISO-40), *Purpureocillium lilacinum* (ISO-48) *Aspergillus niger* (ISO-53), *Penicillium multicolor* (ISO-58), *Penicillium digitatum* (ISO-59), *Penicillium chrysogenum* (ISO-69), *Clonostachys rosea* (ISO-80), *Penicillium citrinum* (ISO-99), *Trichoderma* sp. (ISO-106), *Penicillium citrinum* (ISO-113) and *Trichoderma pseudokoningii* (ISO-116) from the rhizosphere and rhizoplane

regions of selected grass species with potentials of antagonism against two major pathogens of forest tree seedlings (Table 27)

Table 27. Fungal isolates from selected grass species showing prominent *in vitro* antagonistic activity against *Fusarium oxysporum* and *Athelia rolfsii*

Sl. No.	Grass species	Fungal isolates selected
1	*Alloteropsis cimicina*	*Paecilomyces variotii* ISO-07 *Penicillium nigricans* ISO-11 *Talaromyces flavus* ISO-13
2	*Cynodon dactylon*	*Aspergillus glaucus* ISO-20 *Penicillium oxalicum* ISO-28 *Penicillium citrinum* ISO-30 *Trichoderma harzianum* ISO-33 *Trichoderma koningii* ISO-35
3	*Ischaemum indicum*	*Aspergillus niger* ISO-40 *Purpureocillium lilacinum* ISO-48
4	*Oplismenus compositus*	*Aspergillus niger* ISO-53 *Penicillium multicolor* ISO-58 *Penicillium digitatum* ISO-59
5	*Ottochloa nodosa*	*Penicillium chrysogenum* ISO-69
6	*Panicum repens*	*Clonostachys rosea* ISO-79
7	*Paspalum conjugatum*	*Penicillium citrinum* ISO-99 *Trichoderma* sp. ISO-106
8	*Paspalidium flavidum*	*Penicillium citrinum* ISO-113 *Trichoderma pseudokoningii* ISO-116

The type of interaction of antagonists against the pathogens varied widely. For e.g., *Purpureocillium lilacinum* (isolate 48 and 102) exhibited parasitic activity on the hyphae of *F. oxysporum* whereas it developed a clear inhibition zone for *A. rolfsii*. Microscopic observations indicated that interaction between *P. lilacinum* and the two pathogens showed penetration of hyphae in *F. oxysporum* and hyphae thickening and deformation in *A. rolfsii*. In 2016, Wang and others isolated *Purpureocillium lilacinum* from different places of Beijing and Fujian, China and identified leucinostatins a highly bioactive compound and analyzed for its antagonistic potentiality against *Phytophthora*

infestans and *P. capsici*. They observed a significant activity of the compound in preventing the mycelial growth of the test pathogens *in vitro*. Lan *et al*. (2017) reported the efficacy of *Purpureocillium lilacinum* isolated from the soil in Qinling Mountain, Shaanxi Province, China as a good bio-agent inhibiting mycelial growth (*in vitro*) by exhibiting hyphal interactions against various fungal organisms infecting eggplant namely *Mucor piriformis*, *Trichothecium roseum*, *Rhizoctonia solani* and *Verticillium dahliae*. Ali (2020) in Egypt observed mycelial interference by *P. lilacinum* culture filtrate against the onion white rot pathogen *Sclerotium cepivorum*. The potential of *P. lilacinum* as an entomo-pathogen have been reported previously (Liu *et al*., 2014; Barra *et al*., 2015; Hotaka *et al*., 2015).

Likewise *Talaromyces flavus* (isolate 13 and 103) noticed significant mycelial inhibitory activity against the tested pathogens. The efficacy of *Talaromyces* species especially *Talaromyces flavus* in the management of different soil borne pathogens such as *Verticillium dahliae*, *V. albo-atrum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* have been previously reported (Marois *et al*., 1984; Tjamos and Fravel 1997; Menendez and Godeas 1998; Naraghi *et al*., 2006). Naraghi *et al*., 2008 from Golestan province, Iran isolated *Talaromyces flavus* from soil and analyzed against *Verticillium dahliae* causing wilt disease in cotton. Non-volatile extracts of 20 different *T. flavus* isolates resulted in different *in vitro* efficacy against the pathogen with highest activity exhibited by TF-1 and lowest by TF-16. Similarly, Naraghi *et al*., 2010 observed that non-volatile extracts of *Talaromyces flavus* caused different activities against *Verticillium albo-atrum* causing wilt disease in tomato in Tehran and the Western Azarbayjan provinces of Iran. Among 14 isolates studied, extracts of Tf-To-V-24 and Tf-To-U-36 exhibited a significant activity against the pathogen compared with other isolates.

Anand *et al*., (2016) reported 28 fungal strains isolated from marine sediments of coastal areas against human pathogens in Kanyakumari, India. *Talaromyces flavus* SP5 was comparatively more effective against human pathogens namely, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida tropicalis* compared to *Trichoderma gamsii* SP4 and *Aspergillus oryzae* SP6. In addition, studies from Assiut Governorate (Egypt), a novel myco-parasite *Talaromyces pinophilus* strain AUN-1 isolated from healthy onion umbels was found to be effective against onion scape and umbel blight pathogen *Botrytis cinerea in vitro* inhibiting mycelial growth of the pathogen (Abdel-Rahim and Abo-Elyousr, 2018). A combination of mechanisms such as antibiosis (Fravel and Roberts, 1991), parasitism (McLaren *et al*., 1986; McLaren *et al*., 1989; Fahima and Henis, 1990) and competition (Fravel *et al*., 1986; Marois and Fravel, 1983) have been noticed in *Talarmyces* sp. in controlling the pathogens.

The efficacy of *Penicillium* spp. as potential bio-control agents against plant pathogens are well known (Ma *et al*., 2008; Sabuquillo *et al*., 2010). In the current study *Pencillium* spp. showed varied *in vitro* mycelial interference were observed. When *Penicillium chrysogenum* (isolate 69), *P. multicolor* (isolate 58) and *P. nigricans* (isolate 11) exhibited myco-parasitic interactions rest of the selected *Penicillium* spp. namely, *P. citrinum* (isolate 30), *P. digitatum* (isolate 59) and *P. oxalicum* (isolate 28) formed an inhibition zone restricting the growth of the pathogens in culture. Jackson *et al*. (1994) reported that cell free culture filtrates and spore suspensions of different isolates of *Penicillium chrysogenum* (API. S20, AP2. RI6, ZI. S23 and AP2. RI9) isolated from faba bean crops soil and root inhibited mycelial growth of the pathogen *Botrytis fabae* by all the isolates and the significant activity was noticed by the isolate API S20. In 2006

Sabuquillo and colleagues evaluated *Penicillium oxalicum* against tomato wilt causing pathogens *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium dahlia*.

The antagonism of *Pencillium oxalicum* against the rice fungus *Alternaria alternata* was studied by Sempere and Santamarina (2010) in Valencia, Spain and they visualized myco-parasitic interactions resulting disintegration of conidiophores and conidia. Another *Pencillium* species, *Penicillium citrinum* isolated from Brazilian semi-arid sisal plants (stem endophyte) was investigated for its biocontrol potentiality against the sisal bole rot pathogen caused by *Aspergillus welwitschiae* (Damasceno *et al*., 2019). *In vitro* dual culture noticed 65.8% mycelium growth inhibition of the pathogen and in *in plant bioassay* reduced disease incidence up to 90% when inoculated 48 hours before inoculating the pathogen.

Furthermore, in the current study *Aspergillus* spp. namely *A. glaucus* (isolate 20) and *A. niger* (isolate 53) exhibited a significant myco-parasitic interaction against the pathogens. A number of studies corroborating the potency of *Aspergillus* spp. as an important bio-control agent are well known. Melo *et al*., (2006) observed numerous conidia of *Aspergillus terreus* near *Sclerotinia sclerotiorum* hyphae resulting in mycelial deformation thereby inhibiting the growth of the pathogen when analyzed for *in vitro* antagonism. Aydi Ben Abdallah *et al*., (2015) from Tunisia assessed nine *Aspergillus* spp. (*A. niger* CH-1, CH-12, MC-2, *A. terreus* CH-2, MC-8, *A. flavus* MC-5 and *Aspergillus* sp. CH-3, CH-4, CH-8) isolated from soil and compost against *Fusarium* dry rot and pink rot of potato tuber pathogens - *Fusarium sambucinum* and *Phytophthora erythroseptica* respectively *in vitro* and observed isolate CH12 of *A. niger* exhibiting maximum mycelial inhibitory activity against both pathogens. In Wuhan, China, Hu *et al*., (2016) demonstrated a significant myco-parasitic activity of *Aspergillus* sp. ASP-4

against rape seed pathogen *Sclerotinia sclerotiorum* parasitizing hyphae of the pathogen. Likewise, Hidayat *et al*., (2019) found three isolates of *Aspergillus* spp. namely, *A. terrei* (PD2, PD4) and *A. sydowii* (PD5) forming inhibition zones against *Fusarium oxysporum* f. sp. *cubense* inhibiting the growth of the pathogen.

Similarly, *Paecilomyces* sp. namely *Paecilomyces variotii* in *in vitro* dual culture assay found to be effectively inhibiting hyphal growth of the pathogens *Sclerotium rolfsii* and *Pythium aphanidermatum* by parasitic interactions (Perveen *et al*., 2015). These authors recorded an observation on the bio-agent *Paecilomyces lilacinus* which formed a zone of inhibition against the pathogen *Sclerotium rolfsii* and later overgrew the pathogen. Likewise, Shahzad and Ghaffar (1987) observed an inhibition zone formation by the antagonist *Paecilomyces lilacinus* against *Sclerotium oryzae* initially later after 3 days the pathogen over grew the antagonist. The variation in the activity could be attributed to the genetic makeup of the organism.

So the selected 19 candidate fungal isolates were tested for their hyper sensitivity activity by leaf bioassay using teak and mahogany leaves.

c) *In vitro* leaf bioassay

Determining the pathogenicity of antagonists against the selected forest species in question is a prime requisite for further evaluations of their efficacy.

Ten out of 19 fungal isolates *Paecilomyces variotii* (ISO-07), *Talaromyces flavus* (ISO-13), *Aspergillus glaucus* (ISO-20), *Penicillium oxalicum* (ISO-28), *Penicillium citrinum* (ISO-30), *Aspergillus niger* (ISO-53), *Penicillium digitatum* (ISO-59), *Penicillium chrysogenum* (ISO-69), *Penicillium citrinum* (ISO-99) and *Penicillium*

citrinum (ISO-113) resulted hypersensitivity reactions developing disease symptoms on teak and mahogany leaf samples.

The study proved that nine fungal isolates - *Penicillium nigricans* (ISO 11) from *Alloteropsis cimicina*, *Trichoderma harzianum* (ISO 33) and *T. koningii* (ISO 35) from *Cynodon dactylon*, *Aspergillus niger* (ISO 40) and *Purpureocillium lilacinum* (ISO 48) from *Ischaemum indicum*, *Penicillium multicolor* (ISO 58) from *Oplismenus compositus*, *Clonostachys rosea* (ISO-79) from *Panicum repens*, *Trichoderma* sp. (ISO-106) from *Paspalum conjugatum* and *Trichoderma pseudokoningii* (ISO-116) from *Paspalidium flavidum* used for leaf bioassay did not show any symptoms on inoculated leaves (Table 28) (Plate 9).

Table 28. Antagonists selected after *In vitro* leaf Hypersensitivity assay

Sl. No.	Grass species	Antagonistic isolates selected after *in vitro* leaf bioassay
1	*Cynodon dactylon*	*Trichoderma harzianum* ISO-33 *Trichoderma koningii* ISO-35
2	*Paspalum conjugatum*	*Trichoderma* sp. ISO-106
3	*Oplismenus compositus*	*Penicillium multicolor* ISO-58
4	*Paspalidium flavidum*	*Trichoderma pseudokoningii* ISO-116
5	*Alloteropsis cimicina*	*Penicillium nigricans* ISO-11
6	*Ischaemum indicum*	*Aspergillus niger* ISO-40 *Purpureocillium lilacinum* ISO-48
7	*Panicum repens*	*Clonostachys rosea* ISO-79

Numerous biocontrol agents have been detected by *in vitro* studies but only a few were found to be potentially active based on their non-pathogenic nature on the selected hosts. These non-pathogenic antagonists were selected for studies on biocontrol attributes.

PLATE - 9

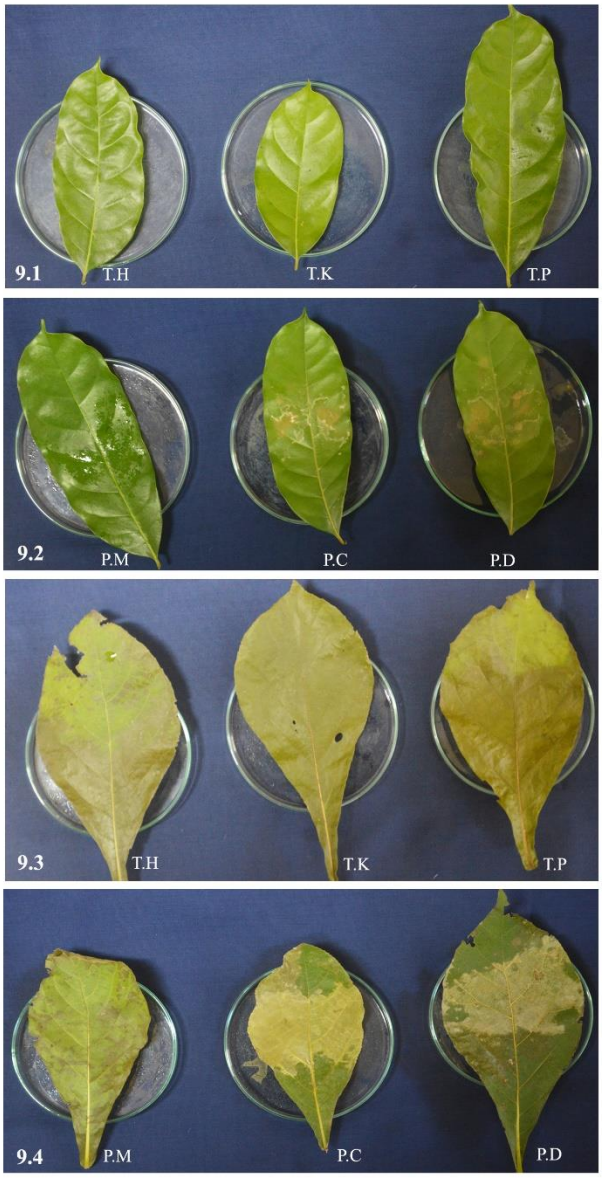
9.1. Detached leaf bio assay showing no symptoms on inoculation of conidial suspensions of *Trichoderma harzianum* (T H), *T. koningii* (T K) and *T. pseudokoningii* (T P) tested in Mahogany leaves.

9.2. Detached leaf bio assay showing no symptoms on inoculation of conidial suspension of *Penicillium multicolor* (P M) but resulted in disease symptoms when tested for *P. citrinum* (P C) and *P. digitatum* (P D) in Mahogany leaves.

9.3. Detached leaf bio assay showing no symptoms on inoculation of conidial suspensions of *Trichoderma harzianum* (T H), *T. koningii* (T K) and *T. pseudokoningii* (T P) tested in Teak leaves.

9.4. Detached leaf bio assay showing no symptoms on inoculation of conidial suspension of *Penicillium multicolor* (P M) but resulted in disease symptoms when tested for *P. citrinum* (P C) and *P. digitatum* (P D) in Teak leaves.

PLATE - 9



Objective – 3

To test the competitive saprophytic, root colonization, biological control and resistance inducing abilities of selected antagonistic fungi against the selected two pathogens

The nine fungal antagonists selected through *in vitro* trials exhibited different antagonistic interactions - parasitism, antibiosis and competition. These fungi namely, *Aspergillus niger* (ISO 40), *Clonostachys rosea* (ISO-79), *Penicillium multicolor* (ISO 58), *Penicillium nigricans* (ISO 11), *Purpureocillium lilacinum* (ISO 48), *Trichderma harzianum* (ISO 33), *T. koningii* (ISO 35), *T. pseudokoningii* (ISO-116) and *Trichoderma* sp. (ISO-106) were also found to be non-pathogenic against the target plant species even at high inoculum density and hence were subjected for further biological control attributes.

a) Competitive saprophytic ability

Broth cultures of all fungal isolates (adjusted to 2 x 10^6 conidial concentrations) were tested for colonization of paddy straw segments after 21 days of incubation. Fungi differed in their ability to saprophytic colonization of paddy straw. *Trichderma harzianum* (ISO 33), *T. koningii* (ISO 35) and *Clonostachys rosea* (ISO-79) colonized up to a depth of 7 cm; *Penicillium multicolor* (ISO 58) and *Purpureocillium lilacinum* (ISO 48) - 6cm; *Aspergillus niger* (ISO 40) and *Trichoderma pseudokoningii* (ISO-116) - 4cm; *Penicillium nigricans* (ISO 11) and *Trichoderma* sp. (ISO-106) - 3cm (Plate 10).

PLATE - 10

10.1. Experimental set up of Competitive Saprophytic ability.

10.2. Re-isolation of bio-agent *Clonostachys rosea* at different depths 2, 3,4,5,6 & 7 cm.

10.3. Re-isolation of bio-agent *Penicillium multicolor* at different depths 2, 3,4,5,6 & 7 cm.

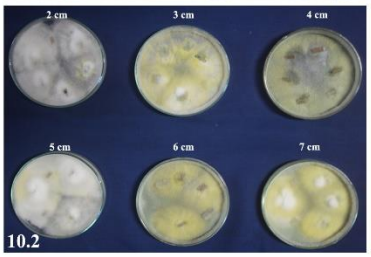
10.4. Re-isolation of bio-agent *Purpureocillium lilacinum* at different depths 2, 3,4,5,6 & 7 cm.

10.5. Re-isolation of bio-agent *Trichoderma harzianum* at different depths 2, 3,4,5,6 & 7 cm.

10.6. Re-isolation of bio-agent *Trichoderma koningii* at different depths 2, 3,4,5,6 & 7 cm.

PLATE - 10


10.1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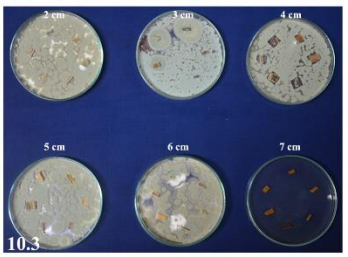
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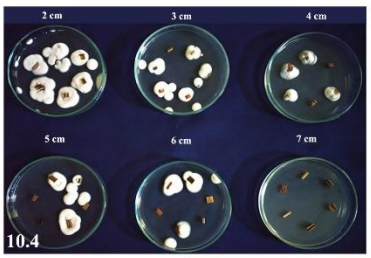
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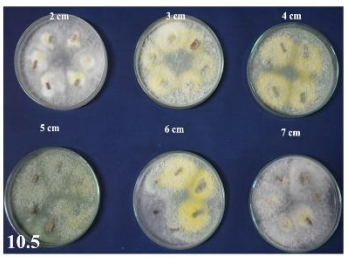
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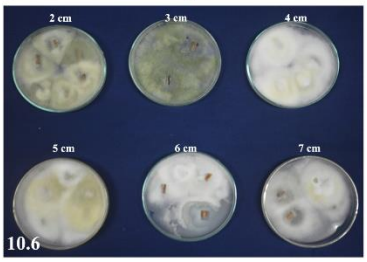
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10.6

Colonization frequency (CF) of fungal isolates on paddy straw segments was also ascertained. It differed between fungi tested. The maximum colonization frequency (100%) was shown by *Trichoderma harzianum* (ISO 33). The CF of other fungal species were *Clonostachys rosea* (ISO-79) – 88%, *T. koningii* (ISO 35) – 88%, *Aspergillus niger* (ISO 40) – 44%, *Purpureocillium lilacinum* (ISO 48) – 38%, *T. pseudokoningii* (ISO-116) – 38%, *Penicillium nigricans* (ISO 11) – 33%, *Penicillium multicolor* (ISO 58) – 22% and *Trichoderma* sp. (ISO-106) - 22%.

It is worth noting that a high saprophytic ability was not always based on high colonization ability. Though *Penicillium multicolor* (ISO 58) and *Purpureocillium lilacinum* (ISO 48) showed a high saprophytic ability but they were weak colonizers. High saprophytic ability was associated with *Trichoderma harzianum* (ISO 33), *T. koningii* (ISO 35) and *Clonostachys rosea* (ISO-79).

Rhizosphere competence of plant species to harness microbes has been demonstrated by various researchers (Papavizas, 1967; Wells *et al*., 1972; Newman and Bowen, 1974; Chao *et al*., 1986) and it is noted that success of a biocontrol agent is determined by its ability to survive in soil in the presence or absence of host tissue. The current study showed the high saprophytic ability of five fungal isolates namely, *Trichoderma harzianum* (ISO 33), *T. koningii* (ISO 35), *Purpureocillium lilacinum* (ISO 48), *Penicillium multicolor* (ISO 58) and *Clonostachys rosea* (ISO-79). These fungi were further tested for their ability to colonize roots of seedlings of teak and mahogany so as to determine the suitability of these fungi as bio-control agents of root disease (Table 29).

Table 29. Competitive saprophytic ability of antagonistic fungal isolates isolated from rhizosphere and rhizoplane regions of selected grass species

Sl. No.	Antagonistic fungal isolates	Saprophytic ability (cm)	Colonization Frequency (%)
1	*Aspergillus niger*	4	44
2	*Clonostachys rosea*	7	88
3	*Penicillium multicolor*	6	22
4	*P. nigricans*	3	33
5	*Purpureocillium lilacinum*	6	38
6	*Trichoderma harzianum*	7	100
7	*T. koningii*	7	88
8	*T. pseudokoningii*	4	38
9	*Trichoderma* sp.	3	22

The selected five antagonists - *Trichoderma harzianum* (ISO 33), *T. koningii* (ISO 35), *Purpureocillium lilacinum* (ISO 48), *Penicillium multicolor* (ISO 58) and *Clonostachys rosea* (ISO-79) and the root pathogens *Fusarium oxysporum* and *Athelia rolfsii* were analyzed for their root colonization abilities in teak and mahogany seedlings (Plate 11).

b) Root colonization ability

Parke (1991) described root colonization as the augmentation of micro-organisms in, on or along the root system. Woeng *et al*., (2000) in his studies on mutant strain *Pseudomonas chlororaphis* PCL 1391 reported that defective root colonization was ineffective against management of *Fusarium* which caused foot and root rot of tomato. So, success of the bio-control agent against a root disease depends on its capability to colonize and establish on the growing root system of the plant.

The present study was carried out in hydroponics system using autoclaved 1% sucrose solution to avoid any contaminations (Plate 12 and 13). Results of the study on

PLATE - 11

11.1. Pure culture of potential fungal biocontrol agent *Clonostachys rosea*.	11.2. Microscopic view of hyphae showing conidiophores and conidia of *Clonostachys rosea*.
11.3. Pure culture of potential fungal biocontrol agent *Penicillium multicolor*.	11.4. Microscopic view of hyphae showing conidiophores and conidia of *Penicillium multicolor*.
11.5. Pure culture of potential fungal biocontrol agent *Purpureocillium lilacinum*.	11.6. Microscopic view of hyphae showing conidiophores and conidia of *Purpureocillium lilacinum*.
11.7. Pure culture of potential fungal biocontrol agent *Trichoderma harzianum*.	11.8. Microscopic view of hyphae showing conidiophores and conidia of *Trichoderma harzianum*.
11.9. Pure culture of potential fungal biocontrol agent *Trichoderma koningii*.	11.10. Microscopic view of hyphae showing conidiophores and conidia of *Trichoderma koningii*.

PLATE - 11

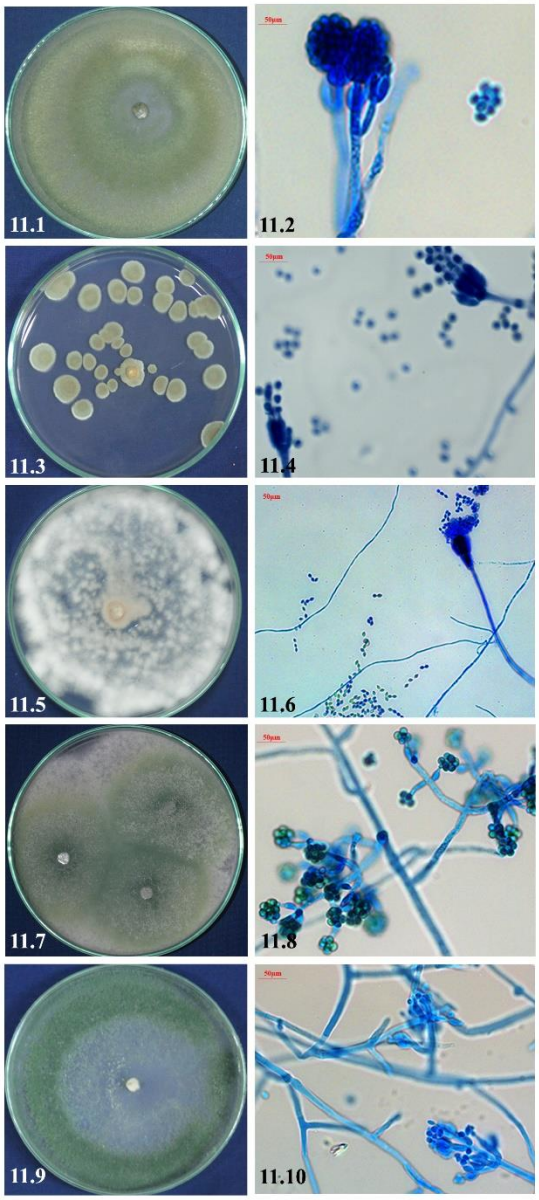


PLATE - 12

12.1. *Fusarium oxysporum* showing colonization of root tissues in mahogany seedlings amended individually in hydroponic system.

12.2. *Athelia rolfsii* showing root surface colonization in mahogany seedlings amended individually in hydroponic system.

12.3. *Clonostachys rosea* showing colonization of root tissues in mahogany seedlings amended individually in hydroponic system.

12.4. *Penicillium multicolor* showing root surface colonization in mahogany seedlings amended individually in hydroponic system.

12.5. *Purpureocillium lilacinum* showing colonization on the root surface in mahogany seedlings amended individually in hydroponic system.

12.6. *Trichoderma harzianum* showing colonization of root tissues in mahogany seedlings amended individually in hydroponic system.

12.7. *Trichoderma koningii* showing colonization of root tissues in mahogany seedlings amended individually in hydroponic system.

PLATE - 12

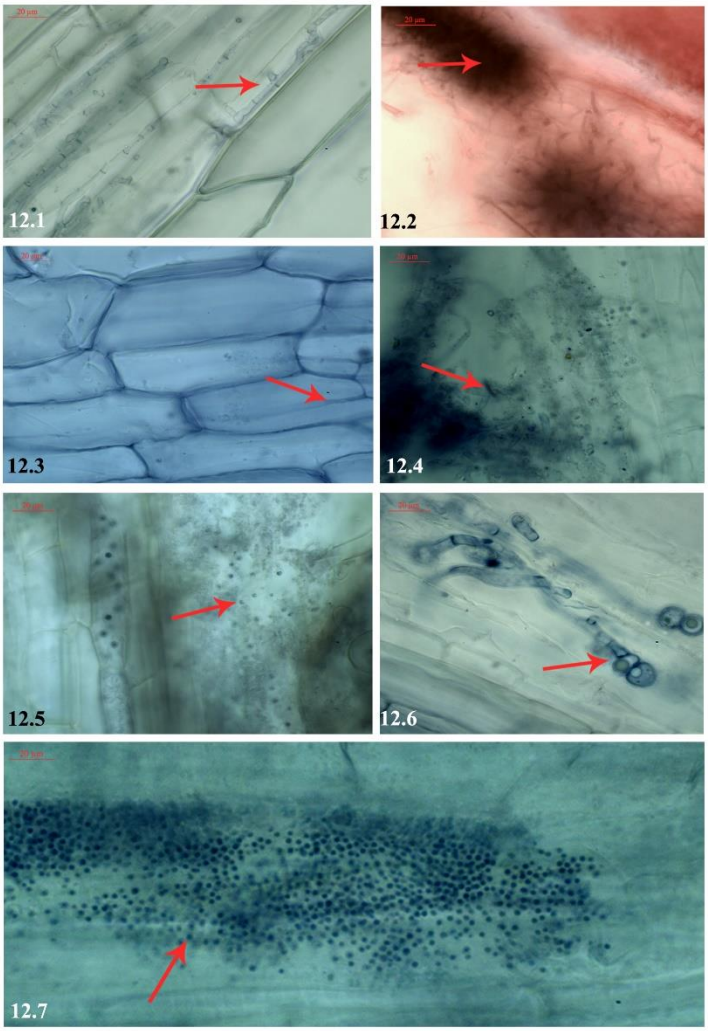


PLATE - 13

13.1. *Fusarium oxysporum* showing colonization of root tissues in teak seedlings amended individually in hydroponic system.

13.2. *Athelia rolfsii* showing root surface colonization in teak seedlings amended individually in hydroponic system.

13.3. *Clonostachys rosea* showing colonization of root tissues in teak seedlings amended individually in hydroponic system.

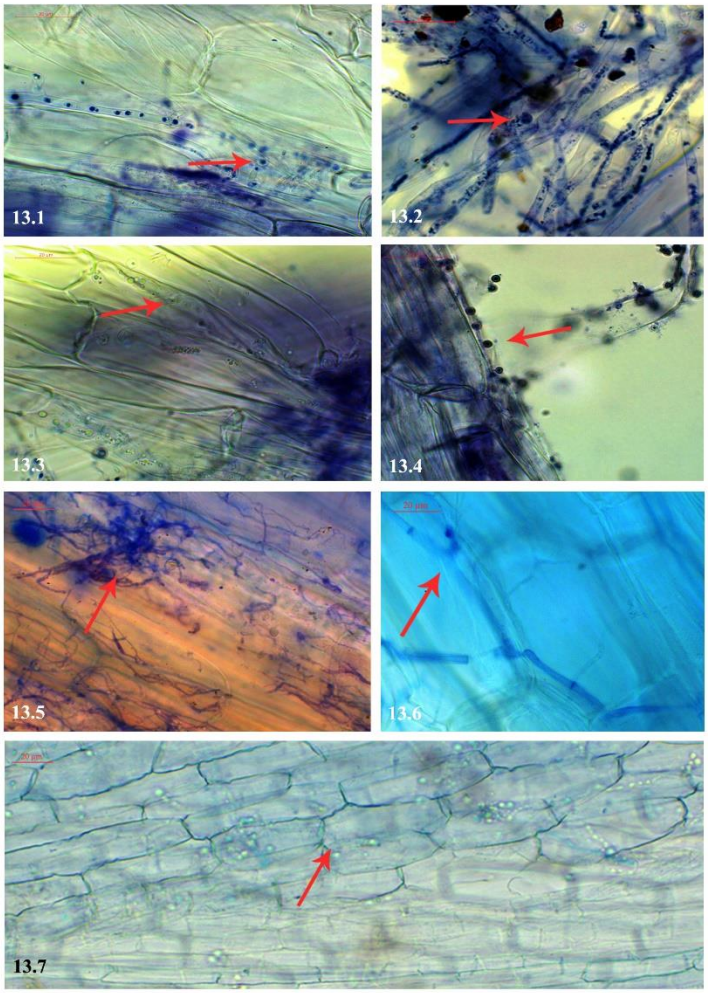
13.4. *Penicillium multicolor* showing root surface colonization in teak seedlings amended individually in hydroponic system.

13.5. *Purpureocillium lilacinum* showing colonization on the root surface in teak seedlings amended individually in hydroponic system.

13.6. *Trichoderma koningii* showing colonization of root tissues in teak seedlings amended individually in hydroponic system.

13.7. *Trichoderma harzianum* showing colonization of root tissues in teak seedlings amended individually in hydroponic system.

PLATE - 13



the root colonizing abilities of *F. oxysporum* in roots of teak and mahogany seedlings showed that it could colonize root cortical tissues extending up to vascular regions and a huge conidial mass was observed over the root surface of seedlings. Jaroszuk-Scisel *et al*., (2008) analyzed colonization of rhizosphere by pathogenic strains of *Fusarium culmorum* in *Secale cereale*. Initial colonization of both strains were similar growing along the root surface and colonizing root tissues but when pathogenic strains colonized vascular stele regions, strains isolated from rhizosphere were unable to do so. In this study when *A. rolfsii*, was tested on both teak and mahogany seedlings, the pathogen exhibited a surface interaction and the mycelium coiled over the root surface delimiting the root interactions and subsequently causing death of root tissues.

In case of *Trichoderma harzianum* (ISO 33), *T. koningii* (ISO 35) and *Clonostachys rosea* (ISO-79), the hyphae colonized the root surface and penetrated the root cortical tissues. *Trichoderma* sp. has been extensively analyzed for root colonization and rhizosphere competency across the globe (McLean *et al*., 2005). Based on their studies, Viterbo *et al*., (2004) indicated that the hyphal penetration is helped by cellulolytic and proteolytic enzymes. In addition, studies by various researchers also iterate the involvement of hydrophobins helping *Trichoderma* spp. in hyphal development and root colonization (Bailey *et al*., 2002; Askolin *et al*., 2005). Fungal attachment by appressoria-like structures is arbitrated by a class I hydrophobin, TasHyd1 (Viterbo and Chet 2006). There are reports that root interaction of *Trichoderma asperellum* in *Cucumis sativus* resulted in the colonization restricting to the first or second layers of cells (Yedidia *et al*., 1999). Colonization by *Clonostachys rosea* on outer

and inner root surfaces of different plant species such as carrot, barley, cucumber and wheat have been reported by various researchers (Roberti *et al*., 2008; Lubeck *et al*., 2002). The fungus has also been attributed to induce defense responses (Chatterton *et al*., 2008)

Tests with *Purpureocillium lilacinum* (ISO 48) and *Penicillium multicolor* (ISO 58) noticed that the fungi moved along the root surface thereby protecting the plants from the pathogen attack. Comparable observations for *Purpureocillium lilacinum* QLP12 strain from soils of Qinling Mountain, Shaanxi Province, in China, were recorded for root colonization of tomato, potato, wheat and cucumber (Lan *et al*., 2017). These results agree with our findings where *Purpureocillium lilacinum* did not show any visible root tissue penetration.

In the next experiment, conidial suspensions of 2 x 10^6 of both the pathogen and the antagonists were tested for their activity and root colonization abilities in hydroponic condition.

Root colonization abilities of selected antagonistic agents against selected pathogens in hydroponic conditions

(i) Root colonization abilities of *Fusarium oxysporum* and bio-agents

In both teak and mahogany seedlings, when 1:1 concentration of pathogen and bio-agent was used in root colonization studies, *Fusarium oxysporum* dominated over bio-agents in root colonization compared to *Penicillium multicolor* (ISO 58) and

Purpureocillium lilacinum (ISO 48). However, *Trichoderma harzianum* (ISO 33), *T. koningii* (ISO 35) and *Clonostachys rosea* (ISO-79), dominated over the pathogen and colonized roots. In the same study, the pathogen colonized root tissues and caused damage in both teak and mahogany seedlings when *P. multicolor* and *P. lilacinum* were used as bio-agents.

When 1:5 concentration of the antagonists were tested for root colonization and antagonistic activity, all the bio-agents were efficient against *F. oxysporum*. In the case of *Trichoderma harzianum, T. koningii* and *Clonostachys rosea,* the bio-agents colonized root tissues prior to the pathogen and apparently served as a shield for preventing the pathogens from causing any infection to the seedlings. Inoculations with *Penicillium multicolor* and *Purpureocillium lilacinum* resulted in formation of numerous conidia over the root surface attached to the root hairs thus exhibiting a surface interaction. Mycelium and conidia of *Fusarium oxysporum* were found deformed in the vicinity of the conidia of *P. multicolor* and *P. lilacinum.*

(ii) Root colonization abilities of *Athelia rolfsii* and bio-agents

When 1:1 concentration was used, the bio-agents *Penicillium multicolor* (ISO 58) and *Purpureocillium lilacinum* (ISO 48) were not found to be effective against the pathogen and also no interactions were observed with the root tissues. However, *Trichoderma harzianum* (ISO 33), *T. koningii* (ISO 35) and *Clonostachys rosea* (ISO-79), colonized the root tissues of both teak and mahogany seedlings and thus effectively managed infection by the pathogen.

When 1:5 concentrations of the pathogen and the antagonist were tried, all the bio-agents were efficient in controlling infection by the pathogen. As in the case of *F. oxysporum*, *Trichoderma harzianum, T. koningii* and *Clonostachys rosea*, colonized root tissues prior to the pathogen and served as a shield preventing the pathogens from root colonization in both the seedlings. In the case of *Penicillium multicolor* and *Purpureocillium lilacinum*, numerous conidia colonized the root surface attaching to the root hairs and exhibited a surface interaction. Mycelium of *Athelia rolfsii* was found to be deformed by *P. multicolor* and *P. lilacinum* (Plate 14-18).

Results of the experiment proved that concentration of the inoculum of the antagonists play a decisive role in managing the pathogen. When the concentration of antagonists was increased to five times, all the bio-agents inhibited hyphal growth of both the pathogens.

Hossain *et al*., (2014) from Gifu University, Japan reported that *Penicillium* GP15-1 strain from zoysia grass rhizosphere noticed an increased suppression activity against the anthracnose pathogen *Colletotrichum orbiculare* in cucumber plants when the inoculum concentration was increased from 0.5% to 1.0% and with an increase in pathogen inoculum the protection provided by *Penicillium* decreased. Nahalkova *et al*., (2008) observed root colonization abilities of pathogenic (Fo47) and non-pathogenic (Fol8) strains of *Fusarium oxysporum* on tomato in hydroponic conditions. Both the strains when analyzed separately for root tissues colonization, only the pathogenic strain invaded the xylem vessels. Whereas, when both the strains applied simultaneously at 1:1

PLATE - 14

14.1. Interaction of *Clonostachys rosea* and *Athelia rolfsii* in roots of teak seedlings at 1:1 concentration (pathogen: Antagonist) showing mycelial deformation of the pathogen *Athelia rolfsii* on the root surface in hydroponic system.

14.2. Interaction of *Clonostachys rosea* and *Athelia rolfsii* in roots of teak seedlings at 1:5 concentration (pathogen: Antagonist) showing colonization of *Clonostachys rosea* in root tissues in hydroponic system.

14.3. Interaction of *Clonostachys rosea* and *Fusarium oxysporum* in roots of teak seedlings at 1:1 concentration (pathogen: antagonist) showing colonization of *Clonostachys rosea* in root tissues.

14.4. Interaction of *Clonostachys rosea* and *Fusarium oxysporum* in roots of teak seedlings at 1:5 concentration (pathogen: antagonist) showing colonization of *Clonostachys rosea* in root tissues.

14.5. Interaction of *Penicillium multicolor* and *Athelia rolfsii* in roots of teak seedlings at 1:1 concentration (pathogen: antagonist) showing colonization of *Athelia rolfsii* on the root surface.

14.6. Interaction of *Penicillium multicolor* and *Athelia rolfsii* in roots of Teak seedlings at 1:5 concentration (pathogen: antagonist) showing mycelial colonization of *Penicillium multicolor* on the root surface and deformation of mycelium of *Athelia rolfsii* in the vicinity of the antagonist.

14.7. Interaction of *Penicillium multicolor* and *Fusarium oxysporum* in roots of teak seedlings at 1:1 concentration (pathogen: antagonist), *Fusarium oxysporum* showing colonization and invasion of root tissues.

14.8. Interaction of *Penicillium multicolor* and *Fusarium oxysporum* in roots of teak seedlings at 1:5 concentration (pathogen: antagonist) showing deformation of *Fusarium oxysporum* mycelium in the vicinity of *Penicillium multicolor* conidia.

PLATE - 14

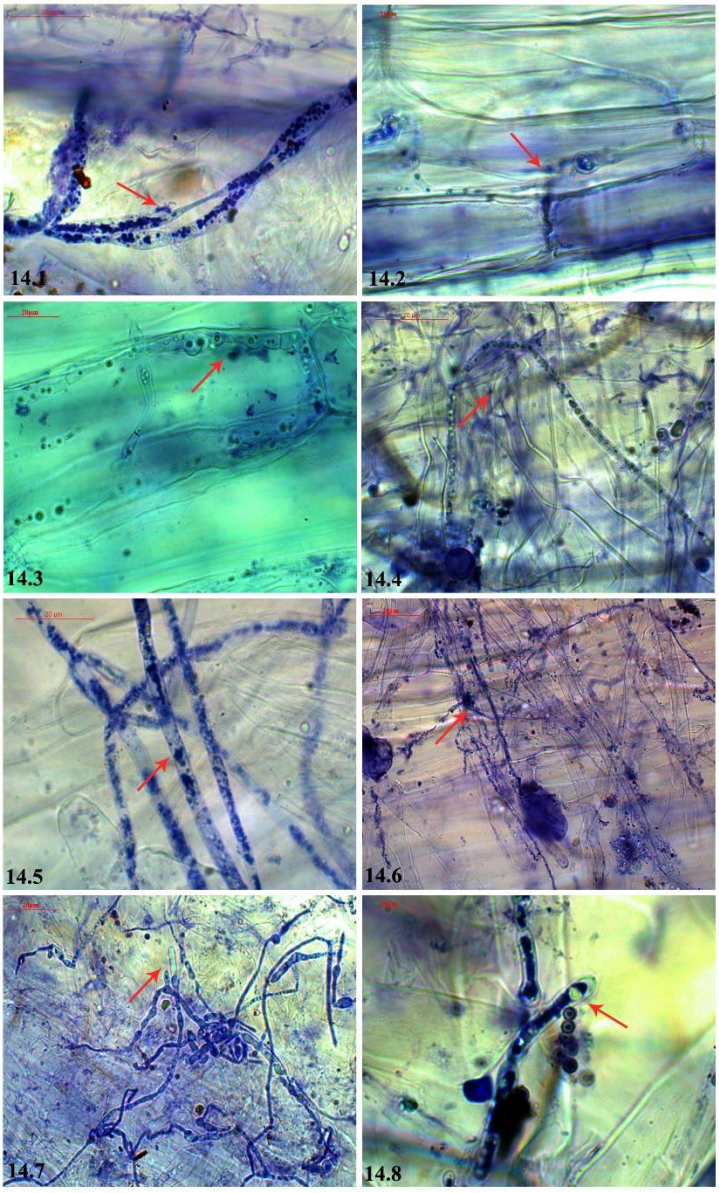


PLATE – 15

15.1. Interaction of *Purpureocillium lilacinum* and *Athelia rolfsii* in roots of teak seedlings at 1:1 concentration (pathogen: antagonist) showing colonization of *Athelia rolfsii* on the root surface.

15.2. Interaction of *Purpureocillium lilacinum* and Pathogen *Athelia rolfsii* in roots of teak seedlings at 1:5 concentration (pathogen: antagonist) showing mycelial colonization of *Purpureocillium lilacinum* on the root surface and deformation of mycelium of *Athelia rolfsii* in the vicinity of the antagonist.

15.3. Interaction of *Purpureocillium lilacinum* and *Fusarium oxysporum* in roots of teak seedlings at 1:1 concentration (pathogen: antagonist), *Fusarium oxysporum* showing colonization and invasion of root tissues.

15.4. Interaction of *Purpureocillium lilacinum* and *Fusarium oxysporum* in roots of teak seedlings at 1:5 concentration (pathogen: antagonist) and conidial mass of *Purpureocillium lilacinum* showing retarded growth of the pathogen.

15.5. Interaction of *Trichoderma harzianum* and *Athelia rolfsii* in roots of teak seedlings at 1:1 concentration (pathogen: antagonist) and *Trichoderma harzianum* showing colonization of root tissues and inhibiting mycelial invasion of the pathogen.

15.6. Interaction of *Trichoderma harzianum* and *Athelia rolfsii* in roots of teak seedlings at 1:5 concentration (pathogen: antagonist) and *Trichoderma harzianum* showing colonization of root tissues and inhibiting mycelial invasion of the pathogen.

15.7. Interaction of *Trichoderma harzianum* and *Fusarium oxysporum* in roots of teak seedlings at 1:1 concentration (pathogen: Antagonist) and *Trichoderma harzianum* showing colonization of root tissues and inhibiting mycelial invasion of the pathogen.

15.8. Interaction of *Trichoderma harzianum* and *Fusarium oxysporum* in roots of teak seedlings at 1:5 concentration (pathogen: Antagonist) and *Trichoderma harzianum* showing colonization of root tissues and inhibiting mycelial invasion of the pathogen.

PLATE - 15

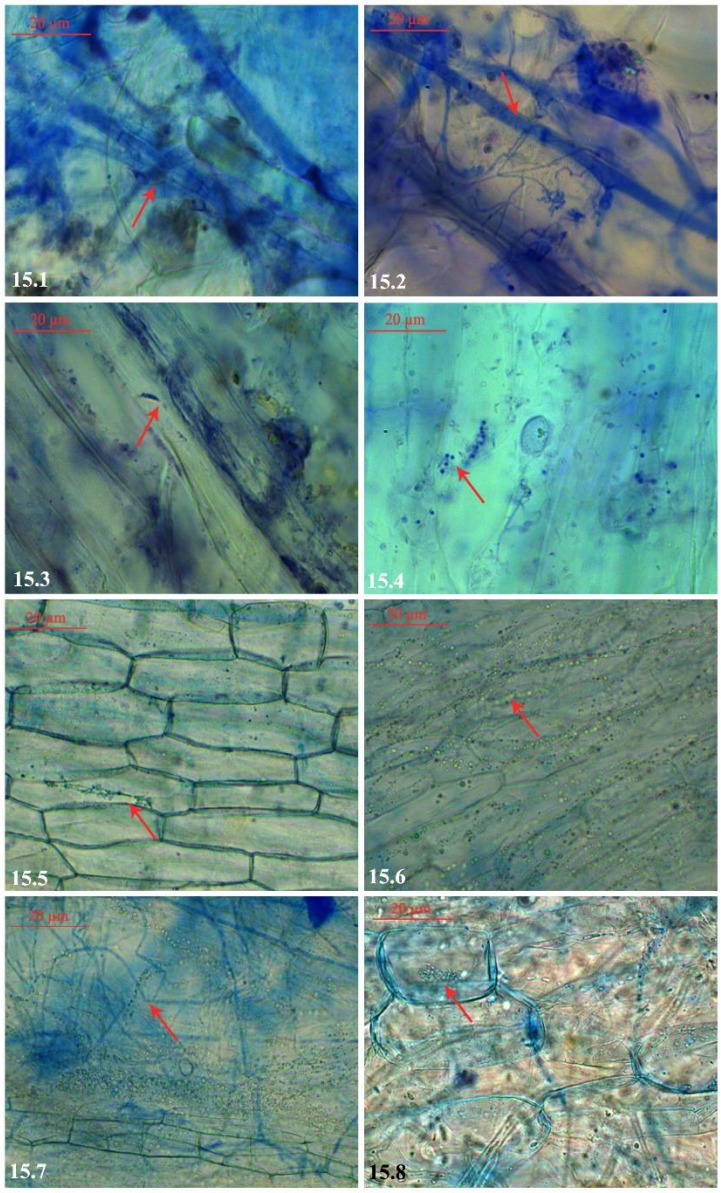

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PLATE - 16

16.1. Interaction of *Trichoderma koningii* and *Athelia rolfsii* in roots of teak seedlings at 1:1 concentration (pathogen: antagonist) and *Trichoderma koningii* showing colonization of root surface and root tissues.

16.2. Interaction of *Trichoderma koningii* and *Athelia rolfsii* in roots of teak seedlings at 1:5 concentration (pathogen: antagonist) and *Trichoderma koningii* showing colonization of root surface and root tissues.

16.3. Interaction of *Trichoderma koningii* and *Fusarium oxysporum* in roots of teak seedlings at 1:1 concentration (pathogen: antagonist) and *Trichoderma koningii* showing colonization of root tissues and inhibition of mycelial invasion of the pathogen.

16.4. Interaction of *Trichoderma koningii* and *Fusarium oxysporum* in roots of teak seedlings at 1:5 concentration (pathogen: antagonist) and *Trichoderma koningii* showing colonization of root tissues and inhibition of mycelial invasion of the pathogen.

16.5. Interaction of *Clonostachys rosea* and *Athelia rolfsii* in roots of mahogany seedlings at 1:1 concentration (pathogen: antagonist) and *Clonostachys rosea* showing root surface and root tissues colonization and inhibiting mycelial growth of the pathogen.

16.6. Interaction of *Clonostachys rosea* and *Athelia rolfsii* in roots of mahogany seedlings at 1:5 concentration (pathogen: antagonist) and *Clonostachys rosea* showing root surface and root tissues colonization and inhibiting mycelial growth of the pathogen.

16.7. Interaction of *Clonostachys rosea* and *Fusarium oxysporum* in roots of mahogany seedlings at 1:1 concentration (pathogen: antagonist) and *Clonostachys rosea* showing root tissues colonization and inhibiting mycelial growth of the pathogen.

16.8. Interaction of *Clonostachys rosea* and *Fusarium oxysporum* in roots of mahogany seedlings at 1:5 concentration (pathogen: antagonist) and *Clonostachys rosea* showing root tissues colonization and inhibiting mycelial growth of the pathogen.

PLATE - 16

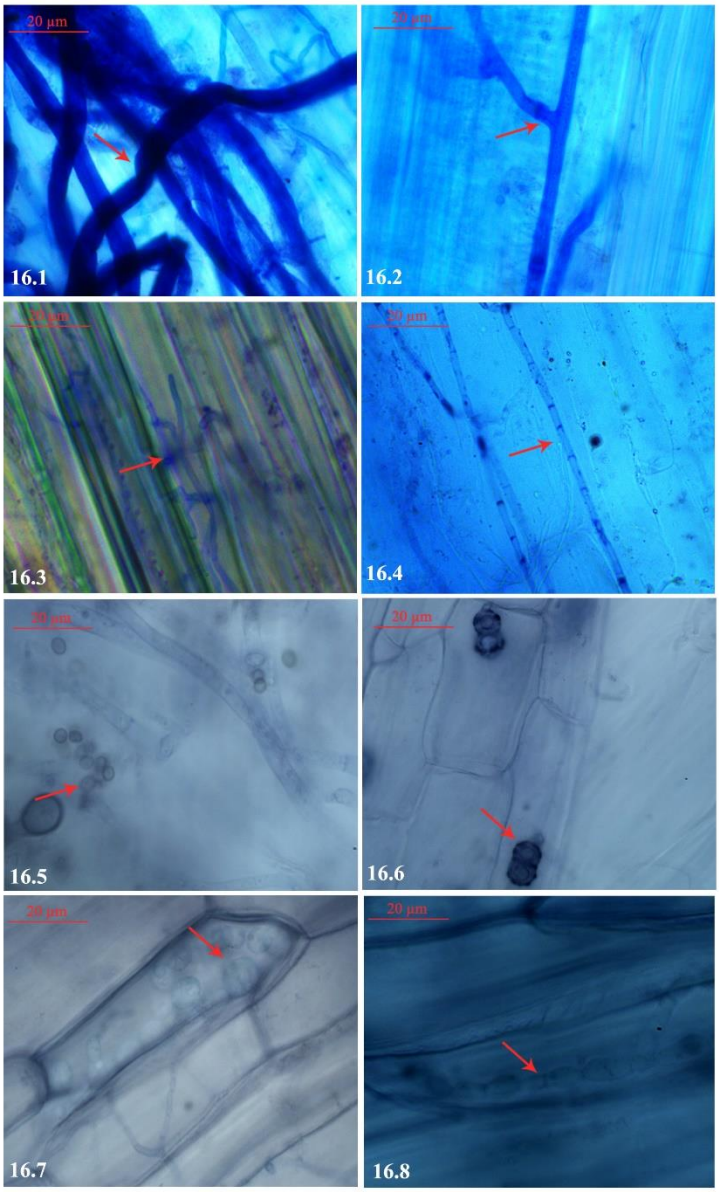
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PLATE - 17

17.1. Interaction of *Penicillium multicolor* and *Athelia rolfsii* in roots of mahogany seedlings at 1:1 concentration (pathogen: antagonist) showing *Athelia rolfsii* root surface colonization.

17.2. Interaction of *Penicillium multicolor* and *Athelia rolfsii* in roots of mahogany seedlings at 1:5 concentration (pathogen: antagonist), conidial mass of *Penicillium multicolor* on the root surface showing retarded mycelial growth of the pathogen.

17.3. Interaction of *Penicillium multicolor* and *Fusarium oxysporum* in roots of mahogany seedlings at 1:1 concentration (pathogen: antagonist) showing *Fusarium oxysporum* root colonization and invasion of root tissues.

17.4. Interaction of *Penicillium multicolor* and *Fusarium oxysporum* in roots of mahogany seedlings at 1:5 concentration (pathogen: antagonist), showing deformation of mycelium of *Fusarium oxysporum* in the vicinity of conidia of *Penicillium multicolor*.

17.5. Interaction of *Purpureocillium lilacinum* and *Athelia rolfsii* in roots of mahogany seedlings at 1:1 concentration (pathogen: antagonist) showing root surface colonization by *Athelia rolfsii*.

17.6. Interaction of *Purpureocillium lilacinum* and *Athelia rolfsii* in roots of mahogany seedlings at 1:5 concentration (pathogen: antagonist) showing root surface colonization of *Purpureocillium lilacinum* and retarded growth of the pathogen.

17.7. Interaction of *Purpureocillium lilacinum* and *Fusarium oxysporum* in roots of mahogany seedlings at 1:1 concentration (pathogen: antagonist) showing root tissue colonization and invasion by *Fusarium oxysporum*.

17.8. Interaction of *Purpureocillium lilacinum* and *Fusarium oxysporum* in roots of mahogany seedlings at 1:5 concentration (pathogen: antagonist), conidial mass of *Purpureocillium lilacinum* on the root surface showing retarded mycelial growth of the pathogen.

PLATE - 17

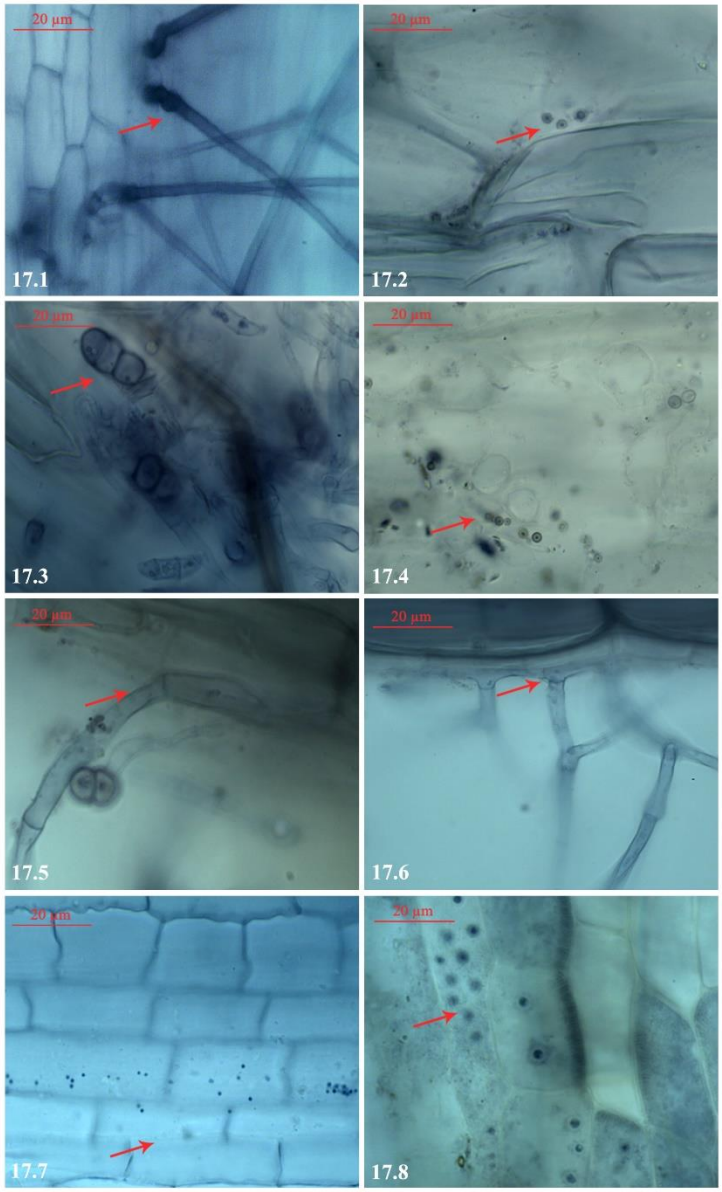
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PLATE - 18

18.1. Interaction of *Trichoderma harzianum* and *Athelia rolfsii* in roots of mahogany seedlings at 1:1 concentration (pathogen: antagonist) showing root tissue colonization of *Trichoderma harzianum* and inhibition of mycelial invasion of the pathogen.

18.2. Interaction of *Trichoderma harzianum* and *Athelia rolfsii* in roots of mahogany seedlings at 1:5 concentration (pathogen: antagonist) showing root tissue colonization of *Trichoderma harzianum* and inhibition of mycelial invasion of the pathogen.

18.3. Interaction of *Trichoderma harzianum* and *Fusarium oxysporum* in mahogany seedlings at 1:1 concentration (pathogen: antagonist) showing root tissue colonization of *Trichoderma harzianum* and inhibition of mycelial invasion of the pathogen.

18.4. Interaction of *Trichoderma harzianum* and *Fusarium oxysporum* in mahogany seedlings at 1:5 concentration (pathogen: antagonist) showing root tissue colonization of *Trichoderma harzianum* and inhibition of mycelial invasion of the pathogen.

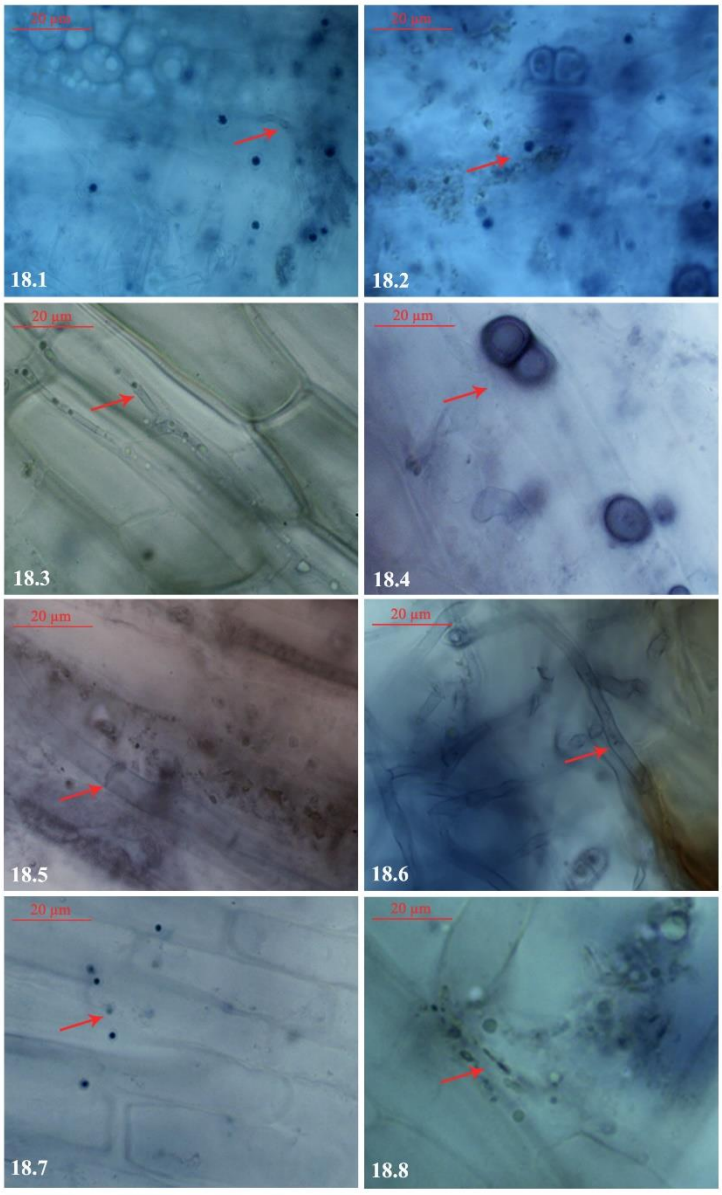
18.5. Interaction of *Trichoderma koningii* and *Athelia rolfsii* in roots of mahogany seedlings at 1:1 concentration (pathogen: antagonist) showing *Trichoderma koningii* root surface and root tissue colonization.

18.6. Interaction of *Trichoderma koningii* and *Athelia rolfsii* in roots of mahogany seedlings at 1:5 concentration (pathogen: antagonist) showing *Trichoderma koningii* root surface and root tissue colonization.

18.7. Interaction of *Trichoderma koningii* and *Fusarium oxysporum* in roots of mahogany seedlings at 1:1 concentration (pathogen: antagonist) showing root tissue colonization of *Trichoderma koningii* and retarded mycelial growth of the pathogen.

18.8. Interaction of *Trichoderma koningii* and *Fusarium oxysporum* in roots of mahogany seedlings at 1:5 concentration (pathogen: antagonist) showing root tissue colonization of *Trichoderma koningii* and retarded mycelial growth of the pathogen.

PLATE - 18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(Fo47:Fol8) and 1:100 (Fo47:Fol8) conidial concentrations, the latter was found effective in controlling the pathogen with maximum colonization at root apex region only. Likewise, Larkin and Fravel (1999) observed dose response could give advantage to the non-pathogenic strains in efficient root colonization and biological management of the pathogenic strains.

Root colonization characteristics of pathogenic and non-pathogenic strains vary among hydroponic and soil systems (Olivain and Alabouvette 1997; Olivain and Alabouvette 1999). In hydroponic system, conidial suspension is the only source and the growth and development of fungi depend on the attachment of conidia on the root surface. Also, when inoculum concentrations were compared in soil and hydroponic systems, it was noted to be lower in hydroponic system. In soil, numerous factors interplay in the development of conidia, germination and spread along the root system. Moreover, in soil, the saprophytic growth of fungi is enhanced by the organic matter from various sources but in hydroponics carbon source available from the mineral nutrient solution is very much limited to support growth of conidia. These differences in the soil and hydroponics system constrain extrapolating data from a hydroponic culture to a soil culture. Nevertheless, the results give a broad indication on the antagonistic abilities of the selected fungi for further trails.

Results of the experiments indicated that the proportion of conidial concentration of 1:5 (pathogen: antagonist) was efficient in controlling the growth of the pathogens tested.

c) Plant growth promotion abilities

The preceding experiments showed that all the five antagonists tested to have abilities to colonize roots in hydroponic conditions. And, of the two concentrations used in co-inoculation studies, a conidial concentration combination of 1:5 (pathogen: antagonists) was efficient in colonizing roots as well as inhibiting growth of the pathogen. This combination was tested *in vivo* to verify the efficacy of bio-agents in soil in terms of plant growth and development.

Antagonists and the pathogens were analyzed for the following response in teak seedlings – preventive and curative actions and abilities for promotion of growth. Root length, shoot length, fresh weight and dry weight of treated and control seedlings were analyzed for each treatment.

1. Effect of antagonistic fungal isolates on growth promotion of teak seedlings inoculated with *Fusarium oxysporum*

(i) Control (untreated with antagonists and pathogen inoculated)

Apparently healthy seedlings of teak showed an average root length of 20.78cm ± 2.49. Seedlings infected by *F. oxysporum* showed a remarkable decrease in root length (16.11cm ± 1.62). Inoculation with antagonists *Clonostachys rosea* (26.33 cm ± 2.29), *Trichderma harzianum* (25.00 cm ± 3.46) and *T. koningii* (22.67 cm ±1.80) significantly increased root length in comparison to un-inoculated control. *Penicillium multicolor* (20.56 cm ± 2.06) and *Purpureocillium lilacinum* (19.78 cm ± 1.39) did not show any effect on root length.

Healthy seedlings of teak showed an average shoot length of 27.89 cm ± 2.57. Inoculation by *F. oxysporum* did not affect shoot length significantly (26.44 cm ± 1.94). Among the antagonists inoculated, *Trichderma harzianum* (32.00 cm ±2.87) resulted in significant increase in shoot length followed by *Clonostachys rosea* (30.33 cm ± 3.31) and *T. koningii* (30.22 cm ± 1.64) in comparison to untreated control. Remaining two antagonists showed no variations in shoot length (Plate 19 and 20).

When fresh weights of seedlings was considered, a significant decrease was observed in the seedlings treated with the pathogen (5.41 g ± 0.58) when compared to untreated control (11.44 g ± 2.86).

All the seedlings treated with the antagonists, fresh weights of *C. rosea* (11.00 g ± 1.15); *P. multicolor* (10.20 g ± 1.16); *P. lilacinum* (10.86 g ± 0.98); *T. harzianum* (11.70 g ± 0.71) and *T. koningii* (10.17 g ± 0.78) were comparable with that of the untreated control. But in dry weights, untreated control exhibited a significant increase (3.47 g ± 1.08) compared to seedlings treated with antagonists *C. rosea* (2.31 g ± 0.17); *P. multicolor* (2.06 g ± 0.55); *P. lilacinum* (2.37 g ± 0.45); *T. harzianum* (2.80 g ± 0.52) and *T. koningii* (2.56 g ± 0.51) and the pathogen (1.88 g ± 0.37).

(ii) Preventive treatment

When preventive treatments were analyzed, seedlings treated with *Penicillium multicolor* (25.44 cm ± 2.01) and *Purpureocillium lilacinum* (22.00 cm ± 2.12) noticed a significant increase in root length compared to the untreated control and other individual

PLATE - 19

19.1. Teak seedlings grown in unamended potting medium.

19.2. Teak seedlings grown in unamended potting medium showing healthy root system.

19.3. Teak seedlings grown in potting medium amended with *Fusarium oxysporum* at 2 x 10^6 conidial suspensions showing wilt symptoms.

19.4. Teak seedlings grown in potting medium amended with *Fusarium oxysporum* at 2 x 10^6 conidial suspensions showing wilt symptoms.

19.5. Teak seedlings grown in potting medium amended with *Athelia rolfsii* showing wilt and rot symptoms.

19.6. Teak seedlings grown in potting medium amended with *Athelia rolfsii* showing wilt and rot symptoms.

19.7. Growth promotion in teak seedlings in potting medium amended with fungal antagonist *Clonostachys rosea* at 2 x 10^6 conidial suspensions.

19.8. Teak seedlings grown in potting medium amended with *Clonostachys rosea* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

PLATE - 19

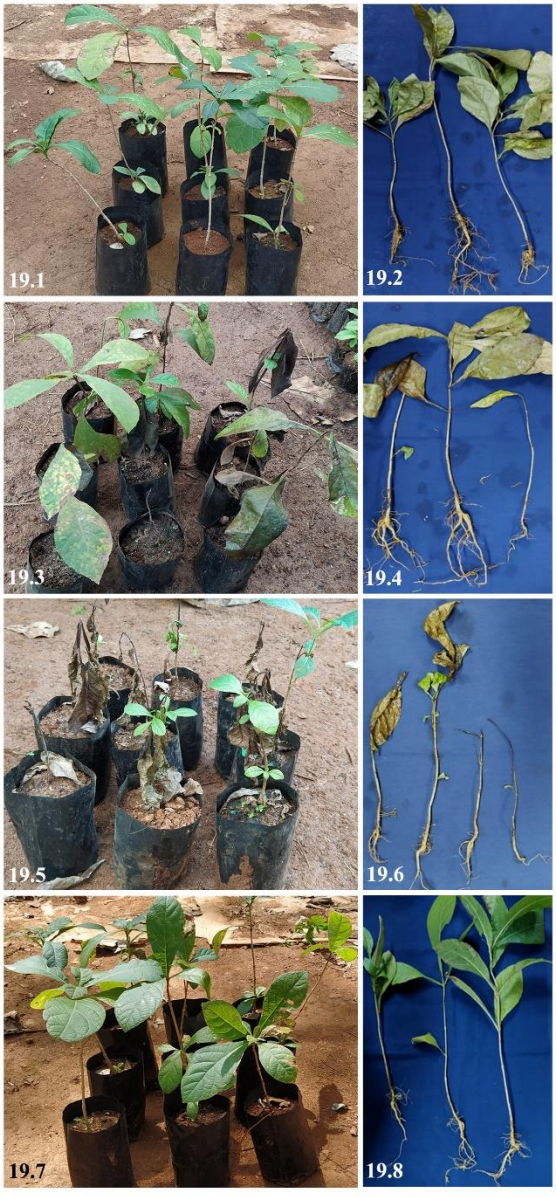

PLATE - 20

20.1. Growth promotion in teak seedlings in potting medium amended with fungal antagonist *Penicillium multicolor* at 2 x 10^6 conidial suspensions.

20.2. Teak seedlings grown in potting medium amended with *Penicillium multicolor* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

20.3. Growth promotion in teak seedlings in potting medium amended with fungal antagonist *Purpureocillium lilacinum* at 2 x 10^6 conidial suspensions.

20.4. Teak seedlings grown in potting medium amended with *Purpureocillium lilacinum* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

20.5. Growth promotion in teak seedlings in potting medium amended with fungal antagonist *Trichoderma harzianum* at 2 x 10^6 conidial suspensions.

20.6. Teak seedlings grown in potting medium amended with *Trichoderma harzianum* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

20.7. Growth promotion in teak seedlings in potting medium amended with fungal antagonist *Trichoderma koningii* at 2 x 10^6 conidial suspensions.

20.8. Teak seedlings grown in potting medium amended with *Trichoderma koningii* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

PLATE - 20

bio-agent treatments. The rest of the bio-agents caused a decrease in root length compared to the respective controls. In the case of shoot length, except for *Purpureocillium lilacinum* (30.89cm ± 2.20), none of the other bio-agents caused any variation in shoot length compared to control.

When fresh weights were analyzed, a significant decrease was observed for all the treatments compared with the untreated control and individual bio-agents treated. Similarly, for dry weights a significant decrease was observed with respect to the untreated control and individual treatments with bio-agents.

(iii) Curative treatment

In curative treatment, all the antagonists noticed increased root length of seedlings which were inoculated with the pathogens. This effect was less pronounced compared with untreated controls. However, antagonists produced no effect on shoot length.

Teak seedlings showed increase in fresh and dry weights when compared to those treated with the pathogen but these were less pronounced when noticed for untreated control and individual bio-agents treated (Table 30 a and b).

Table 30a. Effect of amendment of potting medium with talc formulation of *Clonostachys rosea*, *Penicillium multicolor*, *Purpureocillium lilacinum*, *Trichoderma harzianum* and *Trichoderma koningii* **on root and shoot length of seedlings against seedling root rot and wilt causing pathogen *Fusarium oxysporum* in teak seedlings grown for 180 days in polybag under field condition**

Sl. No.	Selected fungal Bio-agents	Root and Shoot Length of Teak seedlings (cm)[1]									
		Root length					Shoot length				
		C	P	B	BxP	PxB	C	P	B	BxP	PxB
1	*Clonostachys rosea*	20.78± 2.49[2 abc3]	16.11± 1.62[a]	26.33± 2.29[d]	22.67± 4.61[bc]	18.22± 2.59[a]	27.89±2.57[abc]	26.44± 1.94[ab]	30.33±3.31[c]	28.89±2.26[bc]	22.56± 4.82[a]
2	*Penicillium multicolor*			20.56± 2.06[b]	25.44± 2.01[c]	19.56± 2.19[b]			25.44± 2.79 [a]	27.44± 1.87[a]	27.67± 4.06[a]
3	*Purpureocillium lilacinum*			19.78± 1.39[b]	22.00± 2.12[c]	21.33± 2.29[bc]			25.44± 1.13[a]	30.89± 2.20[c]	25.33± 1.59[a]
4	*Trichoderma harzianum*			25.00± 3.46[c]	22.22± 1.94[b]	18.00± 4.01[b]			32.00±2.87[c]	27.67± 2.29[b]	23.67± 1.94[a]
5	*Trichoderma koningii*			22.67±1.80[b]	21.44± 3.67[b]	21.44± 3.12[b]			30.22± 1.64[b]	26.67± 3.20[a]	25.79± 5.38[a]

C- Control, P- Pathogen, B- Biocontrol

[1] Data is an average of three replicates

[2] Standard deviation

[3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

Table 30b. Effect of amendment of potting medium with talc formulation of *Clonostachys rosea*, *Penicillium multicolor*, *Purpureocillium lilacinum*, *Trichoderma harzianum* and *Trichoderma koningii* **on fresh and dry biomass of seedlings against seedling root rot and wilt causing pathogen *Fusarium oxysporum* in teak seedlings grown for 180 days in polybag under field condition**

Sl. No.	Selected fungal Bio-agents	Biomass of Teak seedlings (g)[1]									
		Fresh Weight					Dry weight				
		C	P	B	BxP	PxB	C	P	B	BxP	PxB
1	*Clonostachys rosea*	11.44±2.86[2] cd3	5.41±0.58 a	11.00± 1.15 c	8.08± 1.63 b	7.66± 3.85 b	3.47± 1.08 b	1.88± 0.37 a	2.31± 0.17 a	2.62± 1.12 a	2.02± 0.85 a
2	*Penicillium multicolor*			10.20± 1.16 c	8.39± 1.87 b	7.84± 0.68 b			2.06± 0.55 ab	2.73± 0.92 b	2.39± 0.52 ab
3	*Purpureocillium lilacinum*			10.86± 0.98 b	7.02± 1.51 a	6.72± 1.87 a			2.37± 0.45 a	2.10± 0.50 a	2.05± 0.67 a
4	*Trichoderma harzianum*			11.70± 0.71 c	7.30± 2.31 b	6.93± 1.5 a b			2.80± 0.52 b	2.13± 0.54 a	2.09± 0.64 a
5	*Trichoderma koningii*			10.17± 0.78 bc	8.17± 2.07 b	7.87± 3.28 b			2.56± 0.51 a	2.47± 0.70 a	2.46± 1.21 a

C- Control, P- Pathogen, B- Biocontrol

[1] Data is an average of three replicates

[2] Standard deviation

[3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

2. Effect of antagonistic fungal isolates on growth promotion of teak seedlings inoculated with *Athelia rolfsii*

Results of the untreated control and bio-agents treated are detailed earlier (1 (i)).

(i) Pathogen inoculated

The average root and shoot lengths of teak seedlings inoculated with *A. rolfsii* were noticed 13.22 cm ± 1.20 and 18.22 cm ± 1.30 respectively. The fresh and dry weights of inoculated seedlings were 5.66 g ± 0.45 and 1.88 g ± 0.53, respectively.

(ii) Preventive treatment

Seedlings treated with *Purpureocillium lilacinum* showed a significant increase in root length (26.33 cm ± 3.71) followed by *Trichderma harzianum* (24.22 cm ± 2.91) and *Penicillium multicolor* (23.67 cm ± 4.42) when compared with untreated control and those treated with bio-agents individually. Treatments with *Clonostachys rosea* and *T. koningii* did not increase root length. In respect to shoot length, except *Purpureocillium lilacinum* (32.89 cm ± 3.10), none of the other isolates increased shoot length when compared with untreated control and bio-agent treatments.

Fresh weight of teak seedlings in preventive treatment showed a significant decrease when compared to untreated control and bio-agents treated individually. In the case of dry weights, a significant increase was observed when *T. harzianum* inoculated prior to the pathogen (3.17 g ± 1.18) compared with the individual bio-agent treatments but was less marked with the untreated control. None of the other bio-agents caused any variation in dry weight compared to control.

(iii) Curative treatment

In curative treatment of antagonistic isolates, all the seedlings treated with the pathogen showed a significant increase in root length. Shoot length of seedlings treated with *Purpureocillium lilacinum* (28.22 cm ± 5.72) showed significant improvement in length compared with the other isolates.

Inoculation of bio-agents following pathogen showed no effect in fresh and dry weights when compared to teak seedlings inoculated with the pathogen (Table 31 a and b) (Plate 21 - 25).

3. Effect of antagonistic fungal isolates on growth promotion of mahogany seedlings inoculated with *Fusarium oxysporum*

(i) Control (untreated, treated with pathogen and treated with antagonists)

Apparently healthy seedlings of mahogany noticed average root length of 24.78 cm ± 4.73 compared to *F. oxysporum* infected seedlings which showed significant decrease in root length (18.00 cm ± 0.86). Among the antagonists treated, only *Clonostachys rosea* (27.33 cm ± 2.45) increased root length significantly compared to untreated control.

Shoot length of apparently healthy mahogany seedlings on average was 31.33 cm ± 4.44. Seedligs treated with *Fusarium oxysporum* significantly reduced shoot length (22.44 cm ± 1.81). Treatment by antagonists did not show significant variations in shoot length compared to untreated control.

Table 31a. Effect of amendment of potting medium with talc formulation of *Clonostachys rosea*, *Penicillium multicolor, Purpureocillium lilacinum, Trichoderma harzianum* and *Trichoderma koningii* **on root and shoot length of seedlings against seedling root rot and wilt causing pathogen *Athelia rolfsii* in teak seedlings grown for 180 days in polybag under field condition**

Sl. No.	Selected fungal Bio-agents	Root and Shoot Length of Teak seedlings (cm)[1]									
		Root length					Shootlength				
		C	P	B	BxP	PxB	C	P	B	BxP	PxB
1	*Clonostachys rosea*	20.78± 2.49[2 abc3]	13.22± 1.20[a]	26.33± 2.29[c]	20.78± 3.93[b]	20.22± 2.63[b]	27.89±2.57[bc]	18.22±1.30[a]	30.33±3.31[c]	29.67±3.57[c]	26.67±2.82[b]
2	*Penicillium multicolor*			20.56± 2.06[b]	23.67± 4.42[b]	21.44± 4.44[b]			25.44±2.79[b]	27.22±2.05[b]	25.44±3.01[b]
3	*Purpureocillium lilacinum*			19.78± 1.39[b]	26.33± 3.71[c]	21.44±6.50 [b]			25.44±1.13[b]	32.89± 3.10[c]	28.22±5.72[b]
4	*Trichoderma harzianum*			25.00± 3.46[c]	24.22± 2.91[c]	19.89± 2.26[b]			32.00±2.87[c]	29.89±2.93[bc]	27.44±2.96[b]
5	*Trichoderma koningii*			22.67±1.80[b]	21.78± 1.30[b]	21.11± 3.48[b]			30.22±1.64[c]	29.11±3.82[c]	25.33±3.46[b]

C- Control, P- Pathogen, B- Biocontrol

[1] Data is an average of three replicates

[2] Standard deviation

[3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

Table 31b. Effect of amendment of potting medium with talc formulation of *Clonostachys rosea*, *Penicillium multicolor*, *Purpureocillim lilacinum*, *Trichoderma harzianum* and *Trichoderma koningii* **on fresh and dry biomass of seedlings against seedling root rot and wilt causing pathogen *Athelia rolfsii* in teak seedlings grown for 180 days in polybag under field condition**

Sl. No.	Selected fungal Bio-agents	Biomass of Teak seedlings (g)[1]									
		Fresh Weight					Dry weight				
		C	P	B	BxP	PxB	C	P	B	BxP	PxB
1	*Clonostachys rosea*	11.44±2.86[2] [bc3]	5.66±0.45 [a]	11.00± 1.15 [b]	6.61± 0.57 [a]	6.39± 1.20 [a]	3.47± 1.08[bc]	1.88± 0.53[a]	2.31± 0.17 [a]	2.08± 0.55[a]	1.94± 0.41[a]
2	*Penicillium multicolor*			10.20± 1.16 [c]	7.11± 0.34[b]	4.87± 0.77 [a]			2.06± 0.55[ab]	2.32± 0.70[b]	1.39± 0.86[a]
3	*Purpureocillium lilacinum*			10.86± 0.98[c]	7.70± 1.26[b]	5.93± 2.75 [a]			2.37± 0.45[b]	1.74± 0.24[a]	1.70± 0.84[a]
4	*Trichoderma harzianum*			11.70± 0.71[c]	8.89± 3.47[b]	4.34± 1.38 [a]			2.80± 0.52 [b]	3.17± 1.18 [b]	1.43± 0.64 [a]
5	*Trichoderma koningii*			10.17± 0.78 [b]	6.68± 1.32 [a]	5.77± 2.37 [a]			2.56± 0.51[b]	2.21± 0.74[b]	1.38± 0.73 [a]

C- Control, P- Pathogen, B- Biocontrol

[1] Data is an average of three replicates

[2] Standard deviation

[3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

PLATE - 21

21.1. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	21.2. Teak seedlings amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
21.3. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.	21.4. Teak seedlings amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.
21.5. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	21.6. Teak seedlings amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
21.7. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.	21.8. Teak seedlings amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 21

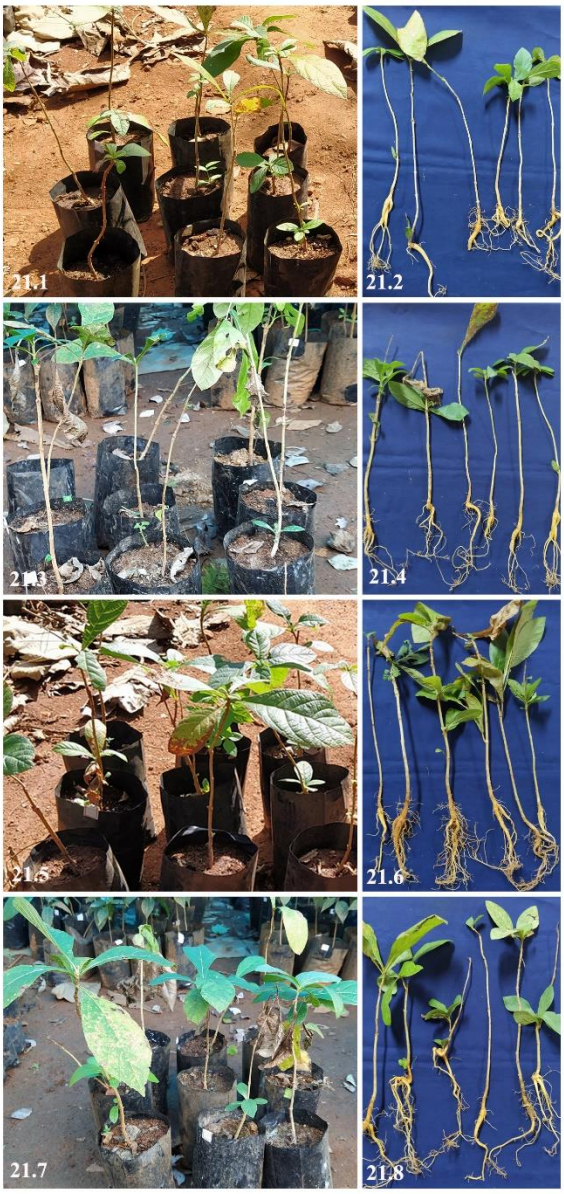

PLATE - 22

22.1. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	22.2. Teak seedlings amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
22.3. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.	22.4. Teak seedlings amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.
22.5. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	22.6. Teak seedlings amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
22.7. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.	22.8. Teak seedlings amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 22

PLATE - 23

23.1. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

23.2. Teak seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

23.3. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

23.4. Teak seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

23.5. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

23.6. Teak seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

23.7. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

23.8. Teak seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 23

PLATE - 24

24.1. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	24.2. Teak seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
24.3. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.	24.4. Teak seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.
24.5. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	24.6. Teak seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
24.7. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.	24.8. Teak seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 24

PLATE - 25

25.1. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	25.2. Teak seedlings amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
25.3. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.	25.4. Teak seedlings amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.
25.5. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	25.6. Teak seedlings amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
25.7. Growth promotion in Teak seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.	25.8. Teak seedlings amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 25

Seedlings amended with *F. oxysporum* showed a significant decrease in fresh weight (3.69 g ± 0.89). Among the antagonist treatments, except for *Trichoderma harzianum* (9.17 g ± 1.20), all the other antagonists caused no influence on fresh weight compared to the untreated control (8.45 g ± 1.52). There was no difference in dry weight between untreated seedlings (2.73 g ± 0.72) and those treated with various antagonists but a significant decrease was observed in seedlings treated with the pathogen (1.65 g ± 0.28) (Plate 26 and 27).

(i) Preventive treatment

In preventive treatments, none of the bio-agents application resulted in significant increase in root or shoot lengths compared to the respective controls.

When fresh and dry weight of seedlings were analyzed, seedlings treated with *Trichoderma harzianum* recorded a significant increase in fresh (10.21 g ± 1.77) and dry weight (3.96 g ± 1.08) compared to controls. None of the other antagonists caused any significant difference in fresh and dry weight of seedlings.

(ii) Curative treatment

In curative treatment, no significant difference was recorded in root length of seedlings treated with the antagonistic fungal isolates when compared to those treated with the pathogen. But, a significant increase was noticed in shoot length of seedlings treated with the bio-agents compared to those treated with the pathogen.

Both fresh and dry weights of seedlings treated with the antagonists showed significant increase when compared to the pathogen treated (Table 32 a and b).

PLATE - 26

26.1. Mahogany seedlings grown in unamended potting medium.

26.2. Mahogany seedlings grown in unamended potting medium showing healthy root system.

26.3. Mahogany seedlings grown in potting medium amended with *Fusarium oxysporum* at 2 x 10^6 conidial suspensions showing wilt symptoms.

26.4. Mahogany seedlings grown in potting medium amended with *Fusarium oxysporum* at 2 x 10^6 conidial suspensions showing wilt symptoms.

26.5. Mahogany seedlings grown in potting medium amended with *Athelia rolfsii* showing wilt and rot symptoms.

26.6. Mahogany seedlings grown in potting medium amended with *Athelia rolfsii* showing wilt and rot symptoms.

26.7. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Clonostachys rosea* at 2 x 10^6 conidial suspensions.

26.8. Mahogany seedlings grown in potting medium amended with *Clonostachys rosea* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

PLATE - 26

PLATE - 27

27.1. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Penicillium multicolor* at 2 x 10^6 conidial suspensions.

27.2. Mahogany seedlings grown in potting medium amended with *Penicillium multicolor* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

27.3. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Purpureocillium lilacinum* at 2 x 10^6 conidial suspensions.

27.4. Mahogany seedlings grown in potting medium amended with *Purpureocillium lilacinum* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

27.5. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Trichoderma harzianum* at 2 x 10^6 conidial suspensions.

27.6. Mahogany seedlings grown in potting medium amended with *Trichoderma harzianum* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

27.7. Growth promotion in mahogany seedlings in potting medium amended with fungal antagonist *Trichoderma koningii* at 2 x 10^6 conidial suspensions.

27.8. Mahogany seedlings grown in potting medium amended with *Trichoderma koningii* at 2 x 10^6 conidial suspensions showing healthy shoot and root.

PLATE - 27

Table 32a. Effect of amendment of potting medium with talc formulation of *Clonostachys rosea*, *Penicillium multicolor, Purpureocillium lilacinum, Trichoderma harzianum* and *Trichoderma koningii* **on root and shoot length of seedlings against seedling root rot and wilt causing pathogen *Fusarium oxysporum* in mahogany seedlings grown for 180 days in polybag under field condition**

Sl. No.	Selected fungal Bio-agents	Root and Shoot Length of Mahagony seedlings (cm)[1]									
		Root length					Shoot length				
		C	P	B	BxP	PxB	C	P	B	BxP	PxB
1	*Clonostachys rosea*	24.78± 4.73[2 abc3]	18.00±0.86[a]	27.33±2.45[b]	19.78±1.64[a]	18.67±2.19[a]	31.33±4.44[abcd]	22.44±1.81[ab]	31.56±2.87[c]	28.78±2.91[bc]	27.33±2.29[b]
2	*Penicillium multicolor*			22.78±2.68[b]	18.89±3.14[a]	18.11±3.99[a]			29.33± 1.59[bc]	30.44±1.94[cd]	27.67± 2.34[b]
3	*Purpureocilliu m lilacinum*			22.11±2.62[b]	18.00±2.12[a]	16.44±2.29[a]			29.00±2.18[c]	28.22±2.20[bc]	28.00±1.59[b]
4	*Trichoderma harzianum*			24.44±1.13[b]	23.33±2.74[b]	18.00±1.79[a]			29.22 ±2.49 [b]	30.11±5.19[b]	29.22±2.08[b]
5	*Trichoderma koningii*			20.89±1.83[b]	20.67±3.39[b]	20.84±3.62[b]			27.78±2.28[b]	28.56±1.74[a]	27.79±4.47[a]

C- Control, P- Pathogen, B- Biocontrol

[1] Data is an average of three replicates

[2] Standard deviation

[3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

Table 32b. Effect of amendment of potting medium with talc formulation of *Clonostachys rosea*, *Penicillium multicolor*, *Purpureocillium lilacinum, Trichoderma harzianum* and *Trichoderma koningii* **on fresh and dry biomass of seedlings against seedling root rot and wilt causing pathogen *Fusarium oxysporum* in mahogany seedlings grown for 180 days in polybag under field condition**

Sl. No.	Selected fungal Bio-agents	Biomass of Mahagony seedlings (g)[1]									
		Fresh Weight					Dry weight				
		C	P	B	BxP	PxB	C	P	B	BxP	PxB
1	*Clonostachys rosea*	8.45±1.52[2 cd3]	3.69±0.89[a]	7.85± 0.79[c]	6.93± 1.15 [c]	5.80± 1.38 [b]	2.73± 0.72[bc]	1.65± 0.28[a]	2.74± 1.18[b]	2.87± 1.12[b]	2.11± 0.51[a]
2	*Penicillium multicolor*			7.67± 0.43[c]	5.38± 1.07[b]	4.07± 1.07[a]			2.24± 0.54[bc]	2.53± 0.55[c]	1.91± 0.52[ab]
3	*Purpureocillium lilacinum*			8.56± 0.51[c]	6.23± 1.47[b]	5.49± 0.67[b]			2.13± 0.45[a]	2.17± 0.59 [a]	2.05± 0.41[a]
4	*Trichoderma harzianum*			9.17± 1.20[c]	10.21±1.77[d]	6.89± 1.21[b]			2.67± 0.49[b]	3.96± 1.08[c]	2.69± 0.73[b]
5	*Trichoderma koningii*			8.12± 0.56 [c]	6.32± 1.18[b]	6.13± 2.03[b]			2.67± 0.61[b]	2.79± 0.64[b]	2.49± 1.05[b]

C- Control, P- Pathogen, B- Biocontrol

[1] Data is an average of three replicates

[2] Standard deviation

[3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

4. Effect of antagonistic fungal isolates on growth promotion of mahogany seedlings inoculated with *Athelia rolfsii*

Results of the untreated control and bio-agents treated are detailed earlier (3 (i)).

(i) Control (seedlings treated with pathogen)

Root and shoot lengths of the seedlings inoculated with the pathogen were 16.78 cm ± 1.99 and 25.11 cm ± 2.37, respectively. A significant decrease in the fresh and dry weight of seedlings was noted in those inoculated upon the pathogen (fresh weight- 2.83 g ± 0.96; dry weight-1.72 g ± 0.37).

(ii) Preventive treatment

None of the antagonistic fungal isolates caused any significant variation in seedling root lengths. In the case of shoot length, except for *P. lilacinum* (31.67 cm ± 3.67) none of the other antagonists noticed any significant influence.

When fresh weights were observed none of the antagonists showed any significant effect compared to the control. But *P. lilacinum* (3.12 g ± 0.91) when applied prior to the pathogen significantly increased the dry weight of the seedlings. Rest of the selected bio-agents exerted no effect on dry weights.

(iii) Curative treatment

In curative treatment against *A. rolfsii*, none of the antagonists caused any significant variation in root and shoot length compared to the respective controls. However, both fresh and dry weight of seedlings exhibited a significant increase when compared to those treated with the pathogen (Table 33 a and b) (Plate 28 - 32).

Table 33a. Effect of amendment of potting medium with talc formulation of *Clonostachys rosea*, *Penicillium multicolor*, *Purpureocillium lilacinum*, *Trichoderma harzianum* and *Trichoderma koningii* **on root and shoot length of seedlings against seedling root rot and wilt causing pathogen *Athelia rolfsii* in mahogany seedlings grown for 180 days in polybag under field condition**

Sl. No.	Selected fungal Bio-agents	Root and Shoot Length of Mahagony seedlings (cm)[1]									
		Root length					Shoot length				
		C	P	B	BxP	PxB	C	P	B	BxP	PxB
1	*Clonostachys rosea*	24.78± 4.73[2 abc3]	16.78± 1.99[a]	27.33± 2.45 [b]	20.00± 3.77 [a]	19.89± 2.31[a]	27.89± 2.57[bc]	18.22± 1.30[a]	30.33±3.31[c]	29.67± 3.57[c]	26.67± 2.82[b]
2	*Penicillium multicolor*			22.78± 2.68[b]	18.22± 1.20[a]	18.00± 2.23 [a]			25.44± 2.79[b]	27.22± 2.05[b]	25.44± 3.01[b]
3	*Purpureocillium lilacinum*			22.11± 2.62[b]	19.11± 1.17[a]	17.78± 1.56[a]			25.44± 1.13[b]	32.89± 3.10[c]	28.22± 5.72[b]
4	*Trichoderma harzianum*			24.44± 1.13 [b]	18.22± 2.22 [a]	18.11± 2.14 [a]			32.00±2.87[c]	29.89± 2.93[bc]	27.44± 2.96[b]
5	*Trichoderma koningii*			20.89±1.83[b]	17.11± 1.83 [a]	16.78± 1.00 [a]			30.22± 1.64[c]	29.11± 3.82[c]	25.33± 3.46[b]

C- Control, P- Pathogen, B- Biocontrol

[1] Data is an average of three replicates

[2] Standard deviation

[3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

Table 33b. Effect of amendment of potting medium with talc formulation of *Clonostachys rosea*, *Penicillium multicolor*, *Purpureocillium lilacinum*, *Trichoderma harzianum* and *Trichoderma koningii* **on fresh and dry biomass of seedlings against seedling root rot and wilt causing pathogen *Athelia rolfsii* in mahogany seedlings grown for 180 days in polybag under field condition**

Sl. No.	Selected fungal Bio-agents	Biomass of Mahagony seedlings (g) [1]									
		Fresh Weight					Dry weight				
		C	P	B	BxP	PxB	C	P	B	BxP	PxB
1	*Clonostachys rosea*	8.45±1.52[2 cd3]	2.83±0.96 [a]	7.85± 0.79[bc]	7.76± 1.25[bc]	6.85± 1.31[b]	2.73± 0.72[b]	1.72± 0.37[a]	2.84± 1.18[b]	2.91± 1.14[b]	2.74± 0.78[b]
2	*Penicillium multicolor*			7.67± 0.43[c]	6.51± 1.65[b]	6.34± 0.68			2.24± 0.54[ab]	2.86± 0.48[b]	2.34± 0.80[ab]
3	*Purpureocillium lilacinum*			8.56± 0.51[d]	6.40± 1.20[c]	4.64± 1.30[b]			2.13± 0.45[a]	3.12± 0.91[c]	1.73± 0.73[a]
4	*Trichoderma harzianum*			9.17± 1.20[d]	7.30± 1.10[c]	4.56± 0.98[b]			2.67± 0.49 [b]	2.71± 0.44[b]	2.22± 0.63[a]
5	*Trichoderma koningii*			8.12± 0.56[bc]	7.16± 1.53[b]	7.13± 1.35[b]			2.67± 0.61[b]	2.41± 0.48[b]	2.23± 0.83[a]

C- Control, P- Pathogen, B- Biocontrol

[1] Data is an average of three replicates

[2] Standard deviation

[3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

PLATE - 28

28.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

28.2. Mahogany seedlings amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

28.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

28.4. Mahogany seedlings amended with conidial suspensions of *Clonostachys rosea* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

28.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

28.6. Mahogany seedlings amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

28.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

28.8. Mahogany seedlings amended with conidial suspensions of *Clonostachys rosea* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 28

PLATE - 29

29.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

29.2. Mahogany seedlings amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

29.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

29.4. Mahogany seedlings amended with conidial suspensions of *Penicillium multicolor* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

29.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

29.6. Mahogany seedlings amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

29.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

29.8. Mahogany seedlings amended with conidial suspensions of *Penicillium multicolor* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 29

PLATE - 30

30.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

30.2. Mahogany seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

30.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

30.4. Mahogany seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

30.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

30.6. Mahogany seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

30.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

30.8. Mahogany seedlings amended with conidial suspensions of *Purpureocillium lilacinum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 30

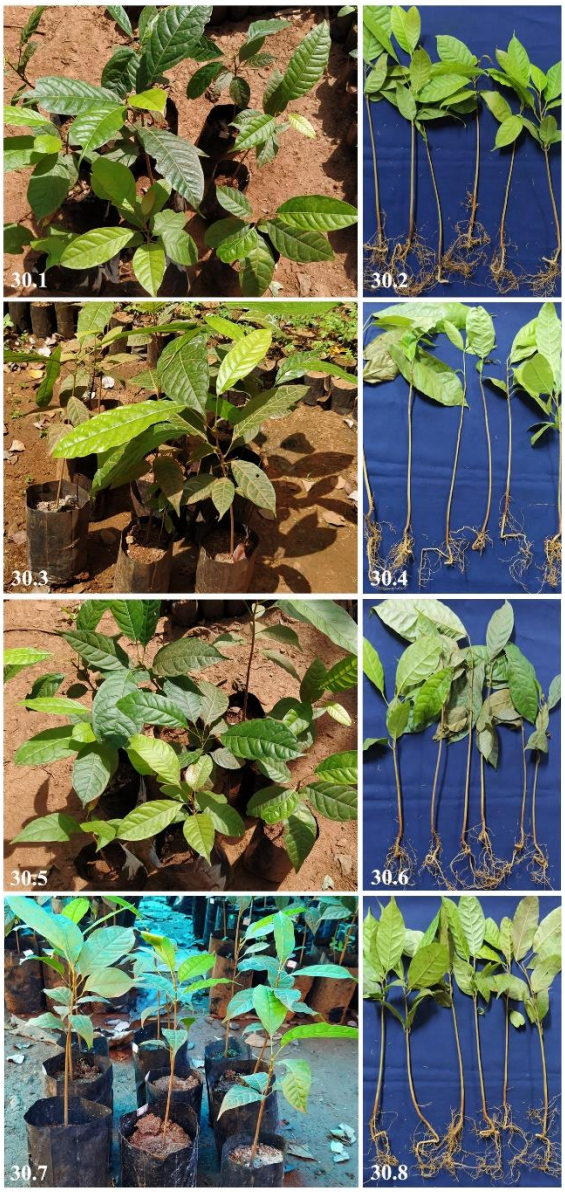

PLATE - 31

31.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	31.2. Mahogany seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
31.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.	31.4. Mahogany seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.
31.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.	31.6. Mahogany seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.
31.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.	31.8. Mahogany seedlings amended with conidial suspensions of *Trichoderma harzianum* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 31

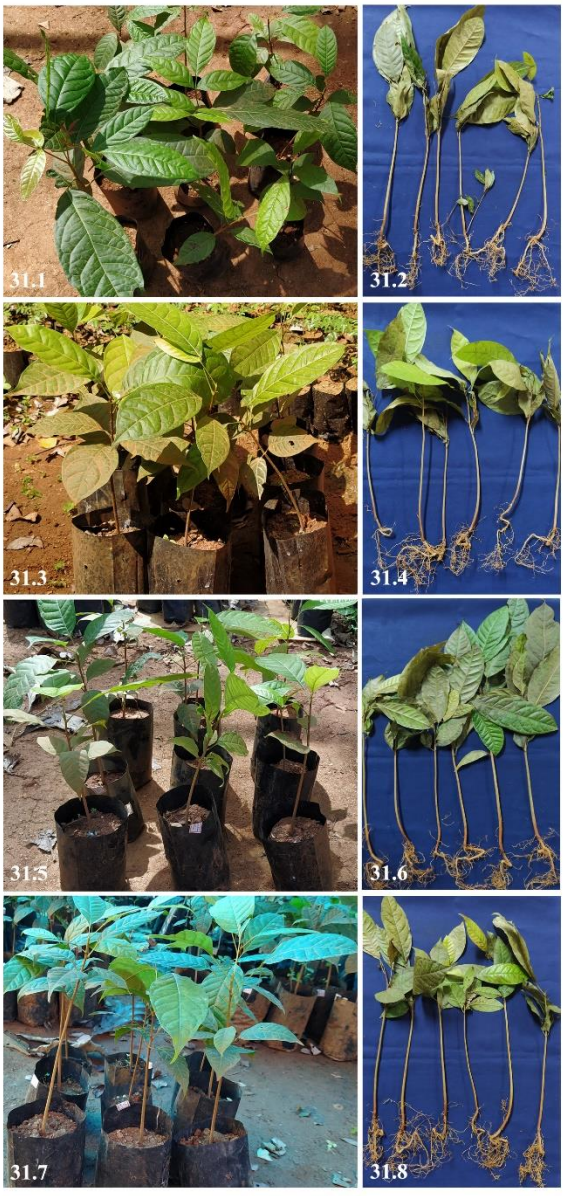

PLATE - 32

32.1. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

32.2. Mahogany seedlings amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

32.3. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration (pathogen: antagonist) in curative treatment.

32.4. Mahogany seedlings amended with conidial suspensions of *Trichoderma koningii* against *Athelia rolfsii* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

32.5. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in preventive treatment.

32.6. Mahogany seedlings amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in preventive treatment showing increased root and shoot length.

32.7. Growth promotion in Mahogany seedlings at 180 days in potting medium amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration (pathogen: antagonist) in curative treatment.

32.8. Mahogany seedlings amended with conidial suspensions of *Trichoderma koningii* against *Fusarium oxysporum* at 1:5 concentration at 180 days (pathogen: antagonist) in curative treatment showing increased root and shoot length.

PLATE - 32

Growth promotion in plant involves mechanisms which enable an increased uptake of mineral nutrients (Azcon and Barea, 1975; Barea *et al*., 1983; Shivanna *et al*., 1994, 1996). A number of studies have shown that an increased fungal colonization of the root and the rhizosphere enhance plant-soil interface which support enhanced absorption of nutrients which results in improved plant growth (Barber and Lynch 1977; Halvorson *et al*., 1990; Sailo and Bagyaraj 2005; Bagyaraj *et al*., 2015; Thilagar and Bagyaraj 2015).

In the present study, selected antagonists showed different influence on the growth of teak and mahogany seedlings. Seedlings of teak showed significant increase in root and shoot length when *Clonostachys rosea*, *Trichoderma harzianum* and *T. koningii* were applied separately but there was no variation in fresh and dry weight compared to controls. Raghu *et al*., (2020) from Mandya district, Karnataka, recently reported the effect of plant beneficial consortium (*Ambispora leptoticha, Azotobacter chroococcum, Trichoderma harzianum*) in the growth of teak seedlings. They reported a 97% increase in dry weight of teak seedlings in response to treatments compared to un-inoculated controls. On the contradictory, the present study showed a decrease in fresh and dry weights of teak seedlings when inoculated with certain fungal antagonists compared to un-inoculated controls. A difference was noted only for mahogany seedlings which showed enhanced root and shoot length when inoculated with *Clonostachys rosea* and enhanced fresh and dry weight when inoculated with *Trichoderma harzianum*. These results confirm to the findings of various researchers who showed that *Trichoderma harzianum* has abilities to promote plant growth especially in agricultural crops (Harman and Bjorkman, 1998; Lo and Lin, 2002; Rini and Sulochana, 2006; Singh and Singh, 2008; Hermosa *et al*., 2012).

In the current study *Trichoderma harzianum* outperformed *T. koningii* in improving all the plant functional attributes emphasizing the influence of conditions like soil environment, inoculum concentration and the strain of *Trichoderma* (Baker, 1988; Kleifeld and Chet, 1992; Ousley *et al*., 1994a, b). Phuwiwat and Soytong (2001) studied influence of *Penicillium notatum* isolated from rhizospheric soil on the growth of Chinese mustard. They recorded a higher plant yield compared to untreated control. And, all plant growth parameters such as height, root length, root diameter, fresh and dry weight of shoot and root, and the total plant dry weight were enhanced in accordance with the inoculum density of *P. notatum*.

The antagonists behaved differently in enhancing seedling growth in preventive and curative treatments in this study. In teak seedlings infected with *Fusarium oxysporum* observed no significant variation in root and shoot length in both the treatments except for treatment with *Penicillium multicolor* and *Purpureocillium lilacinum* where preventive treatment improved root lengths and the latter also promoted shoot length. With regard to fresh and dry weights, curative treatment upon the antagonists resulted in an increase in these features compared to the seedlings inoculated with the pathogen. In seedlings infected by *Athelia rolfsii*, curative treatments promoted growth of seedlings compared to preventive treatments except for *Purpureocillium lilacinum* which exhibited a better activity in the latter. No significant variation was observed in fresh and dry weights in both the treatments compared to the respective controls.

In mahogany seedlings infected by *F. oxysporum* showed comparatively increased shoot length in curative treatments whereas root length noticed no marked difference compared to the respective controls. Also an increase in dry weight for curative

treatments was observed in seedlings treated with *F. oxysporum*. In seedlings infected by *Athelia rolfsii*, there was no significant effect on root and shoot length in both the treatments except for curative treatment which enhanced shoot length. *Purpureocillium lilacinum* incited shoot length in preventive treatments also. Dry weight of seedlings showed a comparative increase in curative treatments but this increase was not apparent in preventive treatments.

Verhaar *et al*., (1997) at Bleiswijk, Netherlands analyzed biocontrol attributes of *Verticillium lecanii* against cucumber powdery mildew pathogen *Sphaerotheca fuliginea*. Their studies stressed the need of timely application of bio-agents in managing the pathogen through preventive and curative treatments. In 2005, Utkhede and Mathur in Ontario, Canada, tested Plant Shield® (*Trichoderma harzianum*), Prestop® (*Gliocladium catenulatum*), Quadra 136 (*Bacillus subtilis*), Root Shield® (*Trichoderma harzianum*), and S33 (*Rhodosporidium diobovatum*) and chemical treatment with Decree® (Cyclohexanecarboxamide) preventively and curatively against tomato stem canker pathogen *Botrytis cinerea* and both the treatments were efficient in reducing the lesions as well as improving plant growth.

In another study, five bio-control agents Predatox® (*Trichoderma* sp.), Ecotrich® (*Trichoderma harzianum*), Trichodelaereo ® (*Trichoderma harzianum*), Nemathel® (*Bacillus* sp.) and conidial suspension of *Trichoderma atroviride* were tested against the pathogen *Oidium eucalypti* in *Eucalyptus benthamii* and preventive and curative treatments effectively managed the pathogen (Bovolini *et al*., 2018). Boughalleb-M'Hamdi *et al*., (2018) in Tunisia, evaluated the efficacy of species of *Aspergillus*, *Penicillium* and *Trichoderma* against four soil-borne fungi affecting melon and

watermelon. Preventive treatments using *A. flavus* and *A. fumigates* reduced disease incidence by *Fusarium oxysporum* and also improved shoot and root dry weight. Likewise, *Penicillium digitatum*, *Trichoderma harzianum* and *Trichoderma viride* were effective against *Macrophomina phaseolina* in water melon. Curative treatments using *Trichoderma erinaceum*, *T. viride* and *A. flavus* was effective against water melon pathogen *F. solani* f. sp. *cucurbitae*. Treatment using *Trichoderma helicum* against *F. oxysporum* f. sp. *niveum* improved growth of the affected water melon plant which indicates the importance of bio-agents in managing plant diseases. Nwauzoma *et al*., (2017) evaluated the efficacy of leaf extracts of *Carica papaya*, *Chromolaena odorata* and *Azadirachta indica* and bio-agent *Trichoderma koningii* (preventive and curative) and kept Dithane M45 at 2% as control for comparison against *Sclerotium rolfsii* causing rot disease of cocoyam cormel plant. Curative treatments resulted in greater efficacy with plant extracts and Dithane M45 while *T. koningii* better controlled the disease in preventive treatments. Also, treatment with plant extracts and *T. koningii* were found more effective in controlling the disease than those treated with Dithane M45.

The antagonistic attributes of the bio-agent in the rhizosphere may be ascribed to the saprophytic ability and biocontrol activity. Parasitic abilities may be limited in the rhizosphere region owing to the exudates released from the host species but it could be efficient in non-rhizosphere region where antibiosis perform better irrespective of the source (Adams and Fravel, 1990; Fravel *et al*., 1992). The present study also proved the efficacy of the treatments using antagonists in the management of the pathogens but was not significantly better compared to the untreated control. However, the treatments were found to improve plant growth which can give some resistance to the plant against the

pathogen (Amaral *et al*., 2017; Chagas *et al*., 2017; Goncalves *et al*., 2018). These results also indicate the ability of antagonists to colonize roots outperforming indigenous microbial population (Parke 1991). Overall, the study indicates that both preventive and curative treatments using efficient biological antagonists are a viable alternative for chemical fungicides.

It is well established that soil micro-flora contributes to the natural fertility of soils which directly or indirectly influence plant growth. Against this background, variation in soil fungal colony forming units (cfu/g) in the rhizopshere of teak and mahogany seedlings in response to treatments with fungal antagonists and pathogens was also studied.

5. Rhizophere mycoflora of teak seedlings amended with different treatments.

Rhizosphere samples from healthy teak seedlings yielded 89 cfu/g (on average), and the seedlings treated with *F. oxysporum* and *A. rofsii* yielded 53 and 61 cfu/g, respectively. When bio-agents were applied individually, on an average *Clonostachys rosea* treated seedlings yielded 34 cfu/g, *Penicillium multicolor* 37 cfu/g, *Purpureocillium lilacinum* 34 cfu/g, *Trichoderma harzianum* and *T. koningii* yielded 26 and 29 cfu/g, respectively. Fungal density in the rhizosphere was decreased in seedlings treated with bio-agents compared to that of untreated control. However, seedlings treated with both the pathogens had a higher density of fungi compared to those treated with the bio-agents. Understandably, rhizosphere of healthy seedling had the highest density of fungi (Table 34a).

Fungal colony forming units were comparatively higher in preventive treatments but the results were not significantly different from those of curative treatments. There was a marked decrease in the cfu/g in seedlings treated with antagonists compared to controls (Table 34b).

Table 34a. Total number of colony forming units of fungi in the rhizosphere of teak seedlings (treated and untreated)

Treatments	Number of fungi - cfu/g[1]
Healthy teak seedlings (Control)	89
Treated with *Fusarium oxysporum*	53
Treated with *Athelia rolfsii*	61
Treated with *Clonostachys rosea*	34
Treated with *Penicillium multicolor*	37
Treated with *Purpureocillium lilacinum*	34
Treated with *Trichoderma harzianum*	26
Treated with *Trichoderma koningii*	29

[1] Data is an average of three replicates

Table 34b. Total number of colony forming units of fungi in the rhizosphere of teak seedlings (preventive and curative treatment)

Organisms	**Preventive and curative treatments of selected biocontrol agents in Teak seedlings**									
	C. rosea		***P. multicolor***		***P. lilacinum***		***T. harzianum***		***T. koningii***	
Treatments	**P**	**C**	**P**	**C**	**P**	**C**	**P**	**C**	**P**	**C**
Fusarium oxysporum	9	5	10	11	21	13	10	7	11	7
Athelia rolfsii	9	4	11	6	11	7	8	6	11	6

C. rosea- Clonostachys rosea; *P. multicolor- Penicillium multicolor*; *P. lilacinum- Purpureocillium lilacinum*; *T. harzianum -Trichoderma harzianum; T. koningii-Trichoderma koningii*

P-Preventive, C- Curative

6. Rhizosphere mycoflora of mahogany seedlings amended with different treatments.

Fungal density in the rhizosphere of healthy mahogany seedlings on average was 35 cfu/g. In contrast, seedlings infested with the pathogens had a higher cfu/g (*F. oxysporum* - 65 cfu/g and *A.rolfsii* - 74 cfu/g) compared to controls. When bio-agents

were applied individually, seedlings treated with *Clonostachys rosea* yielded 16 cfu/g, *Penicillium multicolor* - 18 cfu/g, *Purpureocillium lilacinum*- 26 cfu/g, *Trichoderma harzianum* and *T. koningii* yielded 21 and 24 cfu/g, respectively. The study indicated that the fungal density in the rhizosphere decreased in the case of bio-agent treated seedlings compared to untreated control. However, seedlings treated with the pathogens yielded a higher number of colony forming units compared to untreated controls.

Fungal colony forming units were comparatively higher in preventive treatments but with no significant variation in relation to curative treatments except in seedlings treated with *Purpureocillium lilacinum* which had significantly a higher number of fungal colonies in preventive treatment. Density of fungi was significantly lower in the rhizosphere of seedlings affected by the pathogen compared to healthy seedlings (Table 35 a and b).

Table 35a. Total number of colony forming units of fungi in the rhizosphere of mahogany seedlings (treated and untreated)

Treatments	Number of fungi – cfu/g[1]
Healthy Mahogany seedlings (Control)	35
Treated with *Fusarium oxysporum*	65
Treated with *Athelia rolfsii*	74
Treated with *Clonostachys rosea*	16
Treated with *Penicillium multicolor*	18
Treated with *Purpureocillium lilacinum*	26
Treated with *Trichoderma harzianum*	21
Treated with *Trichoderma koningii*	24

[1] Data is an average of three replicates

Table 35b. Total number of colony forming units of fungi in the rhizosphere of mahogany seedlings (preventive and curative treatment)

Organisms	Preventive and curative treatments of selected biocontrol agents in Mahogany seedlings									
	C. rosea		*P. multicolor*		*P. lilacinum*		*T. harzianum*		*T. koningii*	
Treatments	**P**	**C**	**P**	**C**	**P**	**C**	**P**	**C**	**P**	**C**
Fusarium oxysporum	8	5	9	10	18	9	9	6	4	3
Athelia rolfsii	11	9	10	8	26	13	20	12	14	12

C. rosea- Clonostachys rosea; *P. multicolor- Penicillium multicolor*; *P. lilacinum-Purpureocillium lilacinum*; *T. harzianum -Trichoderma harzianum*; *T. koningii-Trichoderma koningii*

P-Preventive, C- Curative

Naseby *et al*., (2000) observed a characteristic variation of five strains of *Trichoderma* (*Trichoderma harzianum* strains TH1, T4, T12 N47 and *Trichoderma pseudokoningii* strain To10) evaluated for growth parameters as well as soil microflora in pea plant in the presence and absence of *Pythium ultimum* in Surrey, England. They observed a significant increase in wet shoot weight (15%) in the absence of *Pythium* but no significant effect on dry weight. A variation in the activities among the strains was also observed where T4 and N47 significantly increased the root weights by 22% and 8% respectively whilst strains TH1 and N47 resulted in significantly greater root lengths. A significant decrease was observed in plant length and weight when inoculated with *Pythium*. Inoculation of *Trichoderma* strains prior to *Pythium* significantly increased plant lengths and weights except for To10 which had no effect. In addition, a significant increase in soil fungal population was observed in *Pythium* infested plant compared to *Trichoderma* treated which could be attributed to the extent of damage and subsequent nutrient leakage. In 1987, Lynch observed an increase in *Trichoderma* inocula in the absence of *Pythium* is influenced by inoculum concentration.

The soil fungal community is known to be influenced by soil nutrients (Christian *et al*., 2008) and root exudates (Raaijmakers *et al*., 2009). An altered situation in the

rhizosphere influenced by added inoculum of *Trichoderma* may have negative influence of soil mycoflora (Zhang *et al*., 2018a). Increased fungal density in the rhizosphere of pathogen infested plants could be due to the efflux of carbon compounds from damaged roots (Raaijmakers *et al*., 2009). This also serves as an indicator of the extent of damage caused by the pathogen.

i) Resistance Inducing abilities

Biotic and abiotic elements directly or indirectly influence plant growth and development and the involvement of microbes imparting beneficial attributes to the plant have been illustrated (Compant *et al*., 2010). Root exudates comprise several compounds that stimulate beneficial plant-microbe relationship and are orchestrated by complex morpho-molecular expressions (Balestrini and Bonfante, 2005; Hermosa *et al*., 2012; Huang *et al*., 2014). Bio-synthesis of plant metabolites may be influenced by environmental changes (Ramakrishna and Ravishankar, 2011; Chetri *et al*., 2013; Berini *et al*., 2018). However bio-synthesis of metabolites doesn't perform a significant role in the primary life of plant when metabolites are produced in low concentrations corresponding to the different growth stages of the plant (Ncube and van Staden, 2015).

In response to competitive and unfavourable conditions the primary metabolites through various signalling pathways synthesize a number of secondary molecules to cope with the stress and are accumulated in plant cells (Edreva *et al*., 2008; Rejeb *et al*., 2014; Caretto *et al*., 2015; Narayani and Srivastava, 2018). Numerous reports have attributed the role of plant secondary metabolites in protection against various pests and pathogens besides coping up harsh environmental conditions and have led to various *in vitro* and *in vivo* manipulations to establish these roles of secondary metabolites (Zhao *et al*., 2005;

Kim *et al*., 2010; Goyal *et al*., 2012; Selmar and Kleinwachter, 2013). Bryant *et al*., (1983) in his studies showed carbon exchange resulting in an increase in biomass production eliciting plant secondary metabolites in response to stress conditions. Many plant signaling pathways such as salicylic and jasmonic acids, calcium, abscisic acid, polyamines and nitric oxides induce secondary metabolite synthesis in response to various cellular stresses. However, the chemical rationale of the signal transduction system is not clearly understood (Chinnusamy *et al*., 2004; Edreva *et al*., 2008; Ramakrishna and Ravishankar, 2011; Narayani and Srivastava, 2018).

In the present study teak and mahogany seedlings were inoculated with fungal antagonists to evaluate their efficacy in managing the disease and growth and development of the plants. The treated seedlings were evaluated for bio-chemical compounds in response to various treatments and the active compounds with their retention time, area, area percentage, height, height percentage, base m/z and their names were recorded.

GC-MS analysis of Mahogany seedlings for Resistance Inducing Biochemical Compounds

Methanol extracts of untreated mahogany seedlings showed the presence of 22 compounds. Chinasaure (18.02), Glucal (15.88), Hexadecanoic acid (10.03) were the major compounds recorded (Fig 1). In seedlings inoculated with *Fusarium oxysporum* 21 different bio-compounds were identified of which Hexadecanoic acid (19.42) and 9, 12-Octadecadienoic acid (Z, Z) - (18.59) were the major compounds (Fig 2). Whereas seedlings inoculated upon *Athelia rolfsii*, 23 different compounds were obtained among them Hexadecanoic acid (13.34), Phytol (10.99) and Linolelaidic acid, Methyl ester (10.59) were the major compounds (Table 36) (Fig 3) (Appendix 1-3).

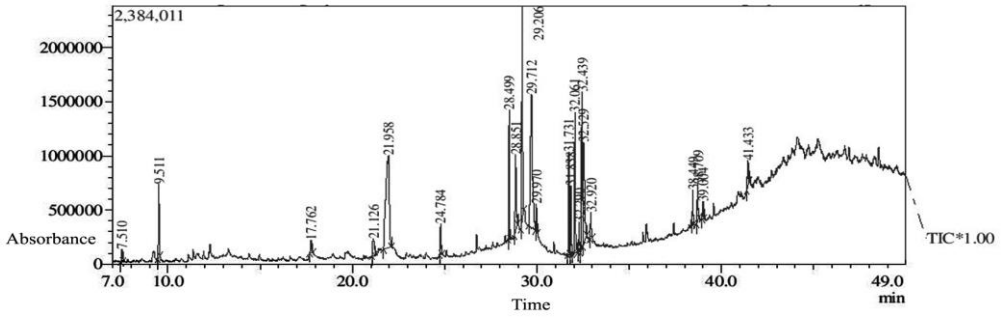

Fig 1.Chromatogram of methanol extract of untreated Mahogany seedlings showing different biochemical compounds by GC-MS analysis

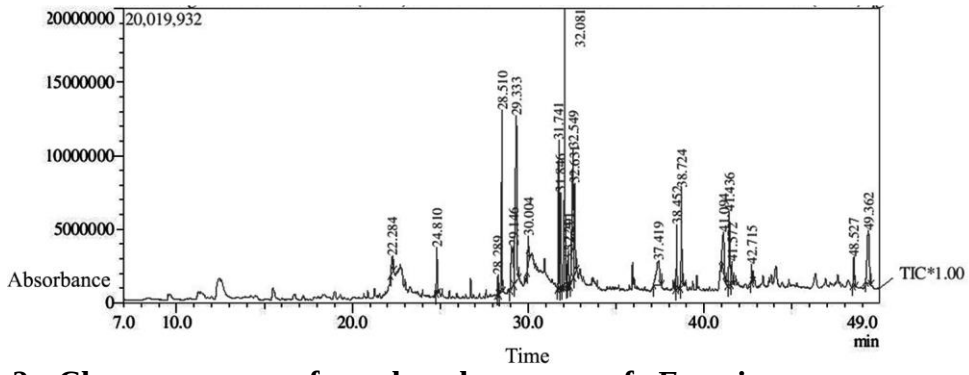

Fig 2. Chromatogram of methanol extract of *Fusarium oxysporum* amended Mahogany seedlings showing different biochemical compounds by GC-MS analysis

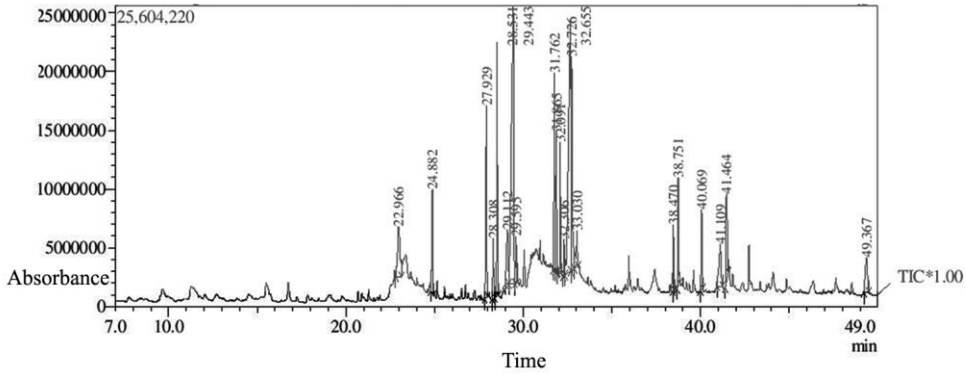

Fig 3. Chromatogram of methanol extract of *Athelia rolfsii* amended Mahogany seedlings showing different biochemical compounds by GC-MS analysis

Table 36. Major biochemical compounds identified in control and pathogen- *Fusarium oxysporum* and *Athelia rolfsii* **treated mahogany seedlings**

Sl. No.	Major biochemical compounds associated in mahogany seedlings	Biochemical compounds produced in mahogany seedlings in control and treated with selected Pathogens		
		Control (22[1])	*Fusarium oxysporum* (21)	*Athelia rolfsii* (23)
1	Chinasaure	18.02	-	-
2	Hexadecanoic acid	10.03	19.42	13.34
3	Glucal	15.88	-	-
4	9,12-Octadecadienoic acid (Z,Z)	3.52	18.59	-
5	Phytol	-	-	10.99
6	Linolelaidic acid, Methyl ester	-	-	10.59

[1] Value in Parenthesis represents total number of Bio-compounds associated with teak seedlings

'-'- Absent

Mahogany seedlings treated upon biocontrol agent, *Clonosatchys rosea* revealed the presence of 22 bio-compounds with Chinasaure (27.59), Glucal (12.59) and Linolenic acid (11.84) representing the major compounds (Fig 4). Seedlings amended with *Penicillium multicolor*, Chinasaure (19.67), gamma.-Sitosterol (13.90), 1, 5-Anhydro-d-mannitol (13.89) and Hexadecanoic acid (10.33) were the major compounds among the 20 different bio-compounds identified (Fig 5).

Seedlings treated with *Purpureocillium lilacinum* produced 19 different compounds with Glucal (27.99), Chinasaure (15.91) and Decanoic acid (11.61) being the major ones (Fig 6). In seedlings treated with *Trichoderma harzianum*, 22 different compounds were obtained of which 1, 5-Anhydro-d-mannitol (25.91) and Chinasaure (15.58) represented the major compounds (Fig 7). Seedlings treated upon *Trichoderma koningii* synthesized 18 different compounds of which Chinasaure (18.86) and Glucal (13.42) were the major among the different compounds identified (Fig 8).

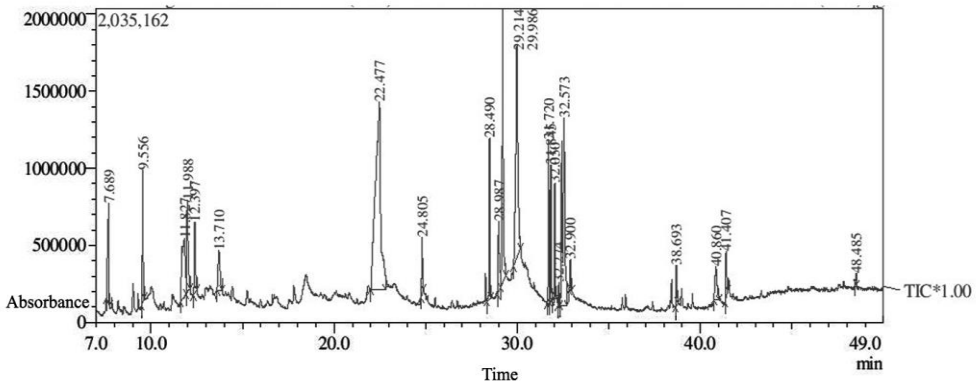

Fig 4. Chromatogram of methanol extract of *Clonostachys rosea* amended Mahogany seedlings showing different biochemical compounds by GC-MS analysis

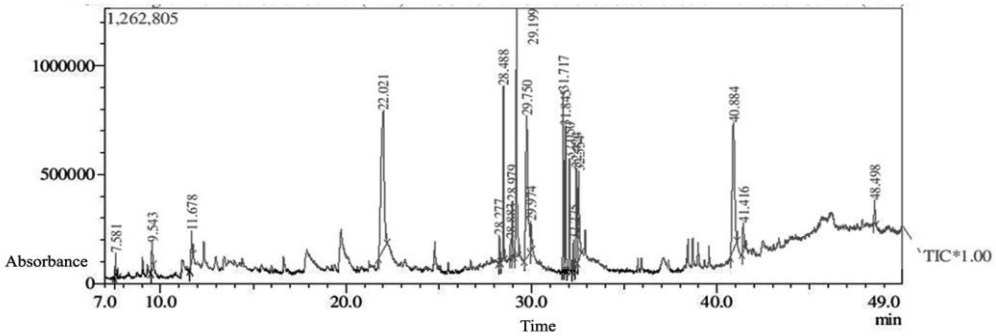

Fig 5. Chromatogram of methanol extract of *Penicillium multicolor* amended Mahogany seedlings showing different biochemical compounds by GC-MS analysis

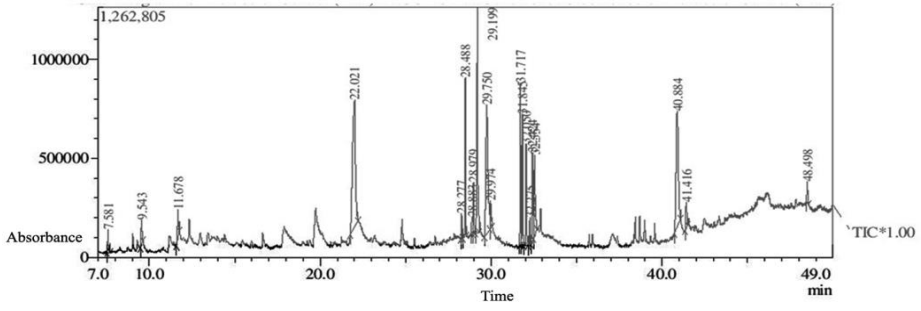

Fig 6. Chromatogram of methanol extract of *Purpureocillium lilacinum* amended Mahogany seedlings showing different biochemical compounds by GC-MS analysis

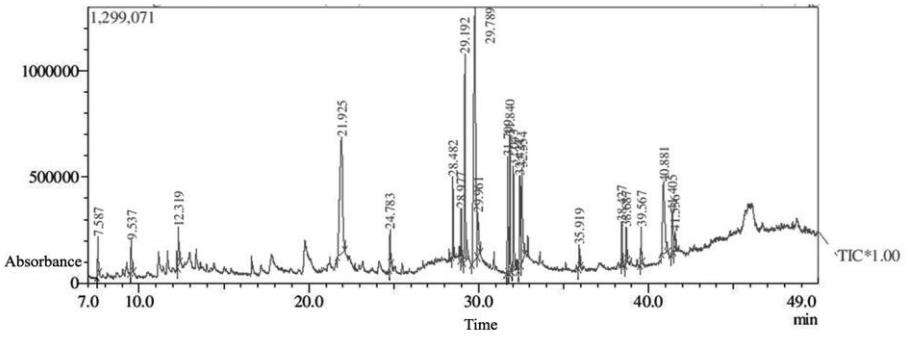

Fig 7. Chromatogram of methanol extract of *Trichoderma harzianum* amended Mahogany seedlings showing different biochemical compounds by GC-MS analysis

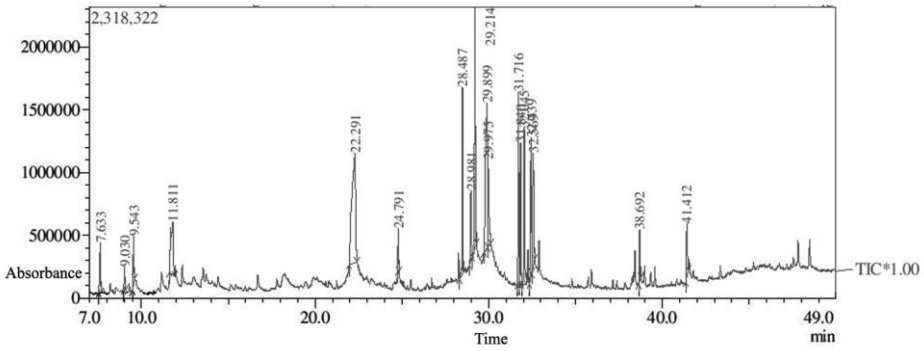

Fig 8. Chromatogram of methanol extract of *Trichoderma koningii* amended Mahogany seedlings showing different biochemical compounds by GC-MS analysis

Preventive and Curative treatments

Seedlings subjected for preventive and curative treatments revealed some variation in the different bio-compounds synthesized as discussed below (Appendix 9-28).

Seedlings treated with *Clonostachys rosea* as a preventive measure against *Fusarium oxysporum* produced 5-Hydroxymethylfurfural (18.03), Chinasaure (17.59) and 9-Octadecenoic acid (Z)- (10.72) as the major compounds. In curative treatment with the same combination, 30 different bio-compounds were identified with beta.-Monolinolein (10.72) and Hexadecanoic acid (9.55) being the major constituents. Chinasaure (32.78),

Glucal (13.87) and Hexadecanoic acid (11.47) were the major compounds identified in preventive treatment involving *Athelia rolfsii.* In curative treatment, Chinasaure (31.89), 5-Hydroxymethylfurfural (21.25) and Propyl butyrate (12.54) were identified as major compounds (Table 37) (Fig 9-12).

Table 37. Major biochemical compounds associated with mahogany seedlings treated with *Clonostachys rosea* individually and in preventive and curative treatments against selected pathogens

Sl. No.	Major biochemical compounds associated in mahogany seedlings	Biochemical compounds produced in mahogany seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		C. rosea treated (22[1])	*Fusarium oxysporum*		*Athelia rolfsii*	
			P (15)	C (30)	P (20)	C (16)
1	Propyl butyrate	-	-	-	-	12.54
2	5-Hydroxymethylfurfural	-	18.03	6.26	2.79	21.25
3	Chinasaure	27.59	17.59	-	32.78	31.89
4	Hexadecanoic acid	7.71	7.98	9.55	11.47	2.65
5	Glucal	12.59	8.27	-	13.87	3.15
6	beta.-Monolinolein	-	-	10.72	-	-
7	9,12-Octadecadienoic acid (Z,Z)	3.00	10.72	-	-	-
8	Linolenic acid	11.84	-	-	-	-

[1] Value in Parenthesis represents total number of Bio-compounds associated with mahogany seedlings
P- Preventive; C-Curative, '-'- Absent

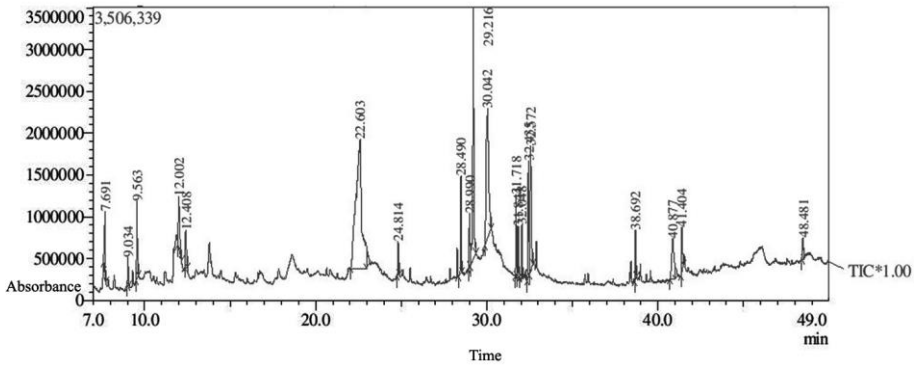

Fig 9. Chromatogram of methanol extract of Mahogany seedlings amended with *Clonostachys rosea* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

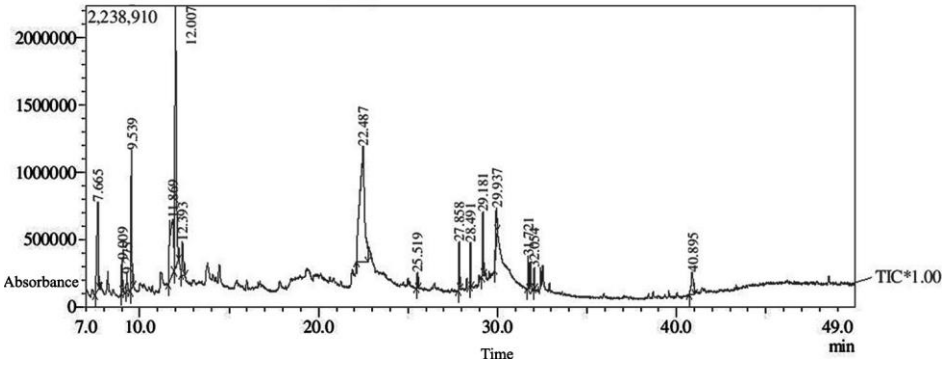

Fig 10. Chromatogram of methanol extract of Mahogany seedlings amended with *Clonostachys rosea* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

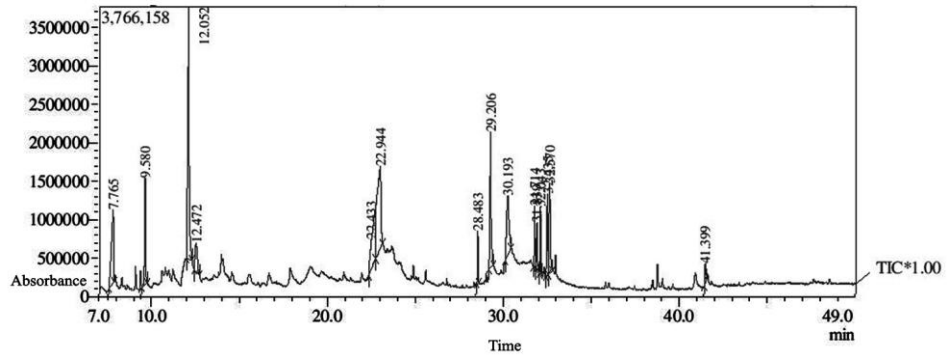

Fig 11. Chromatogram of methanol extract of Mahogany seedlings amended with *Clonostachys rosea* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

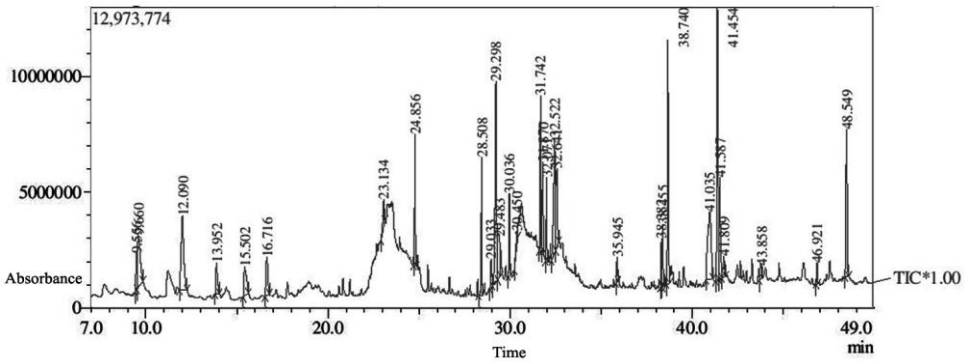

Fig 12. Chromatogram of methanol extract of Mahogany seedlings amended with *Clonostachys rosea* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

Seedlings inoculated with the pathogen *F. oxysporum* and treated with *Penicillium multicolor* yielded 5-Hydroxymethylfurfural (13.55), gamma.-Sitosterol (11.68) and Chinasaure (11.16) as major compounds in preventive and 3-Hydroxy-2,3-dihydromaltol (11.19) and Hexadecanoic acid (9.33) in curative treatments. In seedlings inoculated with *Athelia rolfsii* and treated with *Penicillium multicolor*, Chinasaure (27.70), 1-Deoxy-d-mannitol (14.76) and 5-Hydroxymethylfurfural (12.95) were the major compounds in preventive treatment. While in curative treatment, Chinasaure (29.68) and Glucal (19.04) were the major compounds identified in the seedlings (Table 38) (Fig 13-16).

Table 38. Major biochemical compounds associated with mahogany seedlings treated with *Penicillium multicolor* individually and in preventive and curative treatments against selected pathogens

Sl. No.	Major biochemical compounds associated in mahogany seedlings	Biochemical compounds produced in mahogany seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		***P. multicolor* treated (20[1])**	***Fusarium oxysporum***		***Athelia rolfsii***	
			P (22)	**C (26)**	**P (21)**	**C (20)**
1	5-Hydroxymethylfurfural	-	13.55	7.78	12.59	5.45
2	Chinasaure	19.67	13.84	-	27.70	29.68
3	Hexadecanoic acid	10.33	8.75	9.33	7.38	5.61
4	Glucal	-	8.61	-	-	19.04
5	gamma.-Sitosterol	13.90	11.68	-	-	-
6	9,12-Octadecadienoic acid (Z,Z)	-	0.75	-	2.45	1.99
7	1,5-Anhydro-d-mannitol	13.89	-	-	-	-
8	1-Deoxy-d-mannitol	-	-	-	14.76	-

[1] Value in Parenthesis represents total number of Bio-compounds associated with mahogany seedlings
P- Preventive; C-Curative
'-'- Absent

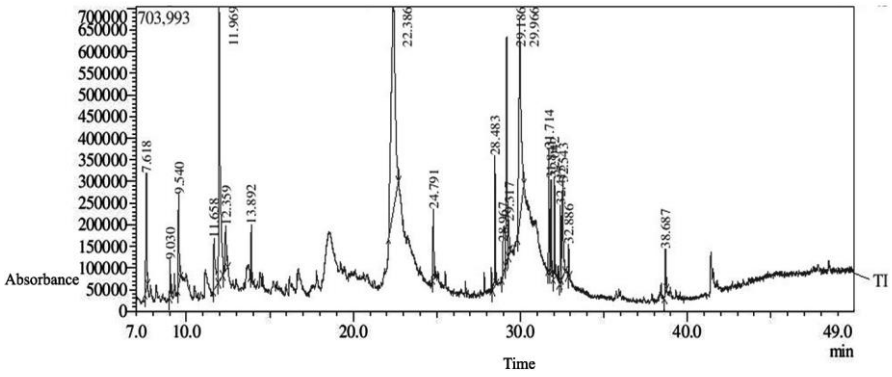

Fig 13. Chromatogram of methanol extract of Mahogany seedlings amended with *Penicillium multicolor* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

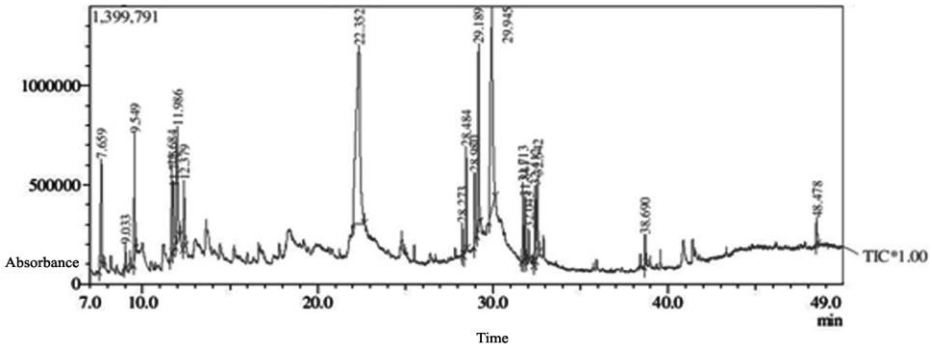

Fig 14. Chromatogram of methanol extract of Mahogany seedlings amended with *Penicillium multicolor* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

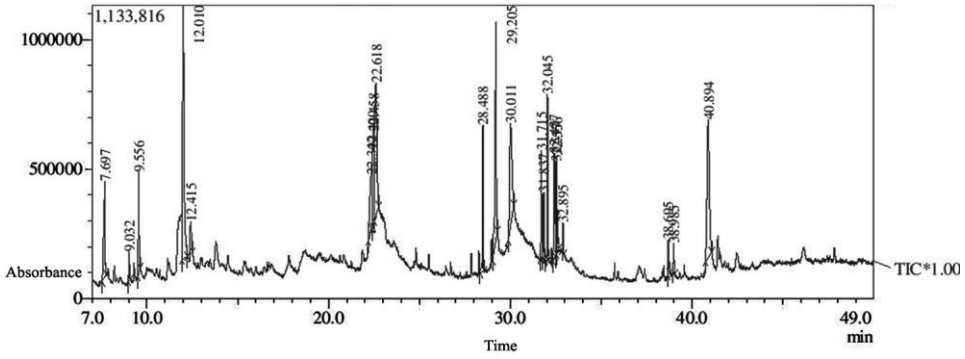

Fig 15. Chromatogram of methanol extract of Mahogany seedlings amended with *Penicillium multicolor* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

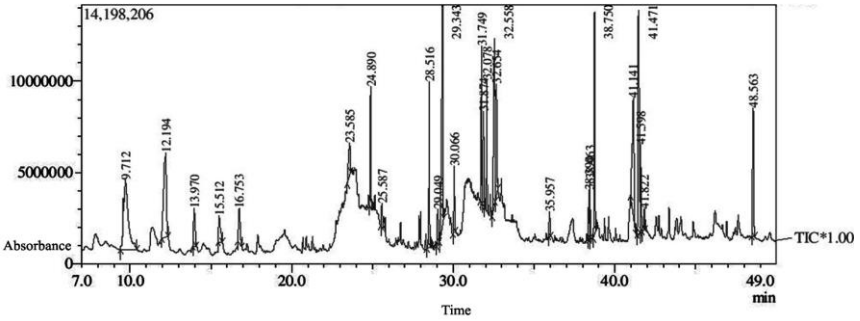

Fig 16. Chromatogram of methanol extract of Mahogany seedlings amended with *Penicillium multicolor* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

Mahogany seedlings inoculated with *F. oxysporum* and the antagonist *Purpureocillium lilacinum* in preventive treatment yielded 5-Hydroxymethylfurfural (39.96) as the major compound followed by Glucal (12.16) whereas in curative treatment of same combination, beta.-Sitosterol (12.25), 5-Hydroxymethylfurfural (9.43) and Hexadecanoic acid (9.15) were identified as the major compounds. In preventive treatment upon *P. lilacinum* against *Athelia rolfsii*, the seedlings yielded 5-Hydroxymethylfurfural (42.99), whereas in curative treatment, Chinasaure (29.02) and Glucal (10.42) were the major bio-compounds identified (Table 39) (Fig 17-20).

Table 39. Major biochemical compounds associated with mahogany seedlings treated with *Purpureocillium lilacinum* individually and in preventive and curative treatments against selected pathogens

Sl. No.	Major biochemical compounds associated in mahogany seedlings	Biochemical compounds produced in mahogany seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		P. lilacinum treated (19[1])	*Fusarium oxysporum*		*Athelia rolfsii*	
			P(17)	C(27)	P (16)	C(21)
1	5 Hydroxymethylfurfural	-	39.96	9.43	42.99	6.88
2	Chinasaure	15.91	-	1.46	-	29.02
3	Hexadecanoic acid	7.71	4.57	9.15	6.53	3.03
4	Glucal	27.99	12.16	-	-	10.42
5	beta.-Monolinolein	-	1.96	7.30	2.27	-
6	9,12-Octadecadienoic acid (Z,Z)	3.00	4.86	5.67	7.94	1.81
7	beta.-Sitosterol	-	-	12.25	-	4.19

[1] Value in Parenthesis represents total number of Bio-compounds associated with mahogany seedlings
P- Preventive; C-Curative, '-'- Absent

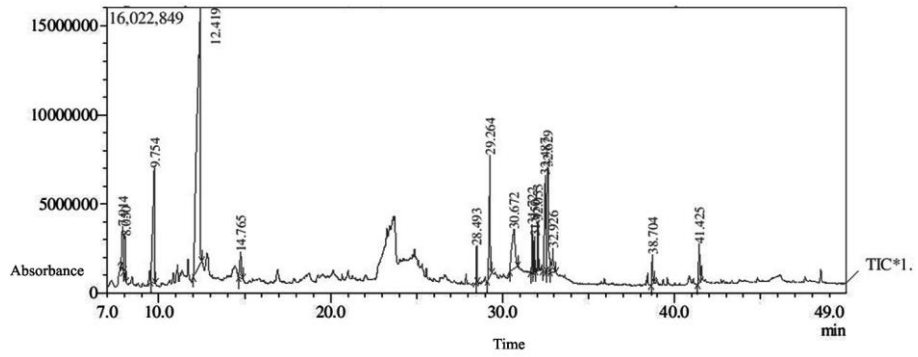

Fig 17. Chromatogram of methanol extract of Mahogany seedlings amended with *Purpureocillium lilacinum* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

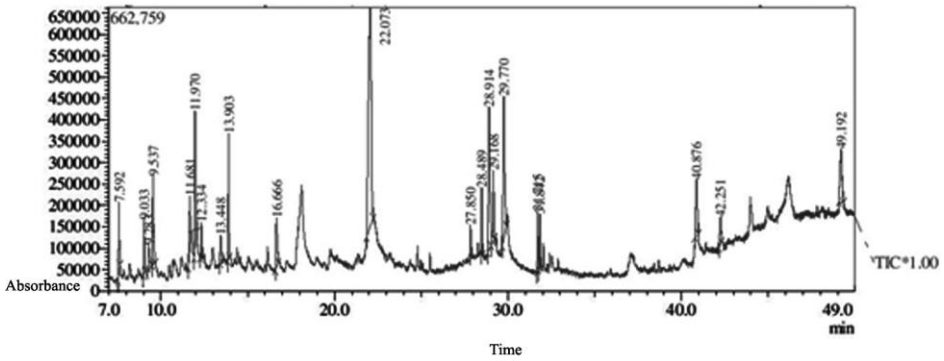

Fig 18. Chromatogram of methanol extract of Mahogany seedlings amended with *Purpureocillium lilacinum* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

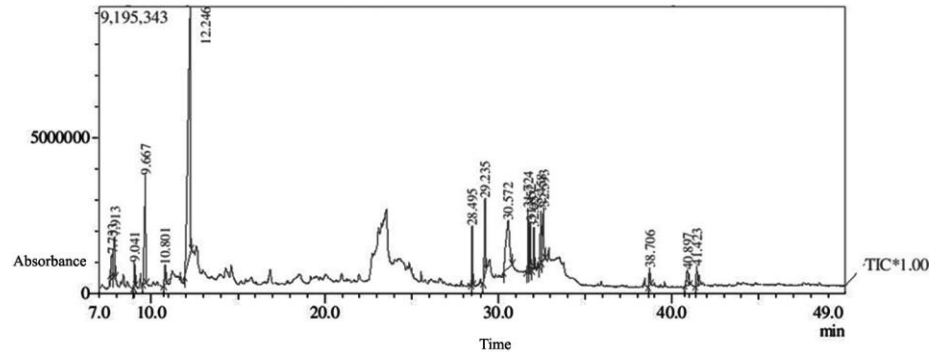

Fig 19. Chromatogram of methanol extract of Mahogany seedlings amended with *Purpureocillium lilacinum* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

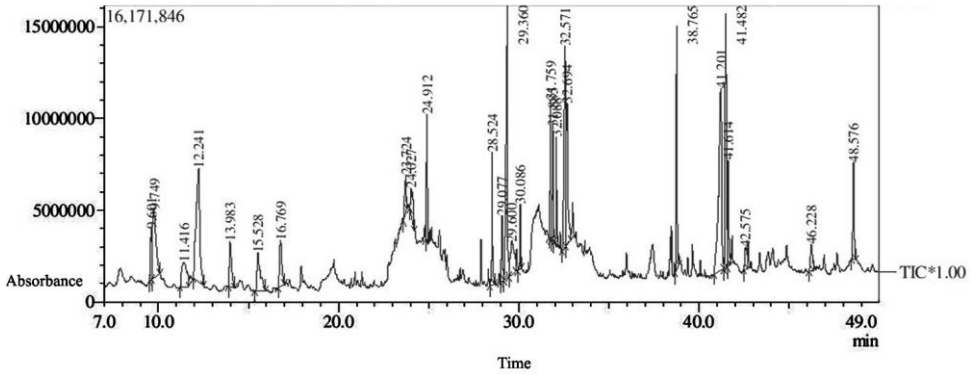

Fig 20. Chromatogram of methanol extract of Mahogany seedlings amended with *Purpureocillium lilacinum* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

Seedlings treated with *Trichoderma harzianum* prior to inoculation with *F. oxysporum* contained 5-Hydroxymethylfurfural (23.88) and Chinasaure (10.04) in higher percentage compared to other bio-compounds whereas in curative application, Gamma.-Sitosterol (13.14) and Hexadecanoic acid (10.96) represented the major bio-compounds. Seedlings treated with combination of *Athelia rolfsii* and *T. harzianum* in preventive treatment produced Chinasaure (26.67) and Glucal (22.08) as the major compounds. In curative treatment of same combination, the seedlings contained Chinasaure (25.63), 1, 5-Anhydro-d-mannitol (14.57) and 5-Hydroxymethylfurfural (13.34) as major compounds (Table 40) (Fig 21-24).

Table 40. Major biochemical compounds associated with mahogany seedlings treated with *Trichoderma harzianum* individually and in preventive and curative treatments against selected pathogens

Sl. No.	Major biochemical compounds associated in mahogany seedlings	Biochemical compounds produced in mahogany seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		T. harzianum treated (22[1])	*Fusarium oxysporum*		*Athelia rolfsii*	
			P (23)	C (28)	P (20)	C (20)
1	5-Hydroxymethylfurfural	-	23.88	3.17	2.79	13.34
2	Chinasaure	15.58	10.04	-	26.67	25.63
3	Hexadecanoic acid	8.05	9.63	10.96	6.45	3.33
4	Glucal	-	7.73	-	22.08	-
5	beta.-Monolinolein	-	1.72	-	-	-
6	9,12-Octadecadienoic acid (Z,Z)	0.86	-	2.69	-	2.79
7	gamma.-Sitosterol	7.27	-	13.14	-	-

[1] Value in Parenthesis represents total number of Bio-compounds associated with mahogany seedlings
P- Preventive; C-Curative
'-'- Absent

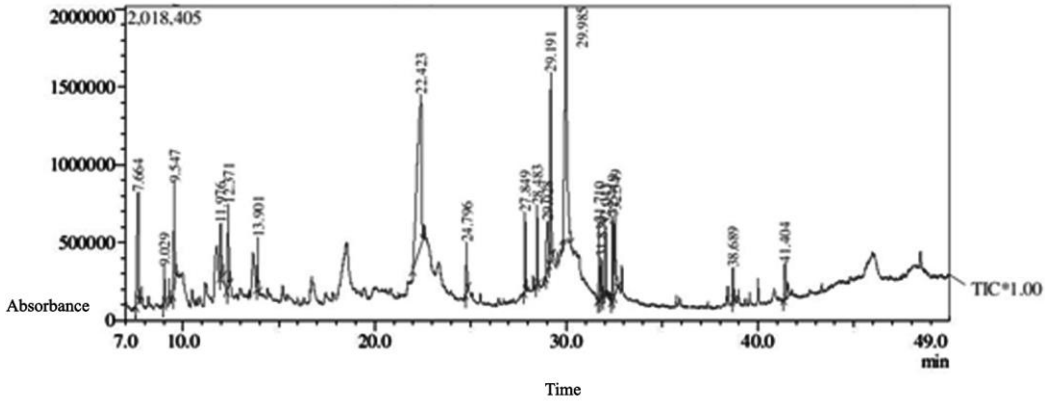

Fig 21. Chromatogram of methanol extract of Mahogany seedlings amended with *Trichoderma harzianum* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

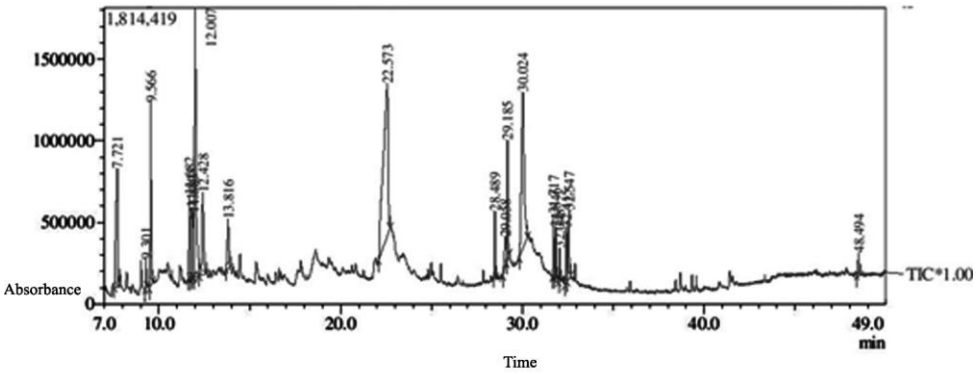

Fig 22. Chromatogram of methanol extract of Mahogany seedlings amended with *Trichoderma harzianum* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

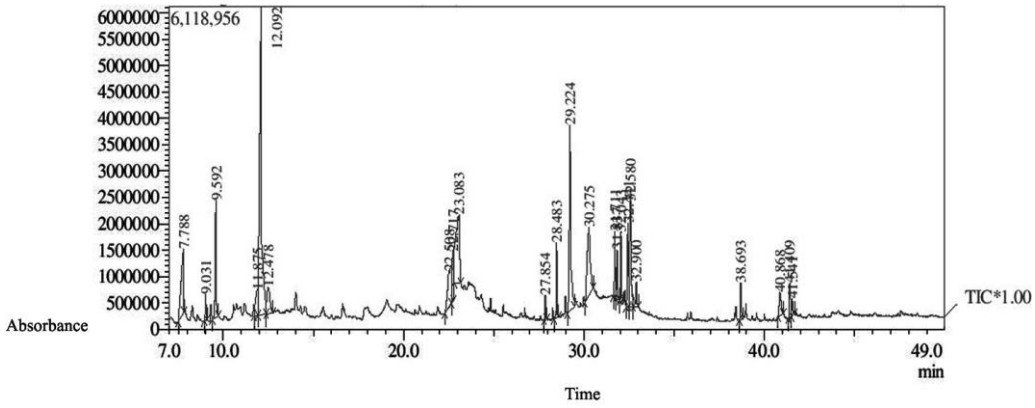

Fig 23. Chromatogram of methanol extract of Mahogany seedlings amended with *Trichoderma harzianum* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

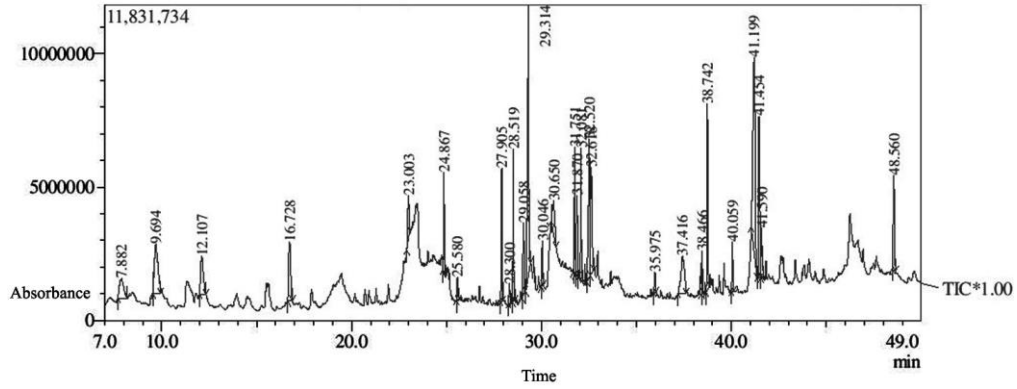

Fig 24. Chromatogram of methanol extract of Mahogany seedlings amended with *Trichoderma harzianum* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

In preventive treatment with *F. oxysporum* and *Trichoderma koningii*, the seedlings were noted to contain Chinasaure (31.55) and Glucal (11.62) as the major compounds where as in curative treatment, Hexadecanoic acid (12.04) was the major compound associated with the seedlings. Seedlings treated with *Athelia rolfsii* and *T. koningii* in preventive treatment yielded Glucal (15.23) and Chinasaure (12.17) as the major compounds and in curative treatment, Chinasaure (24.98) and Glucal (13.26) were the major compounds identified (Table 41) (Fig 25-28).

Table 41. Major biochemical compounds associated with mahogany seedlings treated with *Trichoderma koningii* individually and in preventive and curative treatments against selected pathogens

Sl. No.	Major biochemical compounds associated in mahogany seedlings	Biochemical compounds produced in mahogany seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		T. koningii treated (18[1])	*Fusarium oxysporum*		*Athelia rolfsii*	
			P (18)	C (32)	P (21)	C (20)
1	5-Hydroxymethylfurfural	-	7.35	6.31	4.51	2.97
2	Chinasaure	18.86	31.55	5.74	12.17	24.98
3	Hexadecanoic acid	9.04	5.05	12.04	9.05	6.72
4	Glucal	13.42	11.62	4.86	15.23	13.26
5	beta.-Monolinolein	-	1.13	-	2.11	-
6	9,12-Octadecadienoic acid (Z,Z)	-	-	3.72	-	-

[1] Value in Parenthesis represents total number of Bio-compounds associated with mahogany seedlings
P- Preventive; C-Curative, '-'- Absent

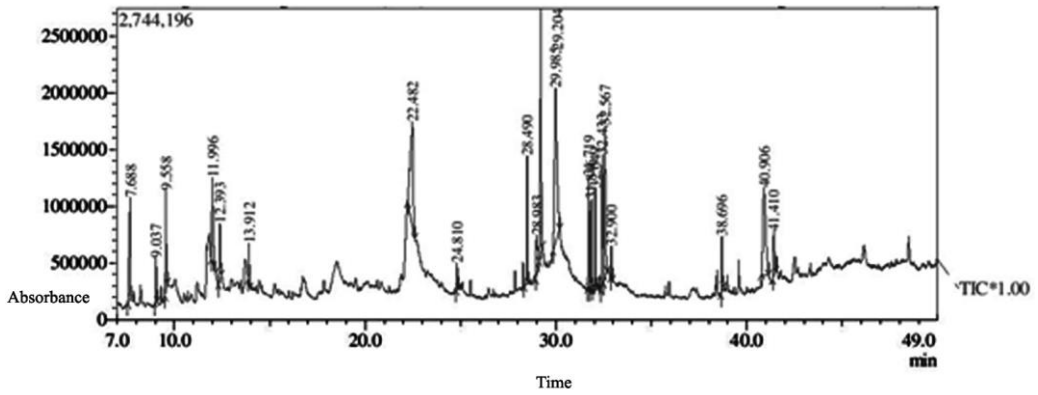

Fig 25. Chromatogram of methanol extract of Mahogany seedlings amended with *Trichoderma koningii* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

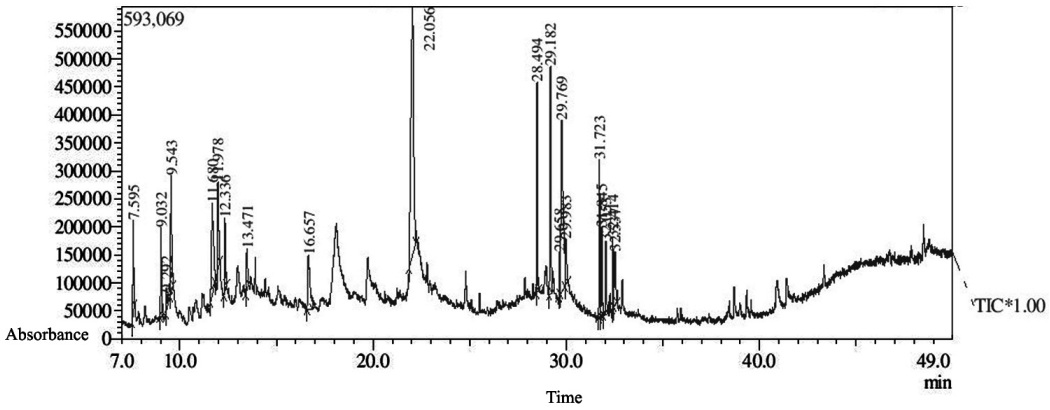

Fig 26. Chromatogram of methanol extract of Mahogany seedlings amended with *Trichoderma koningii* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

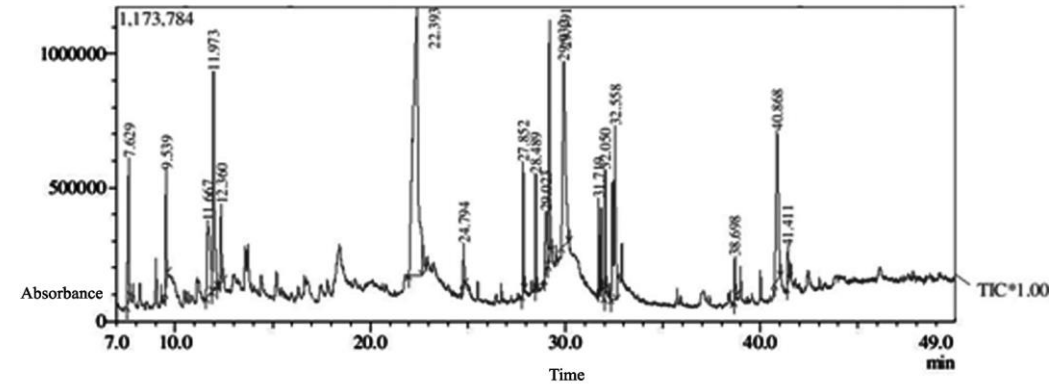

Fig 27. Chromatogram of methanol extract of Mahogany seedlings amended with *Trichoderma koningii* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

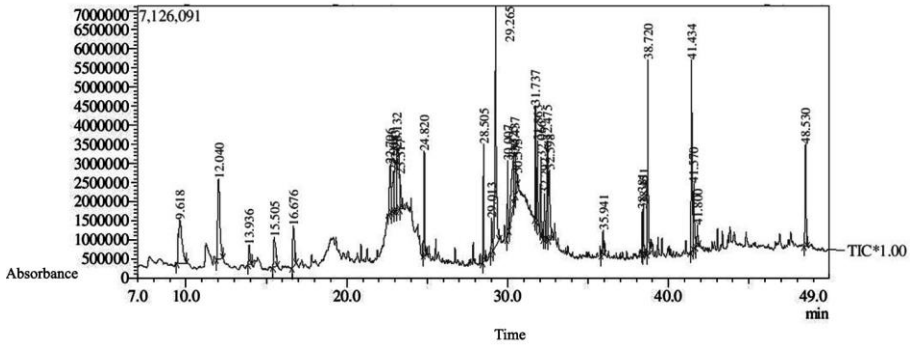

Fig 28. Chromatogram of methanol extract of Mahogany seedlings amended with *Trichoderma koningii* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

GC-MS analysis of teak seedlings for Resistance Inducing Biochemical Compounds

Apparently healthy seedlings of teak were found to contain 18 different compounds with Linoleic acid, methyl ester (20.51), Gamma.-sitosterol (14.29), Phytol (10.44) and Methyl palmitate (10.31) were the major ones (Fig 29). Seedlings affected by *F. oxysporum* yielded 23 different bio-compounds with Linoleic acid, methyl ester (18.06), Hexadecanoic acid (12.62) and Methyl 11, 14-eicosadienoate (11.09) as major compounds (Fig 30). Seedlings infected by *Athelia rolfsii* produced Gamma.-sitosterol (23.92) and Linoleic acid, methyl ester (10.80) as the main compounds of the 23 compounds identified (Table 42) (Fig 31) (Appendix 29-31).

Table 42. Major biochemical compounds identified in control and pathogen-*Fusarium oxysporum* and *Athelia rolfsii* treated teak seedlings

Sl. No.	Major biochemical compounds associated in teak seedlings	Biochemical compounds produced in teak seedlings in control and treated with selected Pathogens		
		Control (18[1])	*Fusarium oxysporum* (23)	*Athelia rolfsii* (23)
1	Chinasaure	3.59	-	-
2	Hexadecanoic acid	9.13	12.62	7.12
3	.gamma.-Sitosterol	14.29	18.59	23.92
4	Linoleic acid, Methyl ester	20.51	18.06	10.80
5	Methyl 11,14-eicosadienoate	-	11.09	-
6	2-Methylindoline	5.64	-	-

[1]Value in Parenthesis represents total number of Bio-compounds associated with teak seedlings, '-'- Absent

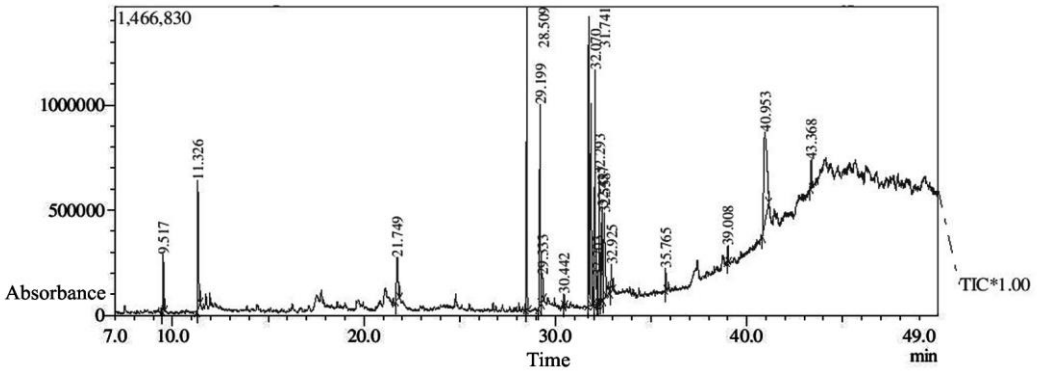

Fig 29. Chromatogram of methanol extract of untreated Teak seedlings showing different biochemical compounds by GC-MS analysis

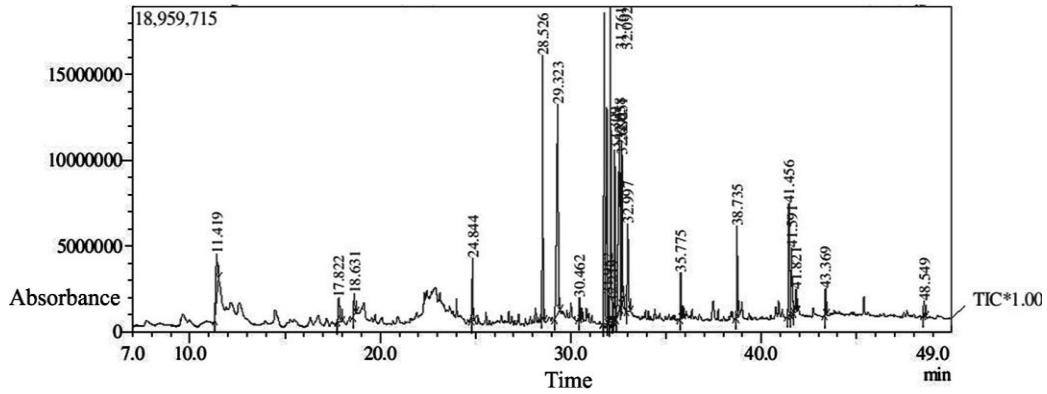

Fig 30. Chromatogram of methanol extract of *Fusarium oxysporum* amended Teak seedlings showing different biochemical compounds by GC-MS analysis

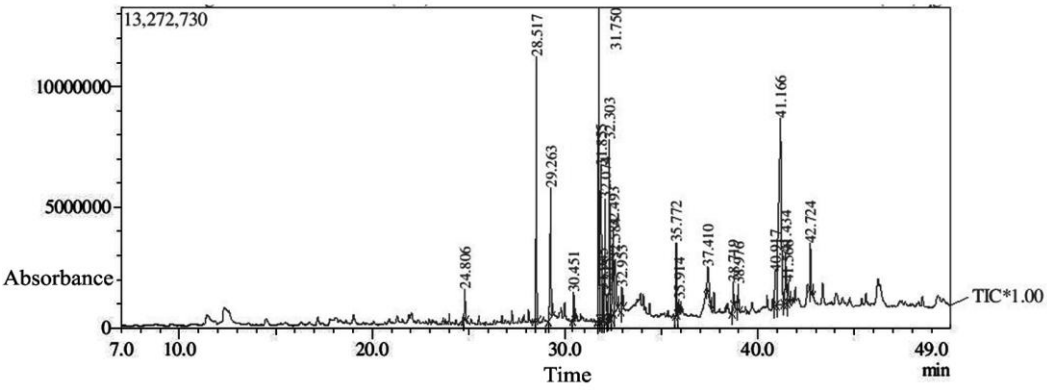

Fig 31. Chromatogram of methanol extract of *Athelia rolfsii* amended Teak seedlings showing different biochemical compounds by GC-MS analysis

Seedlings treated with the antagonists when analyzed individually for bio-chemicals, *Clonosatchys rosea* yielded 19 compounds with Alpha-linolenic acid methyl ester (22.42), Phytol (12.05) and 9, 12-Octadecadienoic acid, methyl ester (11.01) as the major compounds (Fig 32). Seedlings treated with *Penicillium multicolor* produced 19 bio-compounds of which Alpha-linolenic acid methyl ester (19.94) and 9, 12-Octadecadienoic acid, methyl ester (12.85) were the major compounds (Fig 33). Seedlings treated with *Purpureocillium lilacinum* yielded 10 different compounds among them Alpha-linolenic acid methyl ester (32.97), Isophytol, acetate (17.78) and Methyl linolelaidate (15.98) being the major compounds (Fig 34). In the case of *Trichoderma harzianum* treated seedlings, 19 different compounds were obtained, of which Linolenic acid methyl ester (19.91), Guanosine (17.86) and Methyl octadeca-9, 12-dienoate (14.07) represented the major compounds (Fig 35). However, seedlings treated with *Trichoderma koningii* produced 22 different compounds of which Linolenic acid methyl ester (13.57), Methyl octadeca-9, 12-dienoate (12.11) and Methyl palmitate (10.20) being the major compounds (Fig 36) (Appendix 32-36).

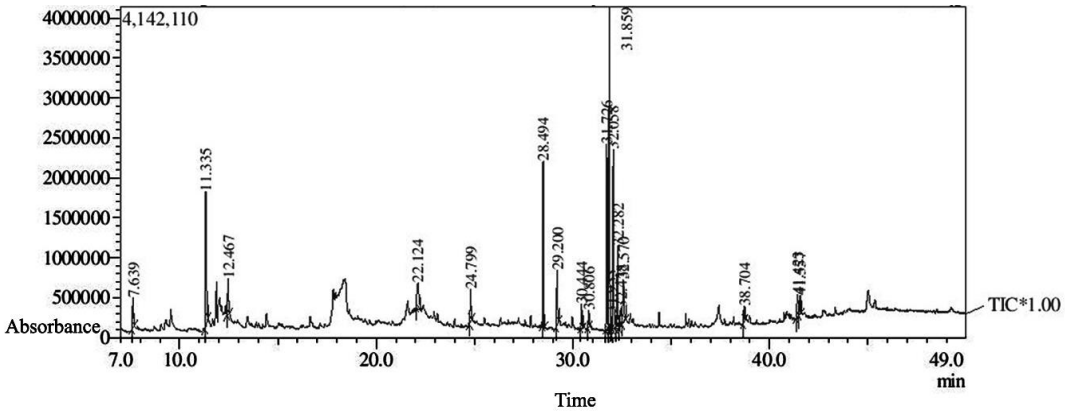

Fig 32. Chromatogram of methanol extract of *Clonostachys rosea* amended Teak seedlings showing different biochemical compounds by GC-MS analysis

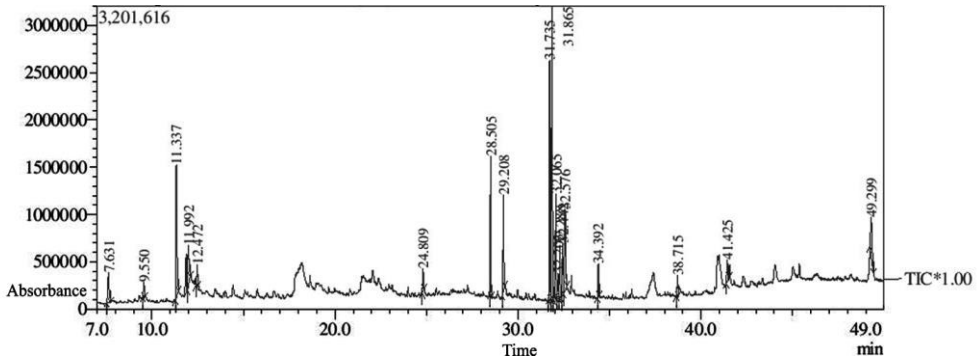

Fig 33. Chromatogram of methanol extract of *Penicillium multicolor* amended Teak seedlings showing different biochemical compounds by GC-MS analysis

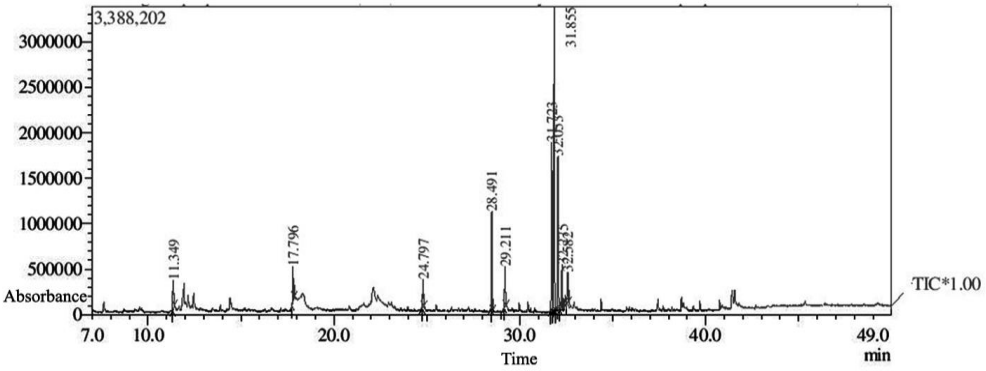

Fig 34. Chromatogram of methanol extract of *Purpureocillium lilacinum* amended Teak seedlings showing different biochemical compounds by GC-MS analysis

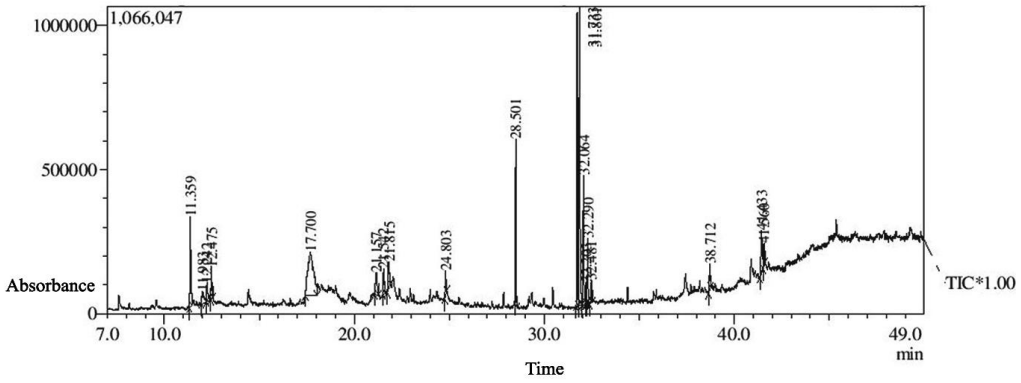

Fig 35. Chromatogram of methanol extract of *Trichoderma harzianum* amended Teak seedlings showing different biochemical compounds by GC-MS analysis

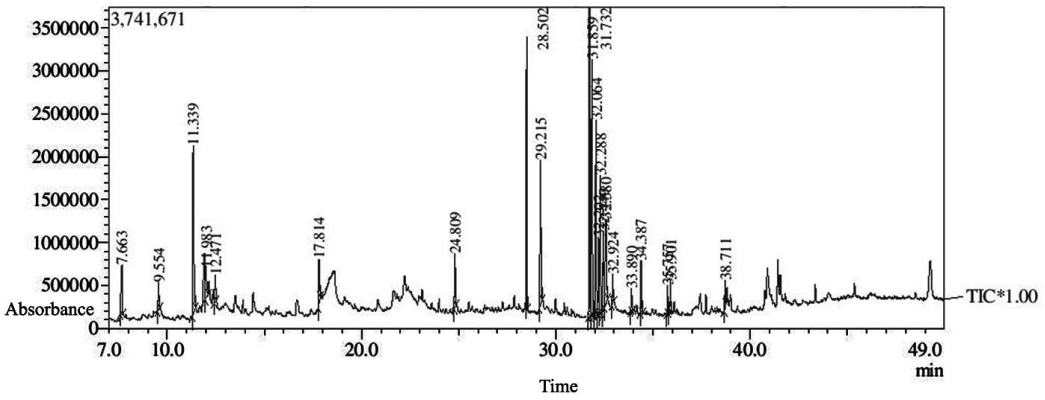

Fig 36. Chromatogram of methanol extract of *Trichoderma koningii* amended Teak seedlings showing different biochemical compounds by GC-MS analysis

Preventive and Curative treatments

In preventive and curative treatments, seedlings upon inoculation with bio-agents and pathogens resulted in variation in different biochemical compounds (Appendix 37-56).

In preventive treatment, Chinasaure (17.86) was the major compound identified in teak seedlings treated with *Clonostachys rosea* against *Fusarium oxysporum*. In curative treatment Alpha-linolenic acid methyl ester (11.07) formed the major compound of the 25 different compounds identified. Seedlings affected by *Athelia rolfsii* which were subjected to preventive treatment using *Clonostachys rosea* produced 5-Hydroxymethylfurfural (14.33) as the major compound. In curative treatment, Pentanoic acid, 3-hydroxy-4-methyl-, methyl ester (10.69), Friedelan-3-one (10.56) and Chinasaure (10.37) were the major compounds recorded (Table 43) (Fig 37-40).

Table 43. Major biochemical compounds associated with teak seedlings treated with *Clonostachys rosea* individually and in preventive and curative treatments against pathogens

Sl. No.	Major biochemical compounds associated in teak seedlings	Biochemical compounds produced in teak seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		C. rosea treated (19[1])	*Fusarium oxysporum*		*Athelia rolfsii*	
			P (25)	C (25)	P (20)	C (30)
1	5-Hydroxymethylfurfural	-	6.04	-	14.33	-
2	Chinasaure	4.35	17.86	8.83	-	10.37
3	Hexadecanoic acid	3.91	6.06	7.30	6.98	4.27
4	2-Methylindoline	9.53	7.33	7.34	12.45	4.92
5	Linoleic acid, Methyl ester	22.42	-	11.07	8.18	7.06
6	9,12-Octadecadienoic acid (Z,Z)	-	5.47	2.83	5.92	4.94

[1] Value in Parenthesis represents total number of Bio-compounds associated with teak seedlings
P- Preventive; C-Curative
'-'- Absent

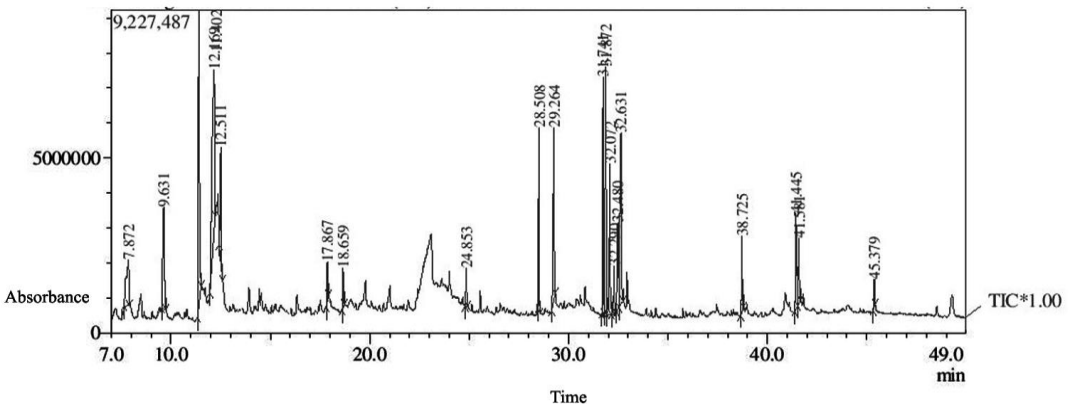

Fig 37. Chromatogram of methanol extract of Teak seedlings amended with *Clonostachys rosea* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

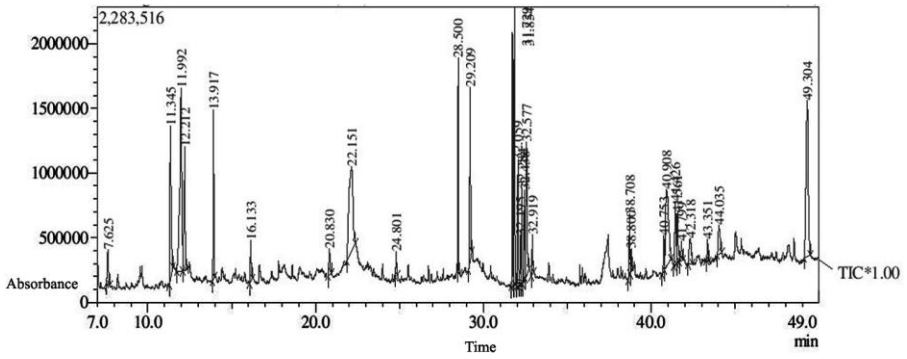

Fig 38. Chromatogram of methanol extract of Teak seedlings amended with *Clonostachys rosea* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

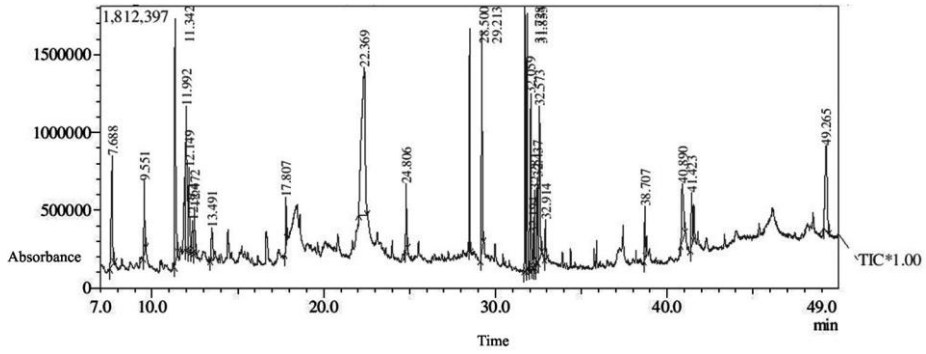

Fig 39. Chromatogram of methanol extract of Teak seedlings amended with *Clonostachys rosea* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

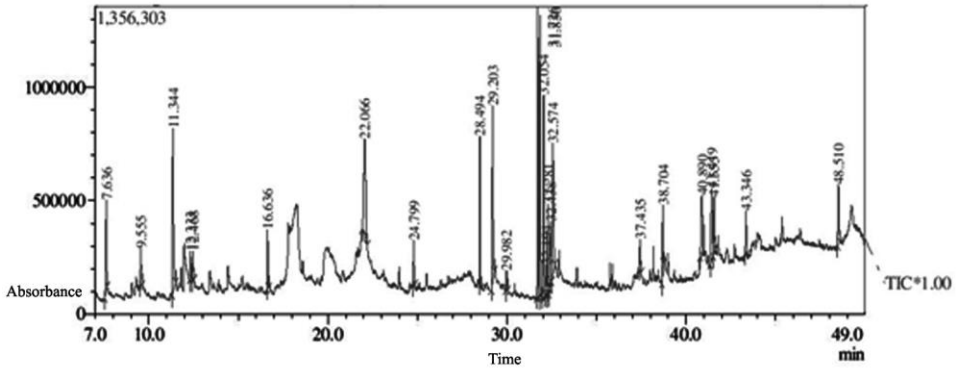

Fig 40. Chromatogram of methanol extract of Teak seedlings amended with *Clonostachys rosea* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

Seedlings affected by *F. oxysporum* when treated with *Penicillium multicolor* in preventive treatment produced Chinasaure (33.81) and 5-Hydroxymethylfurfural (13.01) as major compounds. Chinasaure (17.85) was the major constituent of seedlings in curative treatment as well. In seedlings affected by *A. rolfsii* and treated with *P. multicolor* in preventive treatment identified 2-Methylindoline (12.63) as the major compound. In curative treatment in the same combination 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (9.83) was identified as the main compound (Table 44) (Fig 41-44).

Table 44. Major biochemical compounds associated with teak seedlings treated with *Penicillium multicolor* individually and in preventive and curative treatments against pathogens

Sl. No.	Major biochemical compounds associated in teak seedlings	Biochemical compounds produced in teak seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		***P. multicolor* treated (19[1])**	***Fusarium oxysporum***		***Athelia rolfsii***	
			P (21)	**C (27)**	**P (28)**	**C (31)**
1	5-Hydroxymethylfurfural	3.91	13.01	4.07	-	6.62
2	Chinasaure	-	33.81	17.85	6.92	7.93
3	Hexadecanoic acid	5.78	4.07	7.01	4.80	5.67
4	2-Methylindoline	9.51	7.56	7.55	12.63	8.69
5	Linoleic acid, Methyl ester	19.94	4.06	8.22	6.98	-
6	9,12-Octadecadienoic acid (Z,Z)	12.85	-	5.80	2.56	3.62

[1] Value in Parenthesis represents total number of Bio-compounds associated with teak seedlings
P- Preventive; C-Curative
'-'- Absent

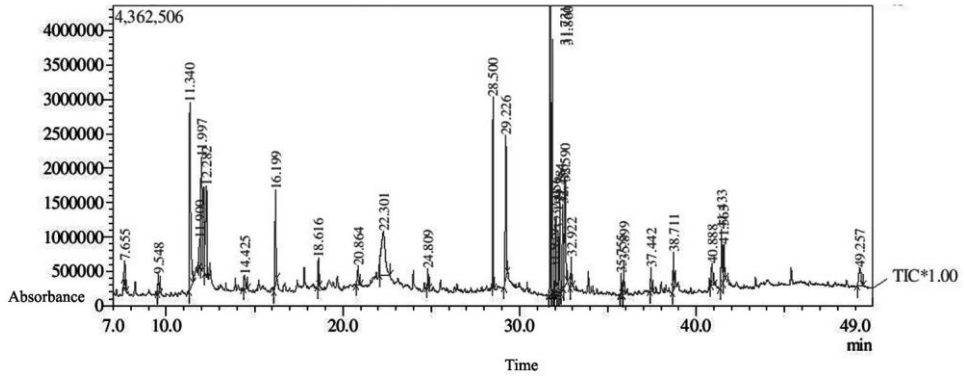

Fig 41. Chromatogram of methanol extract of Teak seedlings amended with *Penicillium multicolor* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

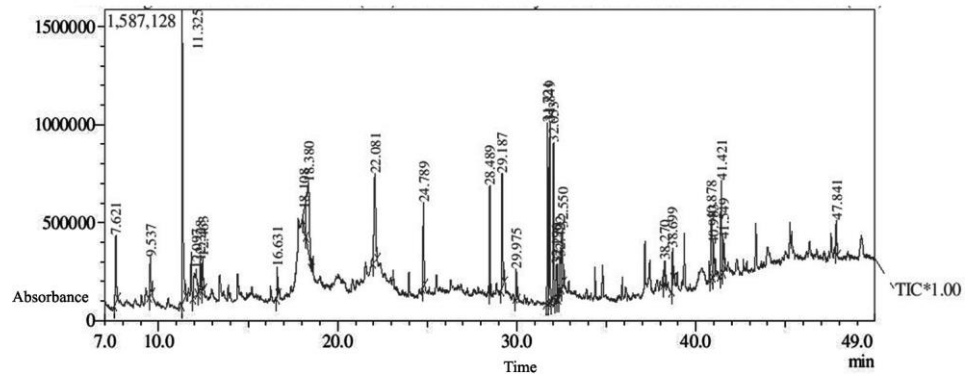

Fig 42. Chromatogram of methanol extract of Teak seedlings amended with *Penicillium multicolor* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

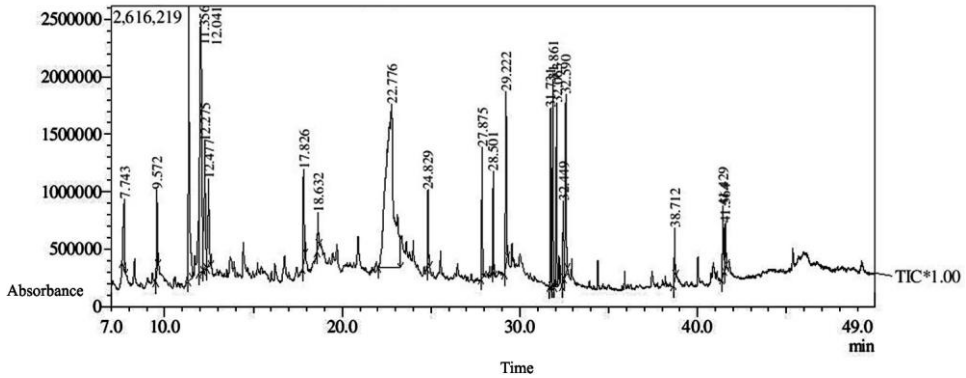

Fig 43. Chromatogram of methanol extract of Teak seedlings amended with *Penicillium multicolor* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

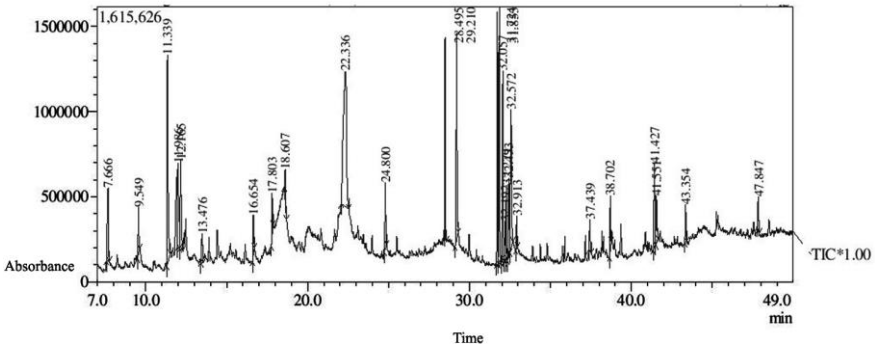

Fig 44. Chromatogram of methanol extract of Teak seedlings amended with *Penicillium multicolor* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

Teak seedlings inoculated with *Purpureocillium lilacinum* in preventive treatment against *F. oxysporum* contained 2-Methylindoline (17.35) and Beta.-Sitosterol (14.80) as the main compounds whereas in curative treatment, the major compounds in seedlings were Chinasaure (12.64) and 2-Methylindoline (12.26). Seedlings with *P. lilacinum* as preventive application against *Athelia rolfsii* contained 2-Methylindoline (11.83) and Alpha-linolenic acid methyl ester (10.74) as major compounds. In curative treatment, the seedlings yielded 5-Hydroxymethylfurfural (23.88) and Methyl 8, 11, 14-heptadecatrienoate (10.65) as main bio-compounds (Fig 45-48) (Table 45).

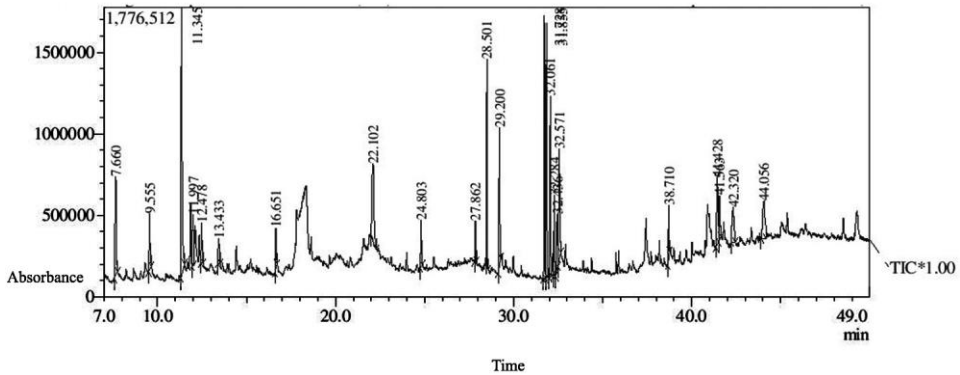

Fig 45. Chromatogram of methanol extract of Teak seedlings amended with *Purpureocillium lilacinum* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

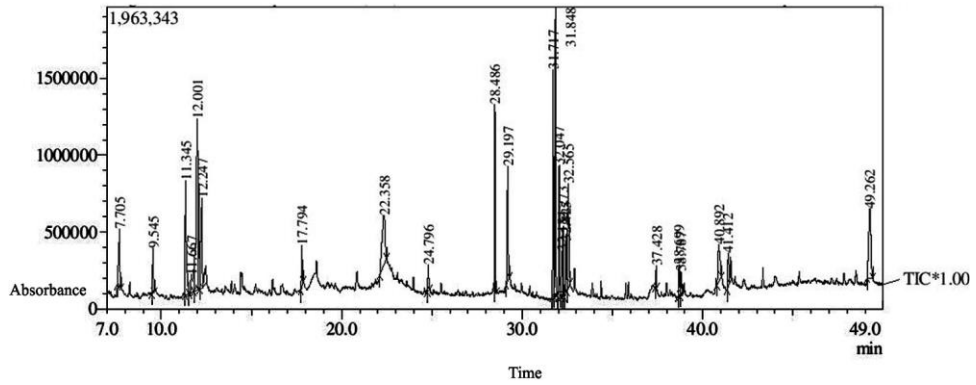

Fig 46. Chromatogram of methanol extract of Teak seedlings amended with *Purpureocillium lilacinum* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

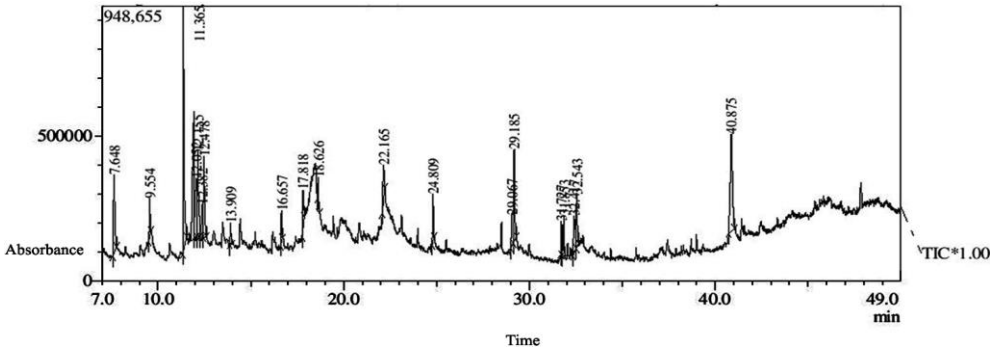

Fig 47. Chromatogram of methanol extract of Teak seedlings amended with *Purpureocillium lilacinum* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

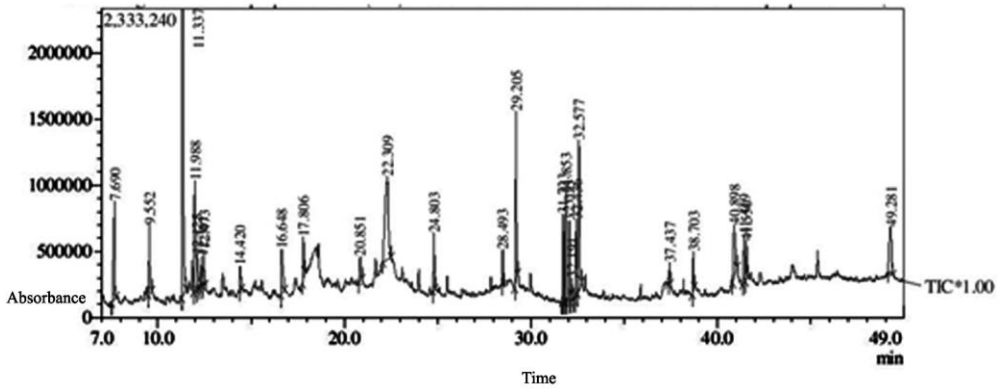

Fig 48. Chromatogram of methanol extract of Teak seedlings amended with *Purpureocillium lilacinum* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

Table 45. Major biochemical compounds associated with teak seedlings treated with *Purpureocillium lilacinum* individually and in preventive and curative treatments against pathogens

Sl. No.	Major biochemical compounds associated in teak seedlings	Biochemical compounds produced in teak seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		P. lilacinum treated (10^1)	*Fusarium oxysporum*		*Athelia rolfsii*	
			P (20)	C (27)	P (23)	C (24)
1	5-Hydroxymethylfurfural	-	-	6.64	2.50	14.52
2	Chinasaure	-	3.14	12.64	7.34	7.01
3	Hexadecanoic acid	-	3.70	6.66	5.28	4.48
4	2-Methylindoline	3.56	17.35	12.26	11.83	5.35
5	Linoleic acid, Methyl ester	32.97	2.50	3.84	10.74	6.52
6	9,12-Octadecadienoic acid (Z,Z)	-	1.48	7.18	8.25	4.92

[1] Value in Parenthesis represents total number of Bio-compounds associated with teak seedlings
P- Preventive; C-Curative
'-'- Absent

Teak seedlings treated with *Trichoderma harzianum* prior to *F. oxysporum* as preventive application, produced 5-Hydroxymethylfurfural (20.69), 2-Methylindoline (10.76) and Chinasaure (10.04) in higher quantities compared to other bio-compounds. In curative application with the same combination, Chinasaure (16.68), Hexadecanoic acid (12.11) and 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (11.49) were the major compounds produced and identified from seedlings. In seedlings infected by *Athelia rolfsii* and treated with *T. harzianum* in preventive treatment, 3, 4-Anhydro-d-galactosan (15.32), Beta.-Sitosterol (12.11) and 2-Methylindoline (11.03) formed the major compounds. In curative treatment, Friedelan-3-one (11.31) and 2-Methylindoline (10.22) represented the major compounds (Table 46) (Fig 49-52).

Table 46. Major biochemical compounds associated with teak seedlings treated with *Trichoderma harzianum* individually and in preventive and curative treatments against pathogens

Sl. No.	Major biochemical compounds associated in teak seedlings	Biochemical compounds produced in teak seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		T. harzianum treated (19[1])	*Fusarium oxysporum*		*Athelia rolfsii*	
			P (20)	C (20)	P (21)	C (23)
1	5-Hydroxymethylfurfural	-	20.69	2.03	-	3.51
2	Chinasaure	2.45	10.04	16.68	-	5.05
3	Hexadecanoic acid	-	5.32	12.11	5.08	6.59
4	2-Methylindoline	6.09	10.76	5.81	11.03	10.22
5	Linoleic acid, Methyl ester	19.91	5.58	8.14	6.09	7.17
6	9,12-Octadecadienoic acid (Z,Z)	-	5.66	11.49	5.59	1.99

[1] Value in Parenthesis represents total number of Bio-compounds associated with teak seedlings
P- Preventive; C-Curative
'-'- Absent

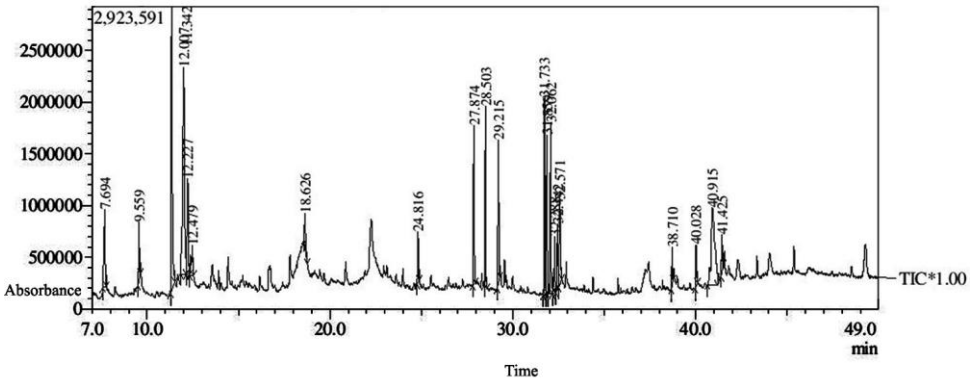

Fig 49. Chromatogram of methanol extract of Teak seedlings amended with *Trichoderma harzianum* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

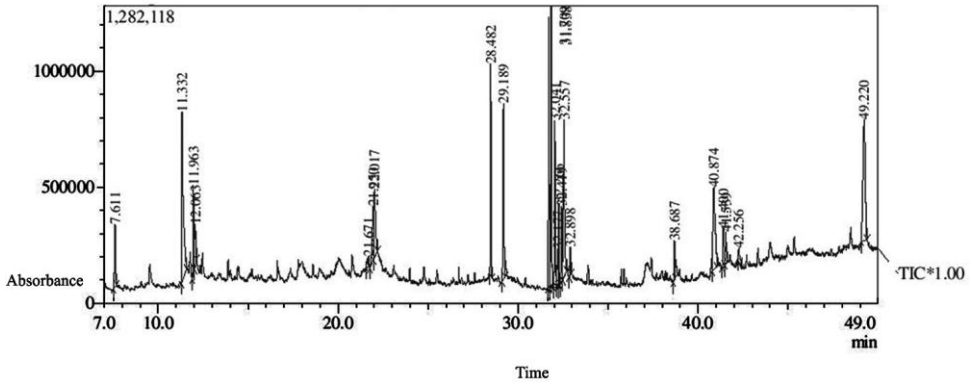

Fig 50. Chromatogram of methanol extract of Teak seedlings amended with *Trichoderma harzianum* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

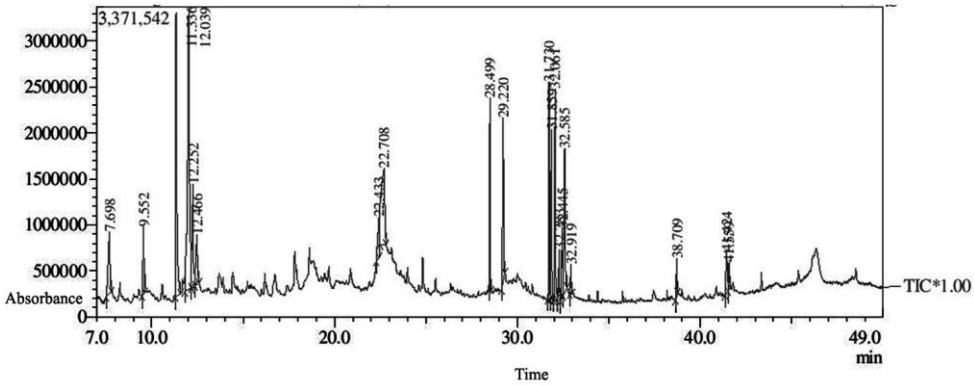

Fig 51. Chromatogram of methanol extract of Teak seedlings amended with *Trichoderma harzianum* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

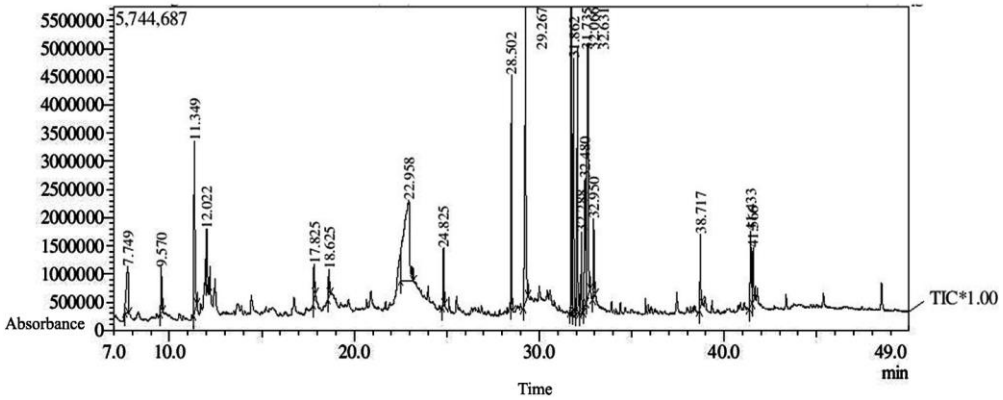

Fig 52. Chromatogram of methanol extract of Teak seedlings amended with *Trichoderma harzianum* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

In preventive treatment, *Trichoderma koningii* was treated against *F. oxysporum*, the seedlings yielded Alpha-copaene-11-ol (38.01), 2-Methylindoline (11.31) and 2, 7-Dimethoxyphenazine 5-oxide (10.23) as major compounds. In curative treatment in the same combination, Chinasaure (36.03), 2-Methylindoline (16.23) and 5-Hydroxymethylfurfural (12.45) were the major compounds. When seedlings infected by *Athelia rolfsii* were subjected to preventive treatment upon *T. koningii*, the major bio-compounds identified in seedlings were 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)- (12.63) and 2-Methylindoline (11.51). In curative treatment, 5-(Hydroxymethyl)-2-(dimethoxymethyl) furan (12.09) and Methyl 8, 11, 14-heptadecatrienoate (11.37) were the major compounds identified (Table 47) (Fig 53-56).

Table 47. Major biochemical compounds associated with teak seedlings treated with *Trichoderma koningii* individually and in preventive and curative treatments against pathogens

Sl. No.	Major biochemical compounds associated in teak seedlings	Biochemical compounds produced in teak seedlings treated with bio-agent and in preventive and curative treatments against selected pathogens				
		***T. koningii* treated (22[1])**	***Fusarium oxysporum***		***Athelia rolfsii***	
			P (12)	**C (18)**	**P (21)**	**C (20)**
1	5-Hydroxymethylfurfural	2.02	5.50	12.45	1.22	1.18
2	Chinasaure	-	9.63	36.03	7.79	14.93
3	Hexadecanoic acid	6.84	3.87	2.49	9.08	5.71
4	2-Methylindoline	8.47	11.31	16.23	11.51	9.69
5	Linoleic acid, Methyl ester	13.57	4.14	-	-	2.00
6	9,12-Octadecadienoic acid (Z,Z)	6.13	3.25	1.22	5.39	-

[1] Value in Parenthesis represents total number of Bio-compounds associated with teak seedlings
P- Preventive; C-Curative
'-'- Absent

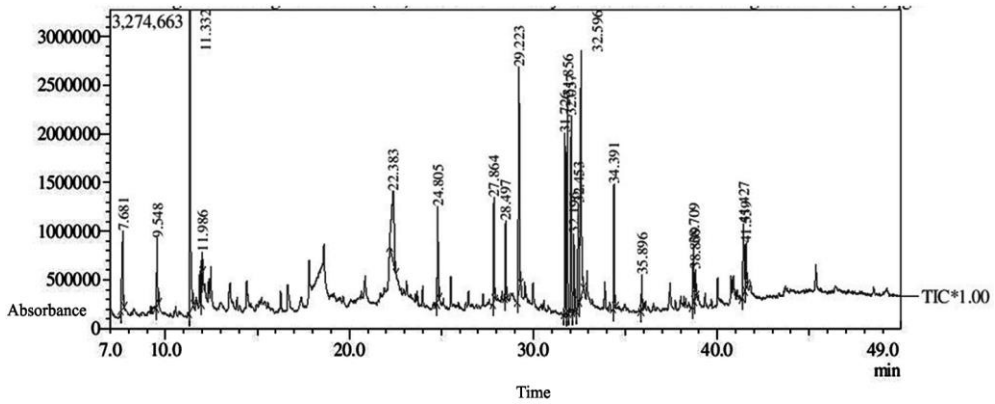

Fig 53. Chromatogram of methanol extract of Teak seedlings amended with *Trichoderma koningii* against *Athelia rolfsii* in preventive treatment showing different biochemical compounds by GC-MS analysis

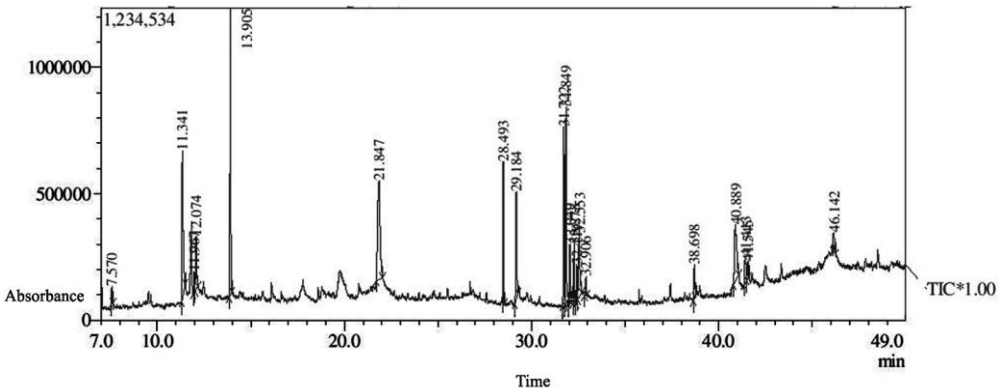

Fig 54. Chromatogram of methanol extract of Teak seedlings amended with *Trichoderma koningii* against *Athelia rolfsii* in curative treatment showing different biochemical compounds by GC-MS analysis

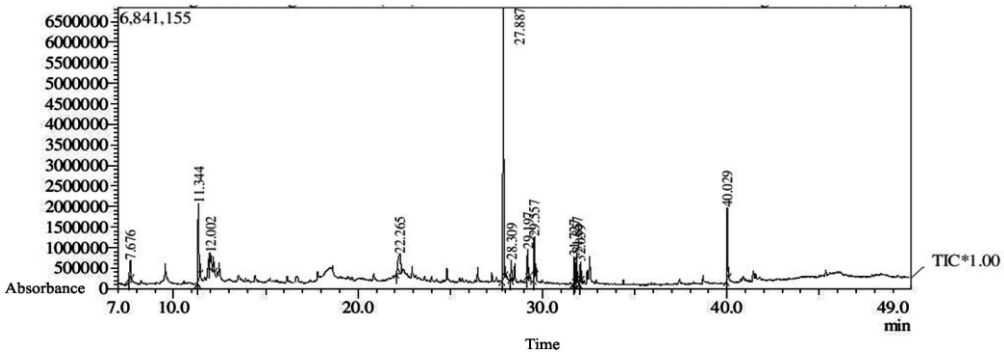

Fig 55. Chromatogram of methanol extract of Teak seedlings amended with *Trichoderma koningii* against *Fusarium oxysporum* in preventive treatment showing different biochemical compounds by GC-MS analysis

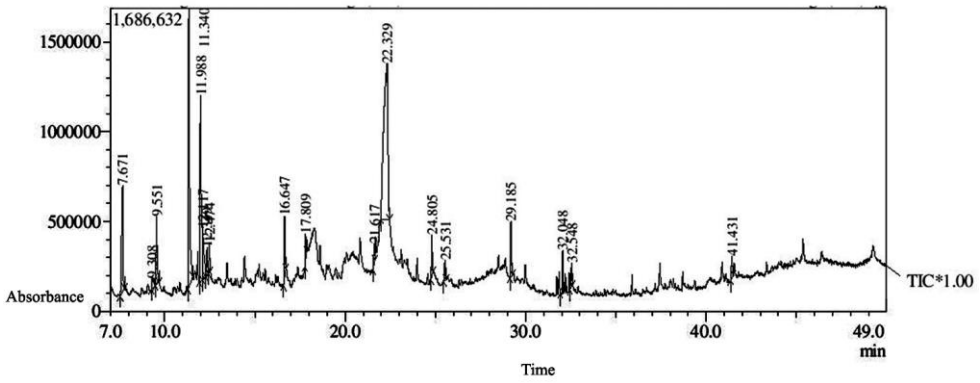

Fig 56. Chromatogram of methanol extract of Teak seedlings amended with *Trichoderma koningii* against *Fusarium oxysporum* in curative treatment showing different biochemical compounds by GC-MS analysis

Analysis of methanol extracts (using GC-MS) of teak and mahogany seedlings subjected to different treatments such as 1) inoculated with the selected pathogens - *F. oxysporum* and *A. rolfsii,* 2) treated with different antagonists as preventive and curative measures against the pathogens and 3) untreated controls revealed the presence of various biochemical compounds in varying concentrations in the seedlings. These results could be attributed to the response to microbe induced signaling system and have been corroborated by studies conducted by various researchers especially in agricultural crops (An *et al*., 2010; Shoresh *et al*. 2010).

Production of isoflavonoid and phytoalexins in soybean and alfalfa, sesquiterpenes in plants of Solanaceae, glucosinolates-myrosinase in plants of Brassicaceae, stilbenes in plants of Vitaceae, isoflavones in plants of Fabaceae and limonoids in plants of Rutaceae and Meliaceae have been reported in response to pathogen defense (Freeman and Beattie, 2008; Goyal *et al*., 2012). Bollina *et al*., (2011)

and Kumaraswamy *et al*., (2011) observed higher concentration of flavonoids and phenyl propanoid using LC-MS in barley cultivars resistant to *Fusarium* head blight.

Seedlings of mahogany noticed an increase in fatty acids when inoculated with *F. oxysporum* and *A. rolfsii* compared to untreated controls. Liu *et al*., (2008) observed in *in vitro* and *in vivo* studies that the fatty acids were highly active against plant pathogens *Alternaria solani*, *Colletotrichum lagenarium*, *Fusarium oxysporum* f. sp. *cucumerinum* and *Fusarium oxysporum* f. sp. *lycopersici*. Similarly, capric acid effectively inhibited *Candida albicans* and (Z)-9-heptadecenoic acid was effective against *Phytophthora infestans* and *Idriella bolleyi* which were attributed to disruption of fungal membrane by the respective compounds (Bergsson *et al*., 2001; Avis and Belanger, 2001). However, in the current study, when seedlings treated with bio-agents in preventive and curative measures against pathogens were analyzed, fatty acids concentration was found to be at par with that of the untreated control.

Analyses of mahogany seedlings treated with antagonists for preventive and curative studies showed the presence of 5-Hydroxymethylfurfural in varying concentrations. Mahogany seedlings treated with *Penicillium multicolor* and *Purpureocillium lilacinum* as preventive measure against both the pathogens yielded a higher concentration of 5-Hydroxymethylfurfural compared to that of curative treatments. Also preventive treatments involving *Clonostachys rosea* and *Trichoderma harzianum* against *F. oxysporum* yielded a higher concentrations of 5-Hydroxymethylfurfural. However, in *A. rolfsii* infected mahogany seedlings, the curative treatment with the above antagonists yielded higher concentrations of the compound. However, seedlings

inoculated with the antagonist *Trichoderma koningii* revealed no variation in the content of 5-Hydroxymethylfurfural when inoculated with *A. rolfsii*. The content of most of the bio-compounds in seedlings varied significantly in response to different treatments.

An increase in sterol content was observed in teak seedlings inoculated with the pathogens *F. oxysporum* and *A. rolfsii* compared to untreated control. Wang *et al*., (2012) in their study highlighted innate ability of phytostreols against bacterial pathogens *Pseudomonas syringae* and *Xanthomonas campestris*. Results of this study showed that bio-agents exerted a differential response against both the pathogens in the presence of antagonists used for preventive and curative purposes.

Teak seedlings subjected to preventive and curative treatments produced varying concentrations of 2-Methylindoline in the presence of all the bio-agents. These seedlings infested with *A. rolfsii* showed a higher concentration of 2-Methylindoline in preventive treatments with all the bio-agents compared to curative treatments as well as healthy seedlings. Teak seedlings inoculated with *F. oxysporum*, *Purpureocillium lilacinum* and *Trichoderma harzianum* yielded a higher concentration of 2-Methylindoline in preventive treatments compared to curative treatments. However, *Trichoderma koningii* resulted in production of higher concentration of 2-Methylindoline in curative treatments. Application of *Clonostachys rosea* and *Penicillium multicolor* did not influence 2-Methylindoline concentration. 2-Methylindoline is a precursor in the synthesis of indoleacetic acid (auxin) and other plant growth substances. Zuniga *et al*., (2013) showed the ability of wild-type *Burkholderia phytofirmans* in the degradation of indole-3-acetic

acid and a subsequent promotion in the growth of *Arabidopsis thaliana* plant roots compared to the mutant strain which failed to exhibit plant growth promotion.

Biochemical compounds produced by mahogany and teak seedlings in response to different treatments gave an insight into the alteration of metabolic pathways responsible for their production and that these may probably assist plants in their defense against plant pathogens. Plant defense response could be constitutive or induced (Harborne, 1990) and for the latter actual damage by the pathogen cause recognition of elicitor compounds which trigger the production and release of toxic secondary metabolites at a distant region or at the site of action (Freeman and Beattie, 2008). Recent studies ascribed spatial and temporal changes in the production of secondary metabolites which also vary with respect to the growth and physiological stages of the plant species (Metlen *et al*., 2009; Grace, 2012; Iason *et al*., 2012; Koricheva and Barton, 2012; Moore *et al*., 2014; Gobbo-Neto 2017; Rai *et al*., 2017). Though higher levels of secondary metabolites indicate higher gene expression and signaling pathways but actual translocation of the biochemical compounds to the storage site plays a decisive role in their activity (Shoji *et al*., 2000; Yang *et al*., 2018).

It is evident from the current study that all the five bio-agents namely *Clonostachys rosea* (ISO-79), *Penicillium multicolor* (ISO 58), *Purpureocillium lilacinum* (ISO 48), *Trichoderma harzianum* (ISO 33) and *T. koningii* (ISO 35), at the concentrations of conidial suspensions evaluated, were effective against both the pathogens *in vitro*. Field studies proved an enhanced growth of mahogany and teak seedlings when treated individually with the antagonists *Clonostachys rosea* and

Trichoderma harzianum. In preventive and curative treatment studies against the pathogens, an increased root and shoot lengths were observed in teak seedlings inoculated with the antagonists *Penicillium multicolor* and *Purpureocillium lilacinum.* However, none of the antagonists had any effect on root and shoot lengths of mahogany seedlings in the conidial concentrations applied. Comparatively, curative treatment was more effective than preventive treatment, and the activity of the bio-agents was dependent on the timely application of the antagonists as well as the right concentration of the conidia. The type of interactions is still unclear as of how the inoculum density is related to the systemic resistance. Advent of modern scientific techniques significantly contribute in deciphering *in vitro* and *in vivo* plant response to stress and defense however a critical evaluation needs to be done so that sufficient information on particular compounds, if not all, could be acquired.

Summary and Conclusions

Plant diseases affect the health of seedlings hindering successful out-planting and growth. Plant diseases, in general, are known to cause 25% crop loss per annum world-wide (Lugtenberg, 2015). Nurseries which are successful in the production of healthy seedlings still follow traditional approaches of producing resistant varieties, modifying cultural practices and use of fungicides. Though use of fungicides is largely effective in controlling nursery diseases, environmental and health concerns from chemical residues outweigh the gains in a big way. Moreover, continuous application of fungicides has resulted in loss of plant vigour and development of resistance in pathogens through genetic shifts in the population (Gunnell *et al*., 2007; Leach and Mumford, 2008). In this situation, research has been ongoing over a long period to develop environmentally benign strategies to fight plant diseases using microbial agents. Microbial agents, usually a fungus, bacterium, or virus, or a consortium of microbes applied to suppress a plant disease is referred to as Biocontrol. Literature is abounding on successful management of various crop diseases using biocontrol.

The potentials of biocontrol agents have previously been ignored in breeding practices (Smith *et al*., 1999) but of late, this perception has changed greatly with the application of genomics. Microorganisms in soil play an important role in various stages of plant growth and development and also form a promising source of biological control agents against plant pathogens. Microorganisms colonize different plant

microenvironments of which high proportions are present in plant-soil interface (Berg *et al*., 2005b).

Soil, a dynamic reservoir of microbes which form a hotspot of microbial interaction with plant roots, performs a wide range of environmental services (Birge *et al*., 2016; Zheng *et al*., 2017). Rhizosphere represents special ecological niches highly influenced by microbe-microbe and microbe-plant interactions in response to chemical signals released by roots (Berendsen *et al*., 2012; van Dam and Bouwmeester, 2016; Verma *et al*., 2016a; Verma *et al*., 2017b; Saleem *et al*., 2018; Yadav *et al*., 2018). The rhizosphere, which abodes a diverse and unique microbial community serves certain plant specific ecological functions and thereby provide a competitive edge over neighbouring species and pathogens. Hence it is considered a potential hub for the isolation of microbes and for use in biocontrol (Lakshmanan *et al*., 2014; Shi *et al*., 2016; Wang *et al*., 2017; Yadav *et al*., 2018).

The beneficial microorganisms associated with the plant habitats are highly active against the pathogens *in vitro* on artificial media (Heimpel and Mills, 2017; van Lenteren *et al*., 2018). Their mode of activity against the pathogens may be direct through hyper-parasitism, competition and antibiosis (Raaijmakers and Mazzola, 2012; Spadaro and Droby, 2016; Ghorbanpour *et al*., 2018) or indirect by inducing resistance (Pieterse *et al*., 2014; Conrath *et al*., 2015). It is mandatory to understand the mode of the bio-agent against the pathogen to select and design biocontrol strategies.

The microbes which harbor the fibrous root system of grasses have been characterized to some extent for their potentials in managing diseases and pests and also promoting plant growth (Hyakumachi *et al*., 1992; 1993a; Shivanna *et al*., 1993; 1994; 1995; 1996; 2005; Meera *et al*., 1993; 1994; 1995). Though a number of microbes are shown to inhibit pathogens *in vitro*, successful inhibition in the field is often limited. Microbes and their biological interactions pose a number of questions such as the nature of plant-microbe association, efficacy of inocula, presence of any novel strains and their mode of activity.

The objectives of the present study were, to isolate and characterize the rhizosphere and rhizoplane fungi of certain grasses of Kerala part of the Western Ghats during different seasons, to assess the antagonistic ability of selected rhizosphere and rhizoplane fungi against selected fungal pathogens causing diseases in forest nursery seedlings *in vitro* and to characterize the competitive saprophytic, root colonization, biological control and resistance inducing abilities of selected antagonistic fungi against selected pathogens in field experiments.

To accomplish objective-1, survey and collection of grass species were carried out in Kerala part of the Western Ghats and three study sites were randomly selected each in northern, central and southern Kerala based on the type of vegetation and altitude. Field visits were made in summer (March-April), rainy (July-August) and winter (November-December) seasons during 2016-2018.

Ten perennial grass species - *Alloteropsis cimicina, Cynodon dactylon, Ischaemum indicum, Oplismenus compositus, Ottochloa nodosa, Panicum repens, Paspalidium flavidum, Paspalum conjugatum, Perotis indica* and *Setaria barbata* were collected and analyzed for rhizosphere and rhizoplane fungi from all the study areas. Fungal isolates were characterized on the basis of colony morphology, conidia and other microscopic features.

A total of 11915.58 fungal isolates from rhizoplane and 975.44 (10^4 dilution factor) cfu g^{-1} of soil from rhizosphere comprising of 94 species belonging to 32 genera and 26 non-sporulating fungi were isolated from the grass species. The density of fungal isolates was higher during winter followed by rainy and summer seasons. Most of the fungal isolates belonged to the division ascomycota and a few to zygomcota. Non sporulating fungi were distributed in varying percentages among the rhizosphere/rhizoplane of grass species. Species of *Absidia, Acremonium, Acrostalagmus, Alternaria, Arthobotrys, Aspergillus, Bipolaris, Cephalosporium, Chaetomium, Cladosporium, Clonostachys, Colletotrichum, Curvularia, Fusarium, Geotrichum, Helminthosporium, Mucor, Myrothecium, Nigrospora, Paecilomyces, Penicillium, Periconia, Pestalotiopsis, Phoma, Phomopsis, Purpureocillium, Rhizopus, Scopulariopsis, Talaromyces, Torula, Trichoderma* and *Verticillium* were isolated from the rhizoplane and rhizopshere but the percentage of occurrence of each species varied among the grass species.

In the present study, the grass rhizoplane regions were dominated by the species of *Aspergillus, Cladosporium, Curvularia, Fusarium, Penicillium* and *Trichoderma*.

Rhizosphere regions of grass speices were found to be dominated by species of the genera *Aspergillus, Cladosporium, Curvularia, Fusarium, Mucor, Paecilomyces, Penicillium* and *Trichoderma*. Higher percentage colonization of fungi in the rhizosphere was observed for *Cynodon dactylon* (Chloridoideae subfamily) and *Ischaemum indicum* followed by *Paspalum conjugatum* (Panicoideae subfamily).Whereas in the grass rhizoplane regions, higher fungal percentage colonization was recorded from *Cynodon dactylon* (Chloridoideae subfamily) and *Paspalum conjugatum* (Panicoideae subfamily).

Diversity indices for rhizosphere and rhizoplane mycoflora of grass species during three seasons noticed a higher diversity (D') for the winter season followed by rainy and summer seasons. Shannon diversity (H') throughout the seasons recorded a significantly high richness of rhizoplane fungi associated with *Alloteropsis cimicina, Cynodon dactylon, Ischaemum indicum, Paspalidium flavidum* and *Perotis indica. Cynodon dactylon* and *Setaria barbata* recorded a higher richness of fungal species in the rhizosphere.

Among the fungal isolates from the grass species, those isolates that exhibited higher frequency of colonization were selected to test the *in vitro* antagonistic ability against root rot and wilt of teak and mahogany seedlings caused by *Fusarium oxysporum* and *Athelia rolfsii*. For objective-2, 16 fungal species from the rhizosphere/rhizoplane of *Alloteropsis cimicina,* 22 from *Cynodon dactylon*, 11 from *Oplismenus compositus,* 10 from *Ottochloa nodosa,* 15 from *Panicum repens,* 10 from *Paspalidium flavidum,* 19 from *Paspalum conjugatum,* 8 from *Perotis indica* and 9 from *Setaria barbata* were selected and analyzed for their antagonistic activity against two root pathogens -

Fusarium oxysporum and *Athelia rolfsii*. *In vitro* dual culture studies revealed differential mycelial interactions between candidate fungal isolates and the pathogens. The mode of antagonism was either by hyphal interactions or by antibiosis, but not all the selected fungal isolates were effective against both the pathogens. The fungi were also evaluated for their efficacy against the pathogens and their hyper-sensitivity activity was conducted by leaf bioassay. Based on this study, *Penicillium nigricans* (ISO-11) from *Alloteropsis cimicina*, *Trichoderma harzianum* (ISO-33) and *Trichoderma koningii* (ISO-35) from *Cynodon dactylon*, *Aspergillus niger* (ISO-40) and *Purpureocillium lilacinum* (ISO-48) from *Ischaemum indicum*, *Penicillium multicolor* (ISO-58) from *Oplismenus compositus*, *Clonostachys rosea* (ISO-79) from *Panicum repens*, *Trichoderma* sp. (ISO-106) from *Paspalum conjugatum* and *Trichoderma pseudokoningii* (ISO-116) from *Paspalidium flavidum* were selected and subjected for further antagonistic abilities.

In objective-3, the selected nine fungal isolates were first observed for saprophytic competency. Of the nine species (after 21 days of incubation), *Trichoderma harzianum, Clonostachys rosea, Trichoderma koningii, Purpureocillium lilacinum* and *Penicillium multicolor* were selected for further studies based on the maximum saprophytic colonization and depth of colonization.

The selected fungi were evaluated for root colonization of the seedlings in hydroponics system. Conidial suspensions of 2 x 10^6 of both the pathogen and the antagonists were tested both individually as well as in combination in two different concentrations 1: 1 (pathogen: antagonist) and 1: 5 (pathogen: antagonists). Of these, 1: 5 (pathogen: antagonists) was effective particularly in inhibiting the mycelial growth of the

pathogen. Among the antagonists, *Clonostachys rosea*, *Trichoderma harzianum* and *Trichoderma koningii* hyphae penetrated root tissues and colonized the cortical layers. The hyphae of *Purpureocillium lilacinum* and *Penicillium multicolor* colonized the surface of the root tissues typically serving as a root shield thereby preventing the entry of the pathogens.

The above five fungi were further tested for their efficacy against the pathogen in field experiments. One month old seedlings of teak and mahogany were drenched with conidial suspensions of both the pathogens as well as antagonists in curative and preventive treatments. Seedling growth in response to treatments was ascertained by measuring root and shoot length and fresh and dry weight. Both the seedlings exhibited variation in growth characteristics in response to treatments and antagonists inoculated. The influence of bio-agents in enhancing plant growth could be attributed to enhanced nutrient uptake, disease suppression or induction of systemic resistance (Contreras-Cornejo *et al*., 2009).

In continuation, testing plant growth parameters, rhizosphere myco-flora of treated teak and mahogany seedlings were also studied. The results showed that the fungal density was high in pathogen inoculated seedlings compared with bio-agents and preventive and curative treatments for both the plants. Irrespective of the inoculum density, a significant decrease in the rhizosphere fungal density for preventive and curative treatments could be attributed to the competition for root colonization and locally induced resistance. Also, seedlings rhizosphere myco-flora was compared for

preventive and curative treatments, preventive treatments harboured a high density of fungi compared to curative treatment.

In the present study, curative treatments were found to be comparatively effective than preventive treatments but it also depend on the timely application of the treatment and physiology of the seedlings.

Bio-chemical analysis of methanol extracts of seedlings subjected to different treatments using GC-MS analysis revealed the presence of a variety of biochemical compounds in varying concentrations in different treatments. These results invited the attention for in depth analysis of phyto-chemical signaling pathways in response to microbial interactions.

The study revealed that the five fungal antagonists were efficient in their capacities in managing as well as improving seedling growth but very much depended upon the timely application and inoculum potential. It is hoped that these results will promote further investigations to develop a successful biocontrol program for the root pathogens of teak and mahogany.

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Appendix

Appendix 1. Bio-chemical compounds in the methanol extract of healthy mahogany seedlings

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.510	372226	0.60	116880	0.81	Thymine	126.05
2	9.511	1944036	3.15	678361	4.71	3-Hydroxy-2,3-dihydromaltol	144.00
3	17.762	496550	0.80	120083	0.83	2,6-Cresotaldehyde	136.05
4	21.126	945104	1.53	156267	1.08	.beta.-D-Glucopyranoside, methyl	60.00
5	21.958	11127113	18.02	851612	5.91	Chinasaure	60.00
6	24.784	903230	1.46	287711	2.00	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
7	28.499	2869324	4.65	1199224	8.32	Methylpalmitate	74.05
8	28.851	2848201	4.61	697305	4.84	3,4-O-Isopropylidene-d-galactose	73.00
9	29.206	6195591	10.03	1977387	13.72	Hexadecanoic acid	73.00
10	29.712	9809700	15.88	1229252	8.53	Glucal	73.00
11	29.970	814831	1.32	258121	1.79	Benzenepropanoic acid, 2,5-dimethoxy-	167.05
12	31.731	2334459	3.78	942411	6.54	linoleic acid, methyl ester	67.00
13	31.838	2176543	3.52	629642	4.37	9-Octadecenoic acid (Z)-, methyl ester	55.00
14	32.061	3946146	6.39	1301361	9.03	Phytol	71.05
15	32.290	702101	1.14	277285	1.92	Methyl stearate	74.00
16	32.439	4647100	7.52	1447817	10.05	Oxacycloheptadec-8-en-2-one	67.00
17	32.529	5379324	8.71	937404	6.50	cis-Vaccenic acid	55.05
18	32.920	804860	1.30	242371	1.68	Octadecanoic acid	55.05
19	38.449	819090	1.33	313578	2.18	Methyl 5,11,14-eicosatrienoate	66.95
20	38.709	1053251	1.71	350525	2.43	Glycerol .beta.-palmitate	57.05
21	39.004	511672	0.83	157433	1.09	Octadecanal	82.10
22	41.433	1055925	1.71	240620	1.67	9-octadecenal, (Z)-	55.05
		61756377	100.00	14412650	100.00		

Appendix 2. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Fusarium oxysporum*

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	22.966	41748390	4.11	4254598	2.03	.beta.-D-Glucopyranoside, methyl	60.00
2	24.882	30368612	2.99	8843894	4.22	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
3	27.929	56642012	5.58	16281285	7.77	Alpha.-copaene-11-ol	59.05
4	28.308	15757024	1.55	5095273	2.43	Methyl palmitoleinate	55.05
5	28.531	58225593	5.73	21638798	10.32	Methylpalmitate	74.05
6	29.112	31743655	3.13	4579204	2.18	trans-13-Octadecenoic acid	55.05
7	29.443	197236615	19.42	23678496	11.30	Hexadecanoic acid	73.00
8	29.595	13460102	1.33	4018689	1.92	Beta.-eudesmol	59.00
9	31.762	46166926	4.55	16820981	8.03	Linoleic acid, methyl ester	67.00
10	31.865	36226631	3.57	11857764	5.66	Methyl 10-octadecenoate	55.05
11	32.091	28544159	2.81	11146086	5.32	Phytol	71.05
12	32.306	7569237	0.75	3055987	1.46	Methyl stearate	74.00
13	32.655	188788636	18.59	21451231	10.23	9,12-Octadecadienoic acid (Z,Z)-	67.05
14	32.726	91239468	8.98	18231593	8.70	Octadec-9-enoic acid	55.05
15	33.030	16894460	1.66	3069620	1.46	Octadecanoic acid	57.05
16	38.470	15212599	1.50	5625695	2.68	Methyl 5,11,14-eicosatrienoate	67.00
17	38.751	29771758	2.93	9231409	4.40	Glycerol .beta.-palmitate	57.05
18	40.069	19766272	1.95	6805380	3.25	4-t-Butyl-2-[4-nitrophenyl]phenol	256.10
19	41.109	28570000	2.81	3572125	1.70	.gamma.-Sitosterol	272.10
20	41.464	35669474	3.51	7391116	3.53	Alpha.-glyceryl linoleate	67.05
21	49.367	26042707	2.56	2952575	1.41	Friedeline	69.05
		1015644330	100.00	209601799	100.00		

Appendix 3. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Athelia rolfsii*

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	22.284	6352403	1.31	1127430	0.96	.beta.-D-Glucopyranoside, methyl	60.00
2	24.810	8515169	1.76	3126851	2.66	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
3	28.289	3357739	0.69	1337639	1.14	trans-13-Octadecenoic acid, methyl ester	55.05
4	28.510	31370040	6.47	12365847	10.53	Methylpalmitate	74.00
5	29.146	25274372	5.22	2687787	2.29	3,4-O-Isopropylidene-d-galactose	101.05
6	29.333	64638720	13.34	11264738	9.59	Hexadecanoic acid	73.00
7	30.004	5895156	1.22	1961858	1.67	Benzenepropanoic acid, 2,5-dimethoxy-	167.05
8	31.741	25183082	5.20	10100561	8.60	Linoleic acid, methyl ester	67.05
9	31.846	23333214	4.82	6532933	5.56	Methyl 10-octadecenoate	55.05
10	32.081	53249588	10.99	18975802	16.16	Phytol	71.05
11	32.224	7047533	1.45	1774629	1.51	Trehalose	73.00
12	32.291	6118712	1.26	2420435	2.06	Methyl stearate	74.05
13	32.549	51314944	10.59	8857142	7.54	Linolelaidic acid, methyl ester	67.00
14	32.631	28215198	5.82	6236140	5.31	cis-Vaccenic acid	55.05
15	37.419	22469628	4.64	1669217	1.42	Stigmasterol	55.00
16	38.452	11340447	2.34	4351671	3.71	Methyl 5,11,14-eicosatrienoate	67.05
17	38.724	20892265	4.31	6737378	5.74	Glycerol .beta.-palmitate	57.05
18	41.094	16670555	3.44	2587207	2.20	.beta.-Sitosterol	55.05
19	41.436	24165363	4.99	4995826	4.25	beta.-Monolinolein	67.05
20	41.572	6885688	1.42	1553777	1.32	Butyl 9,12,15-octadecatrienoate	67.00
21	42.715	3316509	0.68	1204718	1.03	9-Octadecenamide	59.00
22	48.527	8147344	1.68	1975931	1.68	Vitamin E	165.10
23	49.362	30832976	6.36	3587633	3.06	Friedeline	69.05
		484586645	100.00	117433150	100.00		

Appendix 4. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Clonostachys rosea*

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	7.689	3100741	3.48	652335	4.49	Thymine	126.05
2	9.556	2601213	2.92	856690	5.89	Pyranone	144.00
3	11.827	4939673	5.55	375003	2.58	Propyl butyrate	71.00
4	11.988	3101742	3.48	601337	4.14	5-Hydroxymethylfurfural	97.05
5	12.397	1964624	2.21	459771	3.16	Ethanol, 2,2-diethoxy-	103.05
6	13.710	1816008	2.04	256129	1.76	2,4(3H,5H)-Furandione, 3-propyl-	55.00
7	22.477	24577051	27.59	1215289	8.36	Chinasaure	60.00
8	24.805	1175520	1.32	371507	2.56	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
9	28.490	2764455	3.10	1039455	7.15	Methylpalmitate	74.05
10	28.987	2087058	2.34	456041	3.14	Oleic Acid	55.05
11	29.214	6864403	7.71	1775209	12.21	Hexadecanoic acid	73.00
12	29.986	11213006	12.59	1373439	9.45	Glucal	73.00
13	31.720	2675788	3.00	1059708	7.29	9,12-Octadecadienoic acid, methyl ester	67.00
14	31.845	2908059	3.26	886898	6.10	Methyl 8,11,14-heptadecatrienoate	79.05
15	32.050	1795390	2.02	748428	5.15	Phytol	71.05
16	32.274	376661	0.42	147370	1.01	Methyl stearate	74.05
17	32.573	10547865	11.84	1215380	8.36	Linolenic acid	79.05
18	32.900	625663	0.70	200195	1.38	9-Octadecenoic acid (Z)-	55.05
19	38.693	799273	0.90	264032	1.82	Glycerol .beta.-palmitate	57.05
20	40.860	1670441	1.88	215300	1.48	.beta.-Sitosterol	55.05
21	41.407	1232375	1.38	299628	2.06	14-Methyl-8-hexadecyn-1-ol	67.05
22	48.485	231162	0.26	71174	0.49	Vitamin E	165.10
		89068171	100.00	14540318	100.00		

Appendix 5. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Penicillium multicolor*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.581	432909	1.10	115248	1.43	Thymine	126.00
2	9.543	505105	1.29	129923	1.62	1,5-Anhydro-6-deoxyhexo-2,3-diulose	144.00
3	11.678	612228	1.56	143314	1.78	2,3-Dihydro-benzofuran	120.05
4	22.021	7727972	19.67	641200	7.97	Chinasaure	60.00
5	28.277	318816	0.81	127002	1.58	Methyl palmitoleate	55.00
6	28.488	2063553	5.25	802353	9.97	Methylpalmitate	74.05
7	28.883	394167	1.00	87467	1.09	3,4-O-Isopropylidene-d-galactose	73.05
8	28.979	819752	2.09	261200	3.25	Hexadecenoic acid, Z-11-	55.05
9	29.199	4061067	10.33	1140233	14.17	Hexadecanoic acid	73.00
10	29.750	5456699	13.89	650072	8.08	1,5-Anhydro-d-mannitol	73.00
11	29.974	569549	1.45	139443	1.73	(2-Methyl-3-nitrophenyl)methanol	210.00
12	31.717	1951277	4.97	819098	10.18	Methyl octadeca-9,12-dienoate	67.05
13	31.845	2241019	5.70	661865	8.23	Methyl 8,11,14-heptadecatrienoate	79.05
14	32.050	1587484	4.04	513503	6.38	Phytol	71.05
15	32.275	382148	0.97	139546	1.73	Methyl stearate	74.00
16	32.424	1513955	3.85	447636	5.56	Linolelaidic acid, methyl ester	67.05
17	32.554	2201186	5.60	401565	4.99	alpha.-Linolenic acid	79.05
18	40.884	5461371	13.90	583444	7.25	.gamma.-Sitosterol	55.05
19	41.416	598690	1.52	133946	1.67	Z,Z-8,10-Hexadecadien-1-ol	67.00
20	48.498	395516	1.01	106611	1.33	Vitamin E	165.10
		39294463	100.00	8044669	100.00		

Appendix 6. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Purpureocillium lilacinum*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.640	1723586	4.60	451871	7.15	Thymine	126.05
2	9.035	252992	0.68	90793	1.44	Dimethyl dl-malate	103.00
3	9.545	1223224	3.26	416983	6.60	Pyranone	144.00
4	11.681	2825178	7.54	336992	5.33	2,2,7,7-Tetraadeutero-hexamethylene Sulphide	120.05
5	11.989	593922	1.58	108120	1.71	5-Hydroxymethylfurfural	97.00
6	12.365	1691072	4.51	439542	6.96	Ethanol, 2,2-diethoxy-	103.00
7	13.610	646383	1.72	149128	2.36	Heptanoic acid, 6-oxo-	55.00
8	22.117	4350355	11.61	550371	8.71	Decanoic acid	60.00
9	22.240	5963219	15.91	749140	11.85	Chinasaure	60.00
10	24.800	364220	0.97	131271	2.08	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
11	28.495	265633	0.71	104824	1.66	Methyl isopalmitate	74.00
12	28.967	444016	1.18	95012	1.50	3,4-O-Isopropylidene-d-galactose	101.10
13	29.179	1336241	3.57	395220	6.25	Hexadecanoic acid	73.00
14	29.936	10488396	27.99	1133255	17.93	Glucal	73.00
15	31.721	276154	0.74	111870	1.77	9,12-Octadecadienoic acid, methyl ester	67.05
16	31.854	420620	1.12	130318	2.06	Methyl 8,11,14-heptadecatrienoate	79.05
17	32.419	704298	1.88	234540	3.71	Linolelaidic acid, methyl ester	67.05
18	32.544	1059203	2.83	270724	4.28	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.00
19	40.888	2845746	7.59	419394	6.64	.gamma.-Sitosterol	57.05
		37474458	100.00	6319368	100.00		

Appendix 7. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma harzianum*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.587	582904	1.44	182715	2.26	Thymine	126.05
2	9.537	624930	1.55	153892	1.90	Pyranone	144.00
3	12.319	674572	1.67	180491	2.23	Ethanol, 2,2-diethoxy-	103.05
4	21.925	6292334	15.58	549375	6.80	Chinasaure	60.00
5	24.783	487340	1.21	177160	2.19	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
6	28.482	927746	2.30	380580	4.71	Methylpalmitate	74.05
7	28.977	680286	1.68	244805	3.03	Hexadecenoic acid, Z-11-	55.05
8	29.192	3252945	8.05	977010	12.09	Hexadecanoic acid	73.05
9	29.789	10464931	25.91	1188302	14.71	1,5-Anhydro-d-mannitol	73.05
10	29.961	941537	2.33	201796	2.50	(2-Methyl-3-nitrophenyl)methanol	210.05
11	31.709	1425155	3.53	549620	6.80	Methyl octadeca-9,12-dienoate	67.05
12	31.840	2006542	4.97	643680	7.97	Methyl 8,11,14-heptadecatrienoate	79.10
13	32.043	1782465	4.41	493884	6.11	Phytol isomer	71.05
14	32.424	1539913	3.81	432807	5.36	Oxacycloheptadec-8-en-2-one	67.05
15	32.554	2372041	5.87	441569	5.46	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
16	35.919	345621	0.86	108059	1.34	9-Octadecenoic acid (Z)-, methyl ester	55.05
17	38.427	521411	1.29	198606	2.46	Methyl 10,13,16-docosatrienoate	67.05
18	38.687	593148	1.47	171334	2.12	Glycerol .beta.-palmitate	55.05
19	39.567	486828	1.21	172668	2.14	10,12-Docasadiyndioic acid	55.05
20	40.881	2935519	7.27	334797	4.14	.gamma.-Sitosterol	55.05
21	41.405	1001403	2.48	200754	2.48	Beta-monolinolein	67.05
22	41.536	450504	1.12	96469	1.19	Ethyl 6,9,12-hexadecatrienoate	79.05
		40390075	100.00	8080373	100.00		

Appendix 8. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma koningii*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.633	1518178	2.20	397766	2.61	Thymine	126.00
2	9.030	540377	0.78	186838	1.23	Dimethyl dl-malate	103.05
3	9.543	1178055	1.70	390631	2.57	Pyranone	144.00
4	11.811	4222858	6.11	423642	2.78	Butanoic acid, propyl ester	71.00
5	22.291	13041075	18.86	875241	5.75	Chinasaure	60.00
6	24.791	830992	1.20	340523	2.24	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
7	28.487	3996885	5.78	1491936	9.81	Methylpalmitate	74.00
8	28.981	2132232	3.08	563605	3.71	Hexadecenoic acid, Z-11-	55.05
9	29.214	6251690	9.04	1920338	12.62	Hexadecanoic acid	73.05
10	29.899	9281243	13.42	1157948	7.61	Glucal	73.05
11	29.975	2932797	4.24	695294	4.57	1-Butyn-3-one, 1-(6,6-dimethyl-1,2-epoxycyclohexyl)-	149.00
12	31.716	3916264	5.66	1537639	10.11	Methyl octadeca-9,12-dienoate	67.05
13	31.840	3909114	5.65	1129119	7.42	Alpha-linolenic acid methyl ester	79.05
14	32.045	3907556	5.65	1241887	8.16	Phytol	71.05
15	32.439	4003071	5.79	1112941	7.32	Methyl linolelaidate	67.05
16	32.569	4621358	6.68	905247	5.95	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
17	38.692	1183029	1.71	414112	2.72	Glycerol .beta.-palmitate	57.05
18	41.412	1669368	2.41	427082	2.81	Z,Z-8,10-Hexadecadien-1-ol	67.05
		69136142	100.00	15211789	100.00		

Appendix 9. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Clonostachys rosea* x *Athelia rolfsii* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.691	2719943	2.44	769682	4.18	Thymine	126.00
2	9.034	904469	0.81	336136	1.83	Dimethyl dl-malate	103.00
3	9.563	2908885	2.61	919519	5.00	Pyranone	144.00
4	12.002	3112023	2.79	634620	3.45	5-Hydroxymethylfurfural	97.00
5	12.408	2098849	1.88	441757	2.40	3-Acetoxy-3-hydroxypropionic acid, methyl ester	103.05
6	22.603	36514958	32.78	1543095	8.39	Chinasaure	60.00
7	24.814	1389836	1.25	409726	2.23	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
8	28.490	2989301	2.68	1158233	6.30	Methyl palmitate	74.05
9	28.990	1968962	1.77	643407	3.50	trans-13-Octadecenoic acid	55.00
10	29.216	12773390	11.47	3014234	16.38	Hexadecanoic acid	73.00
11	30.042	15452999	13.87	1554116	8.45	Glucal	73.05
12	31.718	2391597	2.15	974592	5.30	Methyl octadeca-9,12-dienoate	67.00
13	31.844	2150644	1.93	609435	3.31	Linolenic acid, methyl ester	79.10
14	32.048	1670289	1.50	641088	3.48	Phytol	71.05
15	32.435	4985844	4.48	1391918	7.57	11,14-Eicosadienoic acid, methyl ester	67.05
16	32.572	7607356	6.83	1492065	8.11	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
17	38.692	1830195	1.64	592501	3.22	Glycerol .beta.-palmitate	57.05
18	40.877	4601304	4.13	486415	2.64	Stigmast-5-en-3-ol, (3.beta.)-	55.00
19	41.404	2165290	1.94	529217	2.88	alpha.-Glyceryl linoleate	67.05
20	48.481	1158995	1.04	255030	1.39	Vitamin E	165.10
		111395129	100.00	18396786	100.00		

Appendix 10. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Clonostachys rosea* x *Athelia rolfsii* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.665	3384710	6.88	656971	8.60	Thymine	126.05
2	9.009	661778	1.35	244930	3.21	Dimethyl dl-malate	103.05
3	9.275	466367	0.95	124247	1.63	Diethylnitrosamine	102.05
4	9.539	3582053	7.29	1036138	13.57	Pyranone	144.05
5	11.869	6165863	12.54	404952	5.30	Propyl butyrate	71.05
6	12.007	10446750	21.25	1945563	25.47	5-Hydroxymethylfurfural	97.05
7	12.393	1341026	2.73	249646	3.27	Ethanol, 2,2-diethoxy-	103.05
8	22.487	15678451	31.89	859284	11.25	Chinasaure	60.05
9	25.519	365199	0.74	116234	1.52	Loliolide	111.10
10	27.858	1019385	2.07	355108	4.65	alpha.-Copaene-11-ol	59.05
11	28.491	773272	1.57	327438	4.29	Methylpalmitate	74.05
12	29.181	1300363	2.65	472122	6.18	Hexadecanoic acid	73.05
13	29.937	1549795	3.15	283426	3.71	Glucal	73.05
14	31.721	608065	1.24	239236	3.13	Methyl octadeca-9,12-dienoate	67.05
15	32.054	377668	0.77	153781	2.01	Phytol, acetate	71.05
16	40.895	1440178	2.93	168105	2.20	.beta.-Sitosterol	81.10
		49160923	100.00	7637181	100.00		

Appendix 11. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Clonostachys rosea* x *Fusarium oxysporum* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.765	7632138	8.09	950055	6.16	Thymine	126.05
2	9.580	5043660	5.35	1387360	9.00	Pyranone	144.05
3	12.052	17008286	18.03	3282356	21.29	5-Hydroxymethylfurfural	97.05
4	12.472	3307923	3.51	376377	2.44	Glycolaldehyde diethyl acetal	103.05
5	22.433	10111165	10.72	384561	2.49	9-Octadecenoic acid (Z)-	60.05
6	22.944	16589952	17.59	1062272	6.89	chinasaure	60.05
7	28.483	1705927	1.81	680732	4.41	Methylpalmitate	74.05
8	29.206	7528804	7.98	1764056	11.44	Hexadecanoic acid	73.05
9	30.193	7800196	8.27	770994	5.00	Glucal	73.05
10	31.714	2035238	2.16	802573	5.20	9,12-Octadecadienoic acid, methyl ester	67.05
11	31.839	2428621	2.57	610619	3.96	Linolenic acid	79.10
12	32.043	2255031	2.39	865491	5.61	Isophytol, acetate	71.05
13	32.435	3790131	4.02	1059044	6.87	Oxacycloheptadec-8-En-2-one	67.05
14	32.570	5988758	6.35	1163294	7.54	alpha.-Linolenic acid	79.05
15	41.399	1094771	1.16	260051	1.69	beta.-Monolinolein	67.10
		94320601	100.00	15419835	100.00		

Appendix 12. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Clonostachys rosea* x *Fusarium oxysporum* in Curative treatments

Peak #	R.Time	Area	Area %	Height	Height %	Name	Base m/z
1	9.556	5361926	1.04	1589471	1.49	3-Hydroxy-2,3-dihydromaltol	144.00
2	9.660	24317855	4.73	2259747	2.12	pyranone	144.00
3	12.090	32157886	6.26	3206612	3.00	5-Hydroxy methyl furfural	97.00
4	13.952	8335680	1.62	1338210	1.25	-(Hydroxymethyl)-2-(dimethoxymethyl) furan	141.05
5	15.502	11522299	2.24	1265752	1.19	Phenol, 2,6-dimethoxy-	154.05
6	16.716	11650124	2.27	1651021	1.55	8-Hydrazino quinoline	142.05
7	23.134	6101824	1.19	1179065	1.10	Methyl .beta.-D-glucoside	60.00
8	24.856	17198790	3.35	5676007	5.32	-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
9	28.508	16321277	3.17	5964908	5.59	Methylpalmitate	74.00
10	29.033	5339958	1.04	1269269	1.19	trans-13-Octadecenoic acid	55.05
11	29.298	49087473	9.55	8674069	8.13	Hexadecanoic acid	73.00
12	29.483	16749906	3.26	1731510	1.62	3,4-O-Isopropylidene-d-galactose	101.05
13	30.036	11037907	2.15	3572571	3.35	-Enzenepropanoic acid, 2,5-dimethoxy-	167.10
14	30.450	4442339	0.86	620544	0.58	3-O-Methyl-d-glucose	73.00
15	31.742	16487818	3.21	6705474	6.28	Methyl octadeca-9,12-dienoate	67.05
16	31.870	13556012	2.64	3913557	3.67	Linolenic acid, methyl ester	79.05
17	32.071	10170005	1.98	3612779	3.38	Phytol	71.05
18	32.522	30515376	5.94	4965251	4.65	11,14-Eicosadienoic acid, methyl ester	67.05
19	32.641	19617009	3.82	3360100	3.15	alpha.-Linolenic acid	79.05
20	35.945	3377663	0.66	1091849	1.02	Ethyl 16-acetylhydroxypalmitate	55.05
21	38.382	5763371	1.12	2065997	1.94	1-Heneicosanol	57.05
22	38.455	7298040	1.42	2673871	2.51	Methyl 5,11,14-eicosatrienoate	67.05
23	38.740	34707565	6.75	10531512	9.87	Glycerol .beta.-palmitate	57.05
24	41.035	37405343	7.28	2913154	2.73	.gamma.-Sitosterol	55.00
25	41.454	55101222	10.72	11771965	11.03	beta.-Monolinolein	67.00
26	41.587	19125744	3.72	4379329	4.10	Butyl 6,9,12-hexadecatrienoate	79.05
27	41.809	4725775	0.92	918254	0.86	1-Glyceryl stearate	98.10
28	43.858	4035735	0.79	573102	0.54	,5-Octadiene, 4,5-diethyl-3,6-dimethyl-	137.10
29	46.921	3574948	0.70	910610	0.85	.gamma.-Tocopherol	151.05
30	48.549	28974778	5.64	6350106	5.95	Vitamin E	165.10
		514061648	100.0	106735666	100.00		

Appendix 13. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Penicillium multicolor* x *Athelia rofsii* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.618	1026588	3.51	267326	5.41	Thymine	126.05
2	9.030	255123	0.87	84661	1.71	Dimethyl dl-malate	71.05
3	9.540	822260	2.81	213384	4.32	,5-anhydro-6-deoxyhexo-2,3-diulose	101.05
4	11.658	890819	3.04	118832	2.40	P-toluylaldehyde	85.05
5	11.969	3788240	12.95	632786	12.81	5-Hydroxymethylfurfural	97.05
6	12.359	631486	2.16	103547	2.10	Dimethyl dl-malate	103.10
7	13.892	303306	1.04	127403	2.58	-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.10
8	22.386	8104735	27.70	475815	9.63	Chinasaure	60.00
9	24.791	576702	1.97	162585	3.29	-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	91.10
10	28.483	823807	2.82	300613	6.08	Methyl isopalmitate	74.05
11	28.967	942152	3.22	79979	1.62	9-octadecenoic acid (Z)-	55.05
12	29.186	2158251	7.38	522879	10.58	Hexadecanoic acid	73.05
13	29.317	274211	0.94	76155	1.54	Dibutyl phthalate	149.05
14	29.966	4320223	14.76	467604	9.46	1-Deoxy-d-mannitol	73.05
15	31.714	716935	2.45	283872	5.74	,12-Octadecadienoic acid, methyl ester	67.10
16	31.841	727573	2.49	214979	4.35	Alpha-linolenic acid Methyl ester	79.10
17	32.042	658978	2.25	233813	4.73	Phytol, acetate	71.10
18	32.415	574514	1.96	177908	3.60	Stearolic acid	67.10
19	32.543	1116592	3.82	217732	4.41	Linolenic acid	79.05
20	32.886	200729	0.69	74029	1.50	11-Dodecyn-1-ol acetate	55.05
21	38.687	350474	1.20	105779	2.14	Glycerol .beta.-palmitate	57.05
		29263698	100.00	4941681	100.00		

Appendix 14. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Penicillium multicolor* x *Athelia rofsii* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.659	2503230	5.10	545956	6.18	Thymine	126.05
2	9.033	329102	0.67	118635	1.34	Dimethyl dl-malate	103.05
3	9.549	1977666	4.03	630228	7.13	Pyranone	144.05
4	11.684	2107571	4.29	444324	5.03	Coumaran	120.10
5	11.775	1826470	3.72	320193	3.62	Butanoic acid, propyl ester	71.00
6	11.986	2675579	5.45	583012	6.60	5-Hydroxymethylfurfural	97.05
7	12.379	1460706	2.97	336708	3.81	Ethanol, 2,2-diethoxy-	103.05
8	22.352	14576703	29.68	899043	10.17	Chinasaure	60.05
9	28.273	370549	0.75	158569	1.79	Methyl palmitoleate	55.05
10	28.484	1353708	2.76	528018	5.98	Methylpalmitate	74.05
11	28.980	1309090	2.67	355010	4.02	Hexadecenoic acid, Z-11-	55.05
12	29.189	2753474	5.61	926396	10.48	Hexadecanoic acid	73.05
13	29.945	9349668	19.04	1025749	11.61	Glucal	73.05
14	31.713	978710	1.99	399105	4.52	9,12-Octadecadienoic acid, methyl ester	67.05
15	31.841	1066427	2.17	320737	3.63	Methyl 8,11,14-heptadecatrienoate	79.05
16	32.043	322502	0.66	158420	1.79	Isophytol, acetate	71.05
17	32.412	1183892	2.41	388799	4.40	Oxacycloheptadec-8-EN-2-one	67.05
18	32.542	1939908	3.95	410260	4.64	alpha.-Linolenic acid	79.05
19	38.690	500236	1.02	156625	1.77	Glycerol .beta.-palmitate	57.05
20	48.478	519550	1.06	131327	1.49	dl-.alpha.-Tocopherol	165.15
		49104741	100.00	8837114	100.00		

Appendix 15. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Penicillium multicolor* x *Fusarium oxysporum* in Preventive treatments

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	7.697	2021972	5.04	367789	4.30	Thymine	55.00
2	9.032	289796	0.72	111589	1.30	Dimethyl dl-malate	71.05
3	9.556	1213273	3.02	390618	4.57	Pyranone	101.10
4	12.010	5435642	13.55	982378	11.48	5-Hydroxymethylfurfural	97.10
5	12.415	496410	1.24	122817	1.44	3-Acetoxy-3-hydroxypropionic acid, methyl ester	103.10
6	22.342	1818276	4.53	220866	2.58	3-Thiepanol	57.05
7	22.400	755392	1.88	305189	3.57	Chinasaure	55.05
8	22.458	2043051	5.09	359647	4.20	Isobutyl isopentyl carbonate	71.05
9	22.618	4479345	11.16	511499	5.98	Chinasaure	60.05
10	28.488	1537421	3.83	565375	6.61	Methyl palmitate	74.05
11	29.205	3509238	8.75	845201	9.88	Hexadecanoic acid	73.05
12	30.011	3455971	8.61	402457	4.71	Glucal	73.05
13	31.715	1103144	2.75	421973	4.93	9,12-Octadecadienoic acid, methyl ester	67.10
14	31.837	1016265	2.53	261078	3.05	8,11,14-Docosatrienoic acid, methyl ester	55.05
15	32.045	1691959	4.22	642814	7.51	Phytol, acetate	71.10
16	32.427	1438054	3.58	407795	4.77	Linolelaidic acid, methyl ester	67.10
17	32.517	965680	2.41	361415	4.23	Octadec-9-enoic acid	55.05
18	32.556	1103111	2.75	376337	4.40	alpha.-Linolenic acid	79.10
19	32.895	301429	0.75	112342	1.31	9-Octadecenoic acid (Z)-	55.05
20	38.695	432632	1.08	135680	1.59	Glycerol .beta.-palmitate	57.10
21	38.985	328377	0.82	115869	1.35	Hexadecanal	57.05
22	40.894	4685616	11.68	533076	6.23	.gamma.-Sitosterol	55.05
		40122054	100.00	8553804	100.00		

Appendix 16. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Penicillium multicolor* x *Fusarium oxysporum* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.712	78818632	11.19	3831572	2.78	3-Hydroxy-2,3-dihydromaltol	144.00
2	12.194	54812789	7.78	4647139	3.37	5-Hydroxymethylfurfural	97.05
3	13.970	13146533	1.87	2047787	1.49	-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.05
4	15.512	11001878	1.56	1472647	1.07	Phenol, 2,6-dimethoxy-	154.05
5	16.753	12969246	1.84	1896920	1.38	4-monochloroanisole	142.05
6	23.585	13430086	1.91	1786495	1.30	.beta.-D-Glucopyranoside, methyl	60.00
7	24.890	21291401	3.02	6634352	4.82	-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
8	25.587	5431893	0.77	1315331	0.95	Loliolide	181.05
9	28.516	24691232	3.51	9100055	6.60	Methylpalmitate	74.05
10	29.049	9653239	1.37	1893944	1.37	Hexadecenoic acid, Z-11-	55.05
11	29.343	65735662	9.33	12077498	8.77	Hexadecanoic acid	73.00
12	30.066	10181071	1.45	3628583	2.63	Benzenepropanoic acid, 2,5-dimethoxy-	167.10
13	31.749	22277655	3.16	8550304	6.21	Methyl octadeca-9,12-dienoate	67.00
14	31.874	20859555	2.96	5158951	3.74	Linolenic acid, methyl ester	79.05
15	32.078	19931155	2.83	7198520	5.22	Phytol	71.05
16	32.558	54347449	7.72	9117129	6.62	11,14-Eicosadienoic acid, methyl ester	67.05
17	32.654	36142683	5.13	6238307	4.53	cis,cis,cis-7,10,13-Hexadecatrienal	55.05
18	35.957	4980030	0.71	1340416	0.97	-Hexadecenoic acid, methyl ester, (Z)-	55.05
19	38.390	7275748	1.03	2683107	1.95	1-Heneicosanol	57.05
20	38.463	8777686	1.25	3289092	2.39	Methyl 5,11,14-eicosatrienoate	67.00
21	38.750	39352614	5.59	12207776	8.86	Glycerol .beta.-palmitate	57.05
22	41.141	48649376	6.91	6388254	4.64	.beta.-Sitosterol	55.05
23	41.471	63969151	9.08	12257842	8.90	alpha.-Glyceryl linoleate	67.05
24	41.598	19173718	2.72	4813392	3.49	Butyl 6,9,12-hexadecatrienoate	67.05
25	41.822	4664075	0.66	1159007	0.84	Octadecanoic acid, 2,3-dihydroxypropyl ester	98.10
26	48.563	32757913	4.65	7040834	5.11	Vitamin E	165.10
		704322470	100.00	137775254	100.00		

Appendix 17. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Purpureocillium lilacinum* x *Athelia rolfsii* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.914	13489216	2.95	2347721	3.80	2−Furoic acid, anhydride with acetic acid	95.00
2	8.050	13525745	2.96	2335746	3.78	Thymine	126.05
3	9.754	47345373	10.36	6433668	10.42	Pyranone	144.00
4	12.419	196461151	42.99	14405425	23.34	5−Hydroxymethylfurfural	97.05
5	14.765	8701301	1.90	1446902	2.34	Heptyl caprylate	115.05
6	28.493	5029571	1.10	2035983	3.30	Methylpalmitate	74.05
7	29.264	29844668	6.53	6821367	11.05	Hexadecanoic acid	73.05
8	30.672	30047623	6.57	2201508	3.57	1,5−Anhydro−d−mannitol	73.05
9	31.722	6961332	1.52	2632119	4.26	Methyl octadeca−9,12−dienoate	67.05
10	31.850	6959686	1.52	1920926	3.11	Alpha−linolenic acid methyl ester	79.05
11	32.053	7829757	1.71	2755631	4.47	Phytol	71.05
12	32.487	29224887	6.39	5368630	8.70	11,14−eicosadienoic acid, methyl ester	67.05
13	32.629	36303691	7.94	5822213	9.43	9,12,15−Octadecatrienoic acid, (Z,Z,Z)−	79.05
14	32.926	9465583	2.07	1340259	2.17	Octadecanoic acid	73.00
15	38.704	5434566	1.19	1666595	2.70	Glycerol .beta.−palmitate	57.05
16	41.425	10380509	2.27	2181360	3.53	.beta.−Monolinolein	67.05
		457004659	100.00	61716053	100.00		

Appendix 18. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Purpureocillium lilacinum* x *Athelia rolfsii* in Curative treatments

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	7.592	546221	2.74	163418	4.40	Thymine	126.10
2	9.033	338212	1.70	125608	3.38	Dimethyl dl-malate	103.05
3	9.283	304961	1.53	53329	1.44	Methylisopropylnitro samine	102.10
4	9.537	904722	4.54	208445	5.61	Pyranone	144.10
5	11.681	631077	3.17	121836	3.28	2,2,7,7-Tetraadeutero-hexamethylene sulphide	120.10
6	11.970	1370278	6.88	323027	8.70	5-Hydroxymethylfurfural	97.05
7	12.334	283537	1.42	83375	2.24	N-propyl N-valerate	103.10
8	13.448	287107	1.44	64957	1.75	Nitro-tert-butyl-acetate	55.05
9	13.903	753560	3.78	294541	7.93	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.10
10	16.666	684615	3.44	116775	3.14	4-Penten-1-ol, trimethylsilyl ether	142.10
11	22.073	5782932	29.02	511698	13.77	Chinasaure	60.05
12	27.850	171879	0.86	72117	1.94	.alpha.-Copaen-11-ol	59.05
13	28.489	378048	1.90	155118	4.18	Methyl isopalmitate	74.05
14	28.914	1852165	9.29	334386	9.00	3,4-O-Isopropylidene-d-galactose	73.05
15	29.168	603782	3.03	163526	4.40	Hexadecanoic acid	73.05
16	29.770	2075533	10.42	331864	8.93	Glucal	73.05
17	31.715	360413	1.81	140887	3.79	9,12-Octadecadienoic acid, methyl ester	67.10
18	31.842	402740	2.02	129882	3.50	Methyl 8,11,14-heptadecatrienoate	79.10
19	40.876	834480	4.19	131724	3.55	.beta.-Sitosterol	57.05
20	42.251	350053	1.76	63899	1.72	Methyl commate C	218.25
21	49.192	1010387	5.07	124439	3.35	Friedelan-3-one	69.10
		19926702	100.00	3714851	100.00		

Appendix 19. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Purpureocillium lilacinum* x *Fusarium oxysporum* in Preventive treatments

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	7.733	5724470	3.13	688347	2.25	Alpha-furoic acid	112.10
2	7.913	7292881	3.99	1267442	4.14	Thymine	126.10
3	9.041	2312702	1.26	788752	2.58	Dimethyl dl-malate	103.05
4	9.667	16887887	9.23	3477345	11.35	Pyranone	144.05
5	10.801	3039890	1.66	620259	2.03	(S)-5-Hydroxymethyl-2[5H]-furanone	84.05
6	12.246	73135930	39.96	7938868	25.92	5-Hydroxymethylfurfural	97.10
7	28.495	4685300	2.56	1877254	6.13	Methylpalmitate	74.05
8	29.235	8371135	4.57	2419917	7.90	Hexadecanoic acid	73.05
9	30.572	22255113	12.16	1536070	5.02	Glucal	73.05
10	31.724	5110428	2.79	1996381	6.52	Methyl octadeca-9,12-dienoate	67.05
11	31.852	5580185	3.05	1552502	5.07	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	79.10
12	32.053	3637407	1.99	1347698	4.40	Phytol	71.05
13	32.458	6661663	3.64	1675926	5.47	11,14-Eicosadienoic acid, methyl ester	67.10
14	32.593	8896978	4.86	1720982	5.62	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
15	38.706	1870924	1.02	569282	1.86	Glycerol .beta.-palmitate	57.05
16	40.897	3960017	2.16	489234	1.60	.gamma.-Sitosterol	55.05
17	41.423	3582343	1.96	660150	2.16	Beta-monolinolein	67.05
		183005253	100.00	30626409	100.00		

Appendix 20. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Purpureocillium lilacinum* x *Fusarium oxysporum* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.601	12024345	1.28	2764782	1.83	3-Hydroxy-2,3-dihydromaltol	144.00
2	9.749	53240593	5.66	3701959	2.44	Pyranone	144.00
3	11.416	25873182	2.75	1322064	0.87	1,2-Benzenediol	110.05
4	12.241	88596464	9.43	6197848	4.09	5-Hydroxymethylfurfural	97.00
5	13.983	20284527	2.16	2457831	1.62	-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.00
6	15.528	26416023	2.81	2050390	1.35	Phenol, 2,6-Dimethoxy-	154.05
7	16.769	22066740	2.35	2505573	1.65	Benzene, 1-chloro-4-methoxy-	142.00
8	23.724	12443174	1.32	2028615	1.34	.beta.-D-Glucopyranoside, methyl	60.00
9	24.027	13688077	1.46	1469997	0.97	Chinasaure	194.00
10	24.912	23805047	2.53	6904743	4.56	-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
11	28.524	21221124	2.26	7224846	4.77	Methylpalmitate	74.00
12	29.077	17536027	1.87	3581919	2.36	trans-13-Octadecenoic acid	55.05
13	29.360	85999710	9.15	14861741	9.81	Hexadecanoic acid	73.00
14	29.600	29248337	3.11	1767517	1.17	3,4-O-Isopropylidene-d-galactose	59.00
15	30.086	11265210	1.20	3545299	2.34	Benzenepropanoic acid, 2,5-dimethoxy-	167.05
16	31.759	19493233	2.07	7539635	4.98	Linoleic acid, methyl ester	67.00
17	31.885	21733532	2.31	6228287	4.11	Methyl linolenate	79.05
18	32.088	18452403	1.96	5904588	3.90	Phytol	71.00
19	32.571	66533576	7.08	10948622	7.23	Oxacycloheptadec-8-EN-2-one	67.00
20	32.694	53267845	5.67	7689363	5.08	,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
21	38.765	44114449	4.69	13291860	8.77	Glycerol .beta.-palmitate	57.00
22	41.201	115170667	12.25	9925711	6.55	.beta.-Sitosterol	55.05
23	41.482	68594351	7.30	13863806	9.15	.beta.-Monolinolein	67.00
24	41.614	23946584	2.55	5787490	3.82	Butyl 6,9,12-hexadecatrienoate	67.00
25	42.575	10433041	1.11	1198285	0.79	Cycloeucalenyl acetate	55.05
26	46.228	11761717	1.25	1459730	0.96	.alpha.-Tocopheryl acetate	165.10
27	48.576	22802505	2.43	5263279	3.47	Vitamin E	165.10
		940012483	100.00	151485780	100.00		

Appendix 21. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma harzianum* x *Athelia rolfsii* in Preventive treatments

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	7.664	3339403	5.70	708357	6.48	Thymine	126.05
2	9.029	680098	1.16	234321	2.14	Dimethyl dl-malate	103.00
3	9.547	2365489	4.03	678120	6.20	pyranone	144.00
4	11.976	1464457	2.50	349220	3.20	5-Hydroxymethylfurfural	97.00
5	12.371	2226380	3.80	537146	4.91	Dimethyl dl-malate	103.05
6	13.901	802699	1.37	341265	3.12	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.00
7	22.423	15636218	26.67	974409	8.92	Chinasaure	60.00
8	24.796	979706	1.67	318012	2.91	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
9	27.849	1434094	2.45	512572	4.69	Alpha-copaen-11-OL	59.05
10	28.483	1213227	2.07	509488	4.66	Methyl palmitate	74.05
11	29.028	2163576	3.69	318748	2.92	3,4-O-Isopropylidene-d-galactose	73.00
12	29.191	3784451	6.45	1209406	11.07	Hexadecanoic acid	73.00
13	29.985	12944607	22.08	1508709	13.80	Glucal	73.00
14	31.710	1204387	2.05	493341	4.51	Methyl 9,12-octadecadienoate	67.05
15	31.830	1035749	1.77	263778	2.41	Linolenic acid, methyl ester	79.05
16	32.043	985871	1.68	457986	4.19	Phytol	71.05
17	32.419	1722032	2.94	535350	4.90	9,12-Octadecadienoic acid	67.05
18	32.549	2780549	4.74	532391	4.87	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
19	38.689	650782	1.11	211637	1.94	Glycerol .beta.-palmitate	57.05
20	41.404	1222116	2.08	235324	2.15	Z,Z-8,10-Hexadecadien-1-ol	67.05
		58635891	100.00	10929580	100.00		

Appendix 22. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma harzianum* x *Athelia rolfsii* in Curative treatments

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	7.721	4629458	6.75	728661	6.72	Thymine	126.10
2	9.301	499762	0.73	175884	1.62	Diethylnitrosamide	56.05
3	9.566	3579100	5.22	1119877	10.33	Pyranone	144.10
4	11.682	2140602	3.12	509846	4.70	2,3-Dihydro-benzofuran	120.10
5	11.803	2550804	3.72	434236	4.00	Methyl 3-hydroxycaproate	61.05
6	11.883	1800216	2.62	397755	3.67	Butanedioic acid, hydroxy-	89.05
7	12.007	9156563	13.34	1650160	15.21	5-Hydroxymethylfurfural	97.05
8	12.428	3749274	5.46	504113	4.65	Ethanol, 2,2-diethoxy-	103.05
9	13.816	2029255	2.96	302596	2.79	Pimelic acid-carboxy-D2	55.05
10	22.573	17585508	25.63	933600	8.61	Chinasaure	60.05
11	28.489	977361	1.42	400037	3.69	Methylpalmitate	74.05
12	29.058	1261276	1.84	188413	1.74	3,4-O-Isopropylidene-d-galactose	73.05
13	29.185	2286222	3.33	733026	6.76	Hexadecanoic acid	73.05
14	30.024	9999968	14.57	959121	8.84	1,5-Anhydro-d-mannitol	73.05
15	31.717	1008938	1.47	387657	3.57	9,12-Octadecadienoic acid, methyl ester	67.05
16	31.846	1137987	1.66	312657	2.88	Linolenic acid methyl ester	79.10
17	32.048	567271	0.83	205952	1.90	Phytol, acetate	71.10
18	32.415	1083308	1.58	339922	3.13	9,12-Octadecadienoic acid, methyl ester, (E,E)-	67.10
19	32.547	1913917	2.79	422495	3.90	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.10
20	48.494	662992	0.97	140025	1.29	dl-.alpha.-Tocopherol	165.15
		68619782	100.00	10846033	100.00		

Appendix 23. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma harzianum* x *Fusarium oxysporum* in Preventive treatments

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	7.788	11244272	5.75	1211097	4.03	Thymine	126.05
2	9.031	1465880	0.75	491491	1.63	Dimethyl dl-malate	103.05
3	9.592	8765828	4.48	2221209	7.38	Pyranone	144.05
4	11.875	4589425	2.35	491027	1.63	Gamma.-decalactone	85.05
5	12.092	46726947	23.88	5850578	19.44	5-Hydroxymethylfurfural	97.05
6	12.478	5860963	3.00	509637	1.69	Glycolaldehyde diethyl acetal	103.05
7	22.508	10026266	5.12	677234	2.25	Methyl .alpha.-D-glucoside	60.00
8	22.717	6139974	3.14	945064	3.14	1,6-Anhydro-.alpha.-d-galactofuranose	73.05
9	23.083	19639331	10.04	1285927	4.27	Chinasaure	60.00
10	27.854	1431917	0.73	468444	1.56	.alpha.-Copaene-11-ol	59.00
11	28.483	3871659	1.98	1424407	4.73	Methylpalmitate	74.05
12	29.224	18846303	9.63	3525487	11.72	Hexadecanoic acid	73.05
13	30.275	15128100	7.73	1284691	4.27	Glucal	73.05
14	31.711	3334474	1.70	1258574	4.18	Linoleic acid, methyl ester	67.05
15	31.837	3602541	1.84	928300	3.09	Alpha-linolenic acid methyl ester	79.10
16	32.043	4022876	2.06	1325154	4.40	Phytol	71.05
17	32.441	6175564	3.16	1585743	5.27	11,14-Eicosadienoic acid, methyl ester	67.05
18	32.580	11595431	5.93	2007902	6.67	alpha.-Linolenic acid	79.10
19	32.900	2491070	1.27	461669	1.53	9-Octadecenoic acid (Z)-	73.05
20	38.693	2596053	1.33	702233	2.33	Glycerol .beta.-palmitate	57.05
21	40.868	3302848	1.69	439927	1.46	.beta.-Sitosterol	81.10
22	41.409	3358185	1.72	665467	2.21	beta.-Monolinolein	67.05
23	41.541	1441463	0.74	327275	1.09	cis,cis,cis-7,10,13-Hexadecatrienal	79.05
		195657370	100.00	30088537	100.00		

Appendix 24. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma harzianum* x *Fusarium oxysporum* in Curative treatments

Peak #	R.Time	Area	Area %	Height	Height %	Name	Base m/z
1	7.882	11320585	2.54	746775	0.80	Thymine	126.05
2	9.694	24850955	5.58	1833780	1.97	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.00
3	12.107	14128279	3.17	1439998	1.55	5-Hydroxymethylfurfural	97.00
4	16.728	16452006	3.69	2219923	2.39	Quinaldine N-oxide	142.05
5	23.003	8145687	1.83	1616463	1.74	beta.-Methylglucoside	60.00
6	24.867	11012973	2.47	3792063	4.08	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
7	25.580	2821732	0.63	852352	0.92	Loliolide	111.05
8	27.905	14481401	3.25	4981059	5.36	Alpha.-copaen-11-ol	59.00
9	28.300	3365533	0.76	720367	0.78	Methyl palmitoleate	55.00
10	28.519	15070987	3.38	5713474	6.15	Methylpalmitate	74.00
11	29.058	10803970	2.43	2695699	2.90	Myristoleic acid	55.05
12	29.314	48803604	10.96	10128208	10.91	Hexadecanoic acid	73.00
13	30.046	5100703	1.15	1705145	1.84	Benzenepropanoic acid, 2,5-dimethoxy-	167.05
14	30.650	11002850	2.47	1290811	1.39	1,5-Anhydro-d-mannitol	73.00
15	31.751	11861421	2.66	4713630	5.08	Methyl octadeca-9,12-dienoate	67.00
16	31.870	11920420	2.68	3019416	3.25	9,12,15-Octadecatrienoic acid, methyl ester	79.05
17	32.081	14497587	3.26	4939599	5.32	Phytol	71.00
18	32.520	27025876	6.07	5168069	5.57	Methyl-11,14-eicosadienoate	67.00
19	32.618	23603282	5.30	3992498	4.30	7-Tetradecenal, (Z)-	55.05
20	35.975	2586434	0.58	776382	0.84	Linoleic acid chloride	57.00
21	37.416	18130429	4.07	1395055	1.50	Stigmasterol	55.05
22	38.466	4142710	0.93	1581011	1.70	Methyl 5,11,14-eicosatrienoate	67.00
23	38.742	21676609	4.87	6972000	7.51	Glycerol .beta.-palmitate	57.05
24	40.059	4891945	1.10	1852459	1.99	4-t-Butyl-2-[4-nitrophenyl]phenol	256.05
25	41.199	58516358	13.14	7279643	7.84	.gamma.-Sitosterol	55.05
26	41.454	27089017	6.08	6036292	6.50	beta.-Monolinolein	67.00
27	41.590	7613099	1.71	1856781	2.00	Butyl 6,9,12-hexadecatrienoate	79.05
28	48.560	14394338	3.23	3547933	3.82	Vitamin E	165.10
		445310790	100.0	92866885	100.00		

Appendix 25. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma koningii* x *Athelia rolfsii* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.688	4448066	5.55	896611	5.26	Thymine	126.05
2	9.037	1058641	1.32	392892	2.30	Dimethyl dl-malate	103.05
3	9.558	2717977	3.39	891524	5.23	Pyranone	144.00
4	11.996	3610304	4.51	747084	4.38	5-Hydroxymethylfurfural	97.00
5	12.393	2190454	2.73	503312	2.95	Ethanol, 2,2-diethoxy-	103.00
6	13.912	857567	1.07	349565	2.05	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.05
7	22.482	9753158	12.17	924317	5.42	Chinasaure	60.00
8	24.810	728732	0.91	233476	1.37	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
9	28.490	3066098	3.83	1130281	6.63	Methylpalmitate	74.05
10	28.983	1997650	2.49	344493	2.02	trans-13-Octadecenoic acid	55.05
11	29.204	7250581	9.05	2165456	12.70	Hexadecanoic acid	73.05
12	29.985	12203647	15.23	1359973	7.97	Glucal	73.00
13	31.719	2501796	3.12	990677	5.81	Methyl octadeca-9,12-dienoate	67.05
14	31.846	2793525	3.49	788513	4.62	AAlpha-linolenic acid methyl ester	79.05
15	32.049	2368306	2.96	893955	5.24	Phytol	71.05
16	32.433	4027346	5.03	1157659	6.79	11,14-Eicosadienoic acid, methyl ester	67.05
17	32.567	6588934	8.22	1340550	7.86	,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.10
18	32.900	880906	1.10	289143	1.70	Z,Z-8,10-Hexadecadien-1-ol	55.05
19	38.696	1534134	1.91	492443	2.89	Glycerol .beta.-palmitate	57.05
20	40.906	7859976	9.81	767726	4.50	.beta.-Sitosterol	57.05
21	41.410	1689472	2.11	397209	2.33	beta.-Monolinolein	67.05
		80127270	100.00	17056859	100.00		

Appendix 26. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma koningii* x *Athelia rolfsii* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.595	551192	3.14	165527	4.50	Thymine	126.05
2	9.032	475782	2.71	158173	4.30	Dimethyl dl-malate	103.05
3	9.292	273077	1.55	34939	0.95	Ethanamine, N-ethyl-N-nitroso-	102.05
4	9.543	863088	4.91	210127	5.71	Pyranone	144.05
5	11.680	1211418	6.89	164328	4.47	2,2,7,7-Tetraadeutero-hexamethylene sulphide	71.00
6	11.978	521830	2.97	156489	4.25	5-Hydroxymethylfurfural	97.05
7	12.336	445392	2.53	130763	3.55	Ethanol, 2,2-diethoxy-	103.05
8	13.471	306503	1.74	73921	2.01	Heptanoic acid, 6-oxo-	55.00
9	16.657	816622	4.65	98323	2.67	Benzene, 1-chloro-2-methoxy-	142.05
10	22.056	4389750	24.98	443271	12.05	Chinasaure	60.00
11	28.494	934849	5.32	376080	10.22	methyl palmitate	74.05
12	29.182	1181051	6.72	407083	11.06	Hexadecanoic acid	73.00
13	29.658	242999	1.38	78178	2.12	1H-indene, 3-methyl-	130.10
14	29.769	2330517	13.26	307502	8.36	Glucal	73.05
15	29.983	391861	2.23	82616	2.25	3-Phenylbutanal	149.05
16	31.723	708657	4.03	280805	7.63	Methyl octadeca-9,12-dienoate	67.05
17	31.845	622845	3.54	158019	4.29	Methyl linolenate	79.10
18	32.053	457690	2.60	131875	3.58	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	71.05
19	32.414	351312	2.00	126047	3.43	Methyl octadeca-9,12-dienoate	67.05
20	32.537	497868	2.83	95435	2.59	cis,cis,cis-7,10,13-Hexadecatrienal	79.05
		17574303	100.00	3679501	100.00		

Appendix 27. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma koningii* x *Fusarium oxysporum* in Preventive treatments

Peak#	R.Time	Area	Area %	Height	Height%	Name	Base m/z
1	7.629	2280673	3.95	534236	6.24	Thymine	126.05
2	9.539	1339014	2.32	435748	5.09	Pyranone	144.05
3	11.667	3278592	5.68	280453	3.28	2,2,7,7-Tetraadeutero-hexamethylene sulphide	85.05
4	11.973	4246018	7.36	817351	9.55	5-Hydroxymethylfurfural	97.05
5	12.360	1201785	2.08	291689	3.41	Methyl 3-(acetyloxy)-3-hydroxypropanoate	103.05
6	22.393	18213771	31.55	1002019	11.70	Chinasaure	60.05
7	24.794	489007	0.85	152550	1.78	Gamma.-Hydroxyisoeugenol	137.10
8	27.852	1334906	2.31	488475	5.71	Alpha-copaen-11-ol	59.05
9	28.489	1058450	1.83	429596	5.02	Methylpalmitate	74.05
10	29.023	1348208	2.34	226139	2.64	Trehalose	73.05
11	29.191	2913300	5.05	891534	10.41	Hexadecanoic acid	73.05
12	29.932	6704782	11.62	688288	8.04	Glucal	73.05
13	31.719	1712422	2.97	345318	4.03	9,12-Octadecadienoic acid, methyl ester	67.05
14	32.050	1255980	2.18	466041	5.44	Phytol, acetate	71.05
15	32.558	4636641	8.03	649321	7.58	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
16	38.698	502172	0.87	160196	1.87	Glycerol .beta.-palmitate	57.05
17	40.868	4556511	7.89	560778	6.55	.beta.-Sitosterol	55.05
18	41.411	652673	1.13	141570	1.65	Beta-monolinolein	67.05
		57724905	100.00	8561302	100.00		

Appendix 28. Bio-chemical compounds in the methanol extract of mahogany seedlings treated with *Trichoderma koningii* x *Fusarium oxysporum* in Curative treatments

Peak #	R.Time	Area	Area %	Height	Height %	Name	Base m/z
1	9.618	17270837	5.58	1139636	1.88	3-Hydroxy-2,3-dihydromaltol	144.00
2	12.040	19541788	6.31	2110740	3.48	5-Hydroxymethylfurfural	97.00
3	13.936	3421039	1.11	517520	0.85	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.05
4	15.505	7008577	2.26	786001	1.30	Phenol, 2,6-dimethoxy-	154.00
5	16.676	7631483	2.47	1082490	1.78	Quinoline, 8-hydrazino-	142.05
6	22.706	8373130	2.70	1293205	2.13	.beta.-D-Glucopyranoside, methyl	60.00
7	22.908	9351534	3.02	1074466	1.77	1,6-Anhydro-.beta.-D-gluco furanose	73.00
8	22.983	8591200	2.78	1206462	1.99	1,7,7-Trimethyl bicyclo [2.2.1]Heptane-2,5-dione	170.10
9	23.132	17771473	5.74	1628838	2.68	Chinasaure	60.00
10	23.317	4467525	1.44	895570	1.48	Cis-p-Mentha-2,8-dien-1-ol	190.05
11	24.820	8604551	2.78	2735296	4.51	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
12	28.505	7982152	2.58	3096531	5.10	Methylpalmitate	74.00
13	29.013	3617685	1.17	911575	1.50	trans-13-Octadecenoic acid	55.05
14	29.265	37283625	12.04	6290341	10.37	Hexadecanoic acid	73.00
15	30.007	5417878	1.75	1956832	3.22	Benzenepropanoic acid, 2,5-dimethoxy-	167.10
16	30.325	15039459	4.86	1284058	2.12	Glucal	73.00
17	30.437	6792222	2.19	1256018	2.07	3-O-Methyl-d-glucose	73.00
18	30.575	3242075	1.05	368710	0.61	Cyclohexyl decanoate	55.00
19	31.737	7776232	2.51	3031967	5.00	Methyl octadeca-9,12-dienoate	67.05
20	31.863	7250348	2.34	2190718	3.61	Linolenic acid, methyl ester	79.05
21	32.066	5995049	1.94	2003106	3.30	Phytol	71.05
22	32.292	3483411	1.13	1198262	1.97	3-O-Methyl-d-glucose	73.00
23	32.475	11695748	3.78	2587272	4.26	Methyl 11,14-Eicosadienoate	67.00
24	32.598	11527730	3.72	1815985	2.99	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
25	35.941	2310323	0.75	655066	1.08	Methyl palmitoleate	55.05
26	38.381	2946469	0.95	1166706	1.92	1-Heneicosanol	57.05
27	38.451	3656502	1.18	1313946	2.17	Methyl 5,11,14-eicosatrienoate	67.05
28	38.720	17026832	5.50	5109544	8.42	Glycerol .beta.-palmitate	57.05
29	41.434	21884015	7.07	5008699	8.25	beta.-Monolinolein	67.05
30	41.570	8005306	2.59	1730413	2.85	Butyl 9,12,15-octadecatrienoate	79.05
31	41.800	2279704	0.74	556980	0.92	Octadecanoic acid, 2,3-dihydroxypropyl ester	98.10
32	48.530	12298769	3.97	2684189	4.42	Vitamin E	165.10
		309544671	100.0	60687142	100.00		

Appendix 29. Bio-chemical compounds in the methanol extract of healthy teak seedlings

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	9.517	732116	2.19	269950	3.13	3-Hydroxy-2,3-dihydromaltol	144.00
2	11.326	1879847	5.64	613525	7.10	2-Methylindoline	118.05
3	21.749	1196333	3.59	203147	2.35	Chinasaure	60.00
4	28.509	3440464	10.31	1441660	16.69	Methyl palmitate	74.00
5	29.199	3046095	9.13	958256	11.09	Hexadecanoic acid	73.00
6	29.333	715391	2.14	122068	1.41	Dibutyl phthalate	149.00
7	30.442	145993	0.44	59987	0.69	Methyl stearate	74.00
8	31.741	6843325	20.51	1376223	15.93	Linoleic acid, methyl ester	67.05
9	32.070	3481562	10.44	1116402	12.93	Phytol	71.05
10	32.203	433884	1.30	131268	1.52	Beta.-methylanthraquinone	222.00
11	32.293	1528250	4.58	611805	7.08	Methyl stearate	74.00
12	32.437	1427805	4.28	448967	5.20	9,12-Octadecadienoic acid	67.00
13	32.558	2336348	7.00	407362	4.72	cis,cis,cis-7,10,13-Hexadecatrienal	79.10
14	32.925	485598	1.46	129176	1.50	Octadecanoic acid	57.00
15	35.765	269170	0.81	104980	1.22	Methyl 18-methylnonadecanoate	74.05
16	39.008	208554	0.63	75991	0.88	Hexadecanal	82.05
17	40.953	4767600	14.29	431849	5.00	.gamma.-Sitosterol	57.05
18	43.368	421551	1.26	134843	1.56	Squalene	69.05
		33359886	100.00	8637459	100.00		

Appendix 30. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Fusarium oxysporum*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	11.419	16688385	3.01	2479308	1.74	2-Methylindoline	118.10
2	17.822	8778884	1.58	1391353	0.98	2,4-Cresotaldehyde	136.10
3	18.631	5199269	0.94	1246578	0.88	4-Chromanol	150.05
4	24.844	10874335	1.96	3639448	2.56	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
5	28.526	40072422	7.22	15316312	10.78	Methylpalmitate	74.00
6	29.323	69998458	12.62	12201733	8.58	Hexadecanoic acid	73.00
7	30.462	4029522	0.73	1243598	0.87	5-Formylsalicylaldehyde	150.00
8	31.761	100165717	18.06	18161808	12.78	Linoleic acid, methyl ester	67.00
9	31.952	3382882	0.61	1598609	1.12	cis-Vaccenic acid methyl ester	55.05
10	32.092	53801326	9.70	18376994	12.93	Phytol	71.05
11	32.219	3539600	0.64	1090024	0.77	beta.-Methylanthraquinone	222.00
12	32.309	25466811	4.59	9973578	7.02	Methyl stearate	74.00
13	32.558	61511420	11.09	9906506	6.97	Methyl 11,14-eicosadienoate	67.00
14	32.651	35862620	6.47	9641524	6.78	Octadec-9-enoic acid	55.05
15	32.692	16518622	2.98	8665910	6.10	alpha.-Linolenic acid	79.05
16	32.997	18251653	3.29	5099193	3.59	Octadecanoic acid	57.05
17	35.775	6763457	1.22	2708021	1.91	Methyl arachate	74.00
18	38.735	16135753	2.91	5329551	3.75	Glycerol .beta.-palmitate	57.05
19	41.456	28883284	5.21	6523561	4.59	beta.-Monolinolein	67.05
20	41.591	15945543	2.87	3852363	2.71	Butyl 9,12,15-octadecatrienoate	79.05
21	41.821	5433193	0.98	1304385	0.92	Glyceryl 1-monostearate	57.05
22	43.369	4067956	0.73	1530870	1.08	Squalene	69.05
23	48.549	3348176	0.60	863216	0.61	Vitamin E	165.10
		554719288	100.00	142144443	100.00		

Appendix 31. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Athelia rolfsii*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	24.806	3349450	1.08	1249019	1.55	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
2	28.517	26165294	8.47	10937537	13.61	Methyl palmitate	74.00
3	29.263	21999837	7.12	5429264	6.76	Hexadecanoic acid	73.00
4	30.451	3013362	0.98	1156769	1.44	Methyl octacosanoate	150.00
5	31.750	33361223	10.80	12967758	16.14	Linoleic acid, methyl ester	67.00
6	31.855	24955860	8.08	6388151	7.95	Methyl petroselinate	55.05
7	31.945	2999656	0.97	1239885	1.54	14-Octadecenoic acid, methyl ester	55.05
8	32.074	14811781	4.80	4998181	6.22	Phytol	71.00
9	32.215	2501837	0.81	775844	0.97	Techtoquinone	222.00
10	32.303	18685037	6.05	7403499	9.21	Methyl stearate	74.00
11	32.493	16143349	5.23	3614331	4.50	Methyl 11,14-eicosadienoate	67.05
12	32.584	12884686	4.17	2191354	2.73	7-Tetradecenal, (Z)-	55.05
13	32.953	2868772	0.93	948941	1.18	Octadecanoic acid	57.05
14	35.772	7938348	2.57	3029696	3.77	Methyl arachate	74.00
15	35.914	1990613	0.64	612499	0.76	Methyl podocarpate	288.10
16	37.410	6581259	2.13	1092946	1.36	Methyl 14-methyl-eicosanoate	74.05
17	38.719	2922657	0.95	1150623	1.43	Glycerol .beta.-palmitate	57.05
18	38.976	2734245	0.89	1020895	1.27	Docosanoic acid, methyl ester	74.05
19	40.917	9572371	3.10	1400281	1.74	4,4-Dimethylandrost-5-en-17-ol	302.20
20	41.166	73873608	23.92	7743804	9.64	.gamma.-Sitosterol	55.05
21	41.434	10114150	3.27	2120721	2.64	Beta-monolinolein	67.05
22	41.568	3517422	1.14	878637	1.09	Butyl 9,12,15-octadecatrienoate	79.05
23	42.724	5856848	1.90	1997684	2.49	9-Octadecenamide	59.00
		308841665	100.00	80348319	100.00		

Appendix 32. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Clonostachys rosea*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.639	1428016	2.66	369037	2.07	Thymine	126.05
2	11.335	5115450	9.53	1690791	9.50	2-Methylindoline	118.10
3	12.467	1350236	2.52	451515	2.54	Cyclopentaneacetic acid, 2-(hydroxymethyl)-3-methyl-, .delta.-lactone	67.05
4	22.124	2336811	4.35	322424	1.81	Chinasaure	60.00
5	24.799	1121071	2.09	406168	2.28	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
6	28.494	4873080	9.08	2071628	11.64	Methyl palmitate	74.05
7	29.200	2100011	3.91	681066	3.83	Hexadecanoic acid	73.00
8	30.444	864294	1.61	298810	1.68	5-Formylsalicylaldehyde	149.00
9	30.806	602235	1.12	210146	1.18	5-Methylorcylaldehyde	165.00
10	31.726	5909853	11.01	2320993	13.04	9,12-Octadecadienoic acid, methyl ester	67.05
11	31.859	12032857	22.42	4034613	22.67	Alpha-linolenic acid methyl ester	79.05
12	31.933	464040	0.86	197444	1.11	11-Octadecenoic acid, methyl ester, (Z)-	55.05
13	32.058	6467332	12.05	2241526	12.59	Phytol	71.05
14	32.282	3052973	5.69	1024267	5.75	Methyl stearate	74.05
15	32.438	895120	1.67	226181	1.27	2-Chloroethyl linoleate	67.05
16	32.570	2336852	4.35	531399	2.99	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
17	38.704	557199	1.04	197352	1.11	Glycerol .beta.-palmitate	57.05
18	41.423	1310287	2.44	284118	1.60	beta.-Monolinolein	67.05
19	41.557	863396	1.61	239824	1.35	Ethyl 9,12,15-octadecatrienoate	79.05
		53681113	100.00	17799302	100.00		

Appendix 33. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Penicillium multicolor*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.631	1636846	3.30	316311	2.04	Thymine	126.00
2	9.550	563658	1.14	163548	1.06	pyranone	144.00
3	11.337	4715859	9.51	1405973	9.08	2-Methylindoline	118.05
4	11.992	1941116	3.91	432515	2.79	5-Hydroxymethylfurfural	97.00
5	12.472	673168	1.36	224493	1.45	7A-Methylhexahydro-1-benzofuran-2(3H)-ONE #	67.05
6	24.809	758834	1.53	242981	1.57	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.00
7	28.505	3600198	7.26	1474110	9.52	Methyl palmitate	74.00
8	29.208	2864584	5.78	1020073	6.59	Hexadecanoic acid	73.00
9	31.735	6373541	12.85	2533739	16.37	9,12-Octadecadienoic acid, methyl ester	67.05
10	31.865	9887941	19.94	3100711	20.03	Alpha-linolenic acid methyl ester	79.05
11	32.065	3546839	7.15	1113430	7.19	Phytol	71.05
12	32.203	1241562	2.50	264738	1.71	9,10-Anthracenedione, 2-methyl-	221.95
13	32.288	1450624	2.93	566225	3.66	Methyl stearate	74.00
14	32.442	1905894	3.84	559428	3.61	Methyl linolelaidate	67.05
15	32.576	3594954	7.25	852421	5.51	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
16	34.392	958282	1.93	347460	2.24	2-Hydroxy-3-[.beta.-iodo-.beta.-isopropylvinyl]-1,4-naphthoquinone	240.95
17	38.715	678198	1.37	221978	1.43	Glycerol .beta.-palmitate	57.05
18	41.425	899933	1.81	234971	1.52	.beta.-Monolinolein	67.05
19	49.299	2300104	4.64	404214	2.61	Friedelan-3-one	69.05
		49592135	100.00	15479319	100.00		

Appendix 34. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Purpureocillium lilacinum*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	11.349	1022212	3.56	323975	3.15	2-Methylindoline	118.15
2	17.796	1250672	4.36	397430	3.86	2,6-Cresotaldehyde	136.15
3	24.797	850445	2.96	312168	3.03	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	91.10
4	28.491	2557759	8.91	1082657	10.52	Methyl palmitate	74.10
5	29.211	1271324	4.43	441499	4.29	Pentadecanoic acid	73.10
6	31.723	4586120	15.98	1865357	18.12	Methyl linolelaidate	67.05
7	31.855	9463281	32.97	3350460	32.55	Alpha-linolenic acid methyl ester	79.10
8	32.053	5102327	17.78	1697097	16.49	Isophytol, acetate	71.10
9	32.275	1515653	5.28	496145	4.82	Methyl stearate	74.05
10	32.582	1079989	3.76	327802	3.18	Alpha-linolenic acid methyl ester	79.10
		28699782	100.00	10294590	100.00		

Appendix 35. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma harzianum*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	11.359	1084428	6.09	314011	6.53	2-Methylindoline	118.05
2	11.983	326839	1.83	42428	0.88	1,3-Cyclopentanedimethanol	66.95
3	12.242	295100	1.66	63155	1.31	tert-Butyldimethylsilyl acetate	117.05
4	12.475	335949	1.89	110226	2.29	13-Tetradece-11-yn-1-ol	67.05
5	17.700	3182126	17.86	151843	3.16	Guanosine	57.00
6	21.157	471707	2.65	77673	1.62	.Alpha.-D-glucopyranoside, methyl	60.00
7	21.542	318561	1.79	87477	1.82	2-Oxovaleric acid, tert-butyldimethylsilyl ester	75.00
8	21.815	435904	2.45	90746	1.89	Chinasaure	60.00
9	24.803	250806	1.41	86386	1.80	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
10	28.501	1433965	8.05	574952	11.96	Methyl palmitate	74.05
11	31.733	2506758	14.07	1018788	21.20	Methyl octadeca-9,12-dienoate	67.05
12	31.861	3548075	19.91	1037775	21.60	Linolenic acid, methyl ester	79.05
13	32.064	1349678	7.57	449634	9.36	Phytol	71.05
14	32.203	233214	1.31	75213	1.57	9,10-Anthracenedione, 2-methyl-	165.05
15	32.290	565869	3.18	228241	4.75	Methyl stearate	74.00
16	32.481	214774	1.21	76968	1.60	1-Ethylsulfanylmethyl-2,8,9-trioxa-5-aza-1-sila-bicyclo[3.3.3]undecane	174.05
17	38.712	281411	1.58	92049	1.92	Glycerol .beta.-palmitate	57.00
18	41.433	717342	4.03	150317	3.13	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	67.05
19	41.560	266262	1.49	77696	1.62	Ethyl linolate	79.10
		17818768	100.00	4805578	100.00		

Appendix 36. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma koningii*

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.663	2134447	2.81	572435	2.28	Thymine	126.00
2	9.554	994350	1.31	322807	1.29	Pyranone	144.00
3	11.339	6442760	8.47	1973444	7.86	2-Methylindoline	118.10
4	11.983	1539351	2.02	361337	1.44	5-Hydroxymethylfurfural	97.00
5	12.471	925838	1.22	316916	1.26	7-Methylhexahydrocyclopenta[C]pyran-3(1H)-ONE #	67.05
6	17.814	1769051	2.33	522678	2.08	2,4-Cresotaldehyde	136.10
7	24.809	1919724	2.52	618488	2.46	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
8	28.502	7759959	10.20	3154889	12.57	Methyl palmitate	74.05
9	29.215	5201345	6.84	1686280	6.72	Hexadecanoic acid	73.00
10	31.732	9207818	12.11	3611679	14.39	Methyl octadeca-9,12-dienoate	67.05
11	31.859	10321250	13.57	2997437	11.94	Linolenic acid, methyl ester	79.05
12	32.064	6641873	8.73	2274194	9.06	Phytol	71.05
13	32.202	2817721	3.71	902757	3.60	9,10-Anthracenedione, 2-methyl-	165.05
14	32.288	4198657	5.52	1614941	6.44	Methyl stearate	74.00
15	32.449	3181201	4.18	923723	3.68	11,14-Eicosadienoic acid, methyl ester	67.05
16	32.580	4663558	6.13	979427	3.90	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
17	32.924	897021	1.18	349852	1.39	Octadecanoic acid	73.05
18	33.890	810726	1.07	276339	1.10	1-Hydroxy-4-methylanthraquinone	237.95
19	34.387	1865719	2.45	604108	2.41	2-Hydroxy-3-[.beta.-iodo-.beta.-isopropylvinyl]-1,4-naphthoquinone	240.95
20	35.757	915536	1.20	333516	1.33	Methyl 18-methylnonadecanoate	74.05
21	35.901	1008864	1.33	377992	1.51	Methyl podocarpate	288.05
22	38.711	834970	1.10	318754	1.27	Glycerol .beta.-palmitate	57.05
		76051739	100.00	25093993	100.00		

Appendix 37. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Clonostachys rosea* x *Athelia rolfsii* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.872	12429189	4.40	1316818	1.92	Thymine	126.05
2	9.631	14346094	5.08	2892442	4.21	Pyranone	144.00
3	11.402	35152406	12.45	8494275	12.37	2-Methylindoline	118.10
4	12.169	40450949	14.33	4643428	6.76	5-Hydroxymethylfurfural	97.05
5	12.511	15464767	5.48	3308395	4.82	-Dimethyl(prop-2-enyl)silyloxypropane	75.05
6	17.867	3455026	1.22	1125726	1.64	2,4-Cresotaldehyde	136.10
7	18.659	3242011	1.15	1152491	1.68	4-Chromanol	150.05
8	24.853	3447361	1.22	1075803	1.57	-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
9	28.508	12622700	4.47	5241758	7.63	Methyl palmitate	74.05
10	29.264	19713004	6.98	4942356	7.20	Hexadecanoic acid	73.05
11	31.741	16714919	5.92	6771482	9.86	,12-Octadecadienoic acid, methyl ester	67.05
12	31.872	23088053	8.18	7061814	10.28	Linolenic acid, methyl ester	79.05
13	32.072	14354142	5.08	4308772	6.27	Phytol	71.05
14	32.290	4505641	1.60	1408299	2.05	Methyl stearate	74.05
15	32.480	11008174	3.90	2449782	3.57	1,14-Eicosadienoic acid, methyl Ester	67.05
16	32.631	23902820	8.46	4895399	7.13	,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
17	38.725	6194189	2.19	2169879	3.16	Glycerol .beta.-palmitate	98.10
18	41.445	12863401	4.56	2727109	3.97	beta.-Monolinolein	67.05
19	41.581	6911692	2.45	1895267	2.76	Butyl 9,12,15-octadecatrienoate	79.05
20	45.379	2506194	0.89	795479	1.16	-Propanone, 1-methyl-1-(2,4,6-trimethoxyphenyl)	195.05
		282372732	100.00	68676774	100.00		

Appendix 38. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Clonostachys rosea* x *Athelia rolfsii* in Curative treatments

Peak #	R.Time	Area	Area %	Height	Height %	Name	Base m/z
1	7.625	1256303	1.19	264916	1.20	Thymine	126.05
2	11.345	5181166	4.92	1203752	5.43	2-Methylindoline	118.10
3	11.992	11247883	10.69	1417217	6.40	Pentanoic acid, 3-hydroxy-4-methyl-, methyl ester	61.05
4	12.212	4621547	4.39	958259	4.33	Dimethyl 2-methoxysuccinate	75.05
5	13.917	3181603	3.02	1307667	5.90	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.05
6	16.133	1047531	1.00	322214	1.45	L-Proline, 5-oxo-, methyl ester	84.10
7	20.830	984523	0.94	204945	0.93	3-Hydroxy-4-methoxybenzoic acid	168.05
8	22.151	10912258	10.37	656882	2.97	Chinasaure	60.05
9	24.801	563686	0.54	188690	0.85	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
10	28.500	4041893	3.84	1655551	7.47	Methylpalmitate	74.05
11	29.209	4499565	4.27	1348325	6.09	Hexadecanoic acid	73.05
12	31.729	5198378	4.94	1962278	8.86	9,12-Octadecadienoic acid, methyl ester	67.05
13	31.854	7430178	7.06	2157176	9.74	Linolenic acid methyl ester	79.05
14	32.059	2940223	2.79	971347	4.38	Isophytol, acetate	71.05
15	32.195	1267923	1.20	381816	1.72	9,10-Anthracenedione, 2-methyl-	222.05
16	32.284	1879667	1.79	751016	3.39	Methyl stearate	74.05
17	32.438	2398043	2.28	700950	3.16	Oxacycloheptadec-8-en-2-one	67.05
18	32.577	4930175	4.68	1027883	4.64	.alpha.-Linolenic acid	79.10
19	32.919	915238	0.87	306505	1.38	Octadecanoic acid	73.05
20	38.708	1395912	1.33	484040	2.19	Glycerol .beta.-palmitate	57.05
21	38.800	669812	0.64	171524	0.77	2-(3-Hydroxyphenyl)-1H-indene-1,3(2H)-dione #	238.05
22	40.753	1132617	1.08	279332	1.26	Sugiol	285.20
23	40.908	7287126	6.92	600694	2.71	.beta.-Sitosterol	55.05
24	41.426	2557054	2.43	492913	2.23	beta.-Monolinolein	67.05
25	41.561	1788426	1.70	389205	1.76	Methyl (Z)-5,11,14,17-eicosatetraenoate	79.05
26	41.799	697794	0.66	150317	0.68	Glycerol .beta.-palmitate	57.05
27	42.318	1761153	1.67	200539	0.91	.beta.-Amyrin	218.15
28	43.351	578998	0.55	163518	0.74	Squalene	69.05
29	44.035	1778230	1.69	227301	1.03	Methyl commate C	218.15
30	49.304	11118194	10.56	1205235	5.44	Friedelan-3-one	69.10
		105263099	100.00	22152007	100.00		

Appendix 39. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Clonostachys rosea* x *Fusarium oxysporum* in Preventive treatments

Peak #	R.Time	Area	Area%	Height	Height %	Name	Base m/z
1	7.688	3765872	4.80	701890	3.88	Thymine	126.05
2	9.551	1599098	2.04	471464	2.61	Pyranone	144.05
3	11.342	5746679	7.33	1577031	8.72	2-Methylindoline	118.10
4	11.992	4735334	6.04	920774	5.09	5-Hydroxymethylfurfural	97.05
5	12.149	2309508	2.95	535556	2.96	Butanedioic acid, methoxy-, dimethyl ester	75.05
6	12.364	1061916	1.35	207414	1.15	3-Acetoxy-3-hydroxy-propionic acid methyl ester	103.05
7	12.472	1184629	1.51	302645	1.67	7-Methylhexahydrocyclopenta[C]pyran-3(1H)-one #	67.05
8	13.491	1176169	1.50	213446	1.18	2-Butoxy-4-methyl-[1,3,2]dioxaborinane	55.00
9	17.807	1001451	1.28	320698	1.77	2,4-Cresotaldehyde	136.10
10	22.369	14007185	17.86	950743	5.26	Chinasaure	60.00
11	24.806	1335232	1.70	424645	2.35	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
12	28.500	3390261	4.32	1430584	7.91	methyl palmitate	74.05
13	29.213	4753468	6.06	1422018	7.86	Hexadecanoic acid	73.00
14	31.728	4289650	5.47	1701870	9.41	9,12-Octadecadienoic acid, methyl ester	67.05
15	31.853	5448742	6.95	1651722	9.13	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	79.05
16	32.059	3341816	4.26	1129239	6.24	Phytol	71.05
17	32.194	745772	0.95	224079	1.24	9,10-Anthracenedione, 2-methyl-	222.00
18	32.281	1241371	1.58	503503	2.78	Methyl stearate	74.00
19	32.437	2010596	2.56	562715	3.11	Methyl linolelaidate	67.05
20	32.573	4407684	5.62	977371	5.40	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
21	32.914	760735	0.97	253631	1.40	Octadecanoic acid	73.05
22	38.707	1010623	1.29	341352	1.89	Glycerol .beta.-palmitate	57.10
23	40.890	2709307	3.46	370520	2.05	.beta.-Sitosterol	55.05
24	41.423	1576615	2.01	347353	1.92	.beta.-Monolinolein	67.05
25	49.265	4797931	6.12	547616	3.03	Friedelan-3-one	69.05
		78407644	100.00	18089879	100.00		

Appendix 40. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Clonostachys rosea* x *Fusarium oxysporum* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.636	1570859	4.25	402326	3.83	Thymine	126.05
2	9.555	648797	1.75	162651	1.55	pyranone	144.05
3	11.344	2712239	7.34	726542	6.91	2-Methylindoline	118.10
4	12.333	369749	1.00	125086	1.19	Ethanol, 2,2-diethoxy-	103.05
5	12.468	351604	0.95	127245	1.21	13-Tetradece-11-yn-1-ol	67.05
6	16.636	752168	2.03	260969	2.48	Para-chloroanisole	142.05
7	22.066	3263748	8.83	432264	4.11	Chinasaure	60.00
8	24.799	596227	1.61	193124	1.84	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
9	28.494	1674136	4.53	657952	6.26	Methyl palmitate	74.05
10	29.203	2698260	7.30	776272	7.38	Hexadecanoic acid	73.00
11	29.982	283323	0.77	85247	0.81	4-Allylphenol	210.10
12	31.726	3134173	8.48	1284642	12.22	Methyl octadeca-9,12-dienoate	67.05
13	31.856	4091641	11.07	1243639	11.83	Alpha-linolenic acid methyl ester	79.05
14	32.054	2722162	7.36	890404	8.47	Phytol	71.05
15	32.194	475714	1.29	133799	1.27	Techtoquinone	165.10
16	32.281	1034012	2.80	404311	3.85	Methyl stearate	74.00
17	32.438	1047303	2.83	284214	2.70	9,12-Octadecadienoic acid	67.05
18	32.574	2433358	6.58	577894	5.50	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
19	37.435	486813	1.32	142148	1.35	Anthraquinone-1-carboxylic acid	235.00
20	38.704	847412	2.29	304494	2.90	Glycerol .beta.-palmitate	57.05
21	40.890	1686089	4.56	271112	2.58	.gamma.-Sitosterol	131.10
22	41.419	1525699	4.13	324357	3.08	.beta.-Monolinolein	67.05
23	41.555	901017	2.44	249540	2.37	Butyl 9,12,15-octadecatrienoate	79.05
24	43.346	530268	1.43	191962	1.83	Squalene	69.05
25	48.510	1135496	3.07	262242	2.49	Vitamin E	165.10
		36972267	100.00	10514436	100.00		

Appendix 41. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Penicillium multicolor* x *Athelia rolfsii* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height %	Name	Base m/z
1	7.621	1432950	3.45	342348	3.30	Thymine	126.05
2	9.537	734535	1.77	199986	1.93	Pyranone	144.05
3	11.325	5245286	12.63	1503061	14.49	2-Methylindoline	118.10
4	12.097	1417056	3.41	134658	1.30	3-Dimethylsilyloxytridecane	75.05
5	12.328	703848	1.70	158231	1.52	3-Acetoxy-3-hydroxypropionic acid, methyl ester	103.05
6	12.463	538502	1.30	170259	1.64	13-Tetradece-11-yn-1-ol	67.05
7	16.631	433354	1.04	150734	1.45	para-Chloroanisole	142.05
8	18.108	1009590	2.43	76629	0.74	2,2-Di(hydroxymethyl)butyl allyl ether	57.00
9	18.380	3741088	9.01	363168	3.50	Guanosine	57.00
10	22.081	2872985	6.92	447309	4.31	Chinasaure	60.00
11	24.789	1325705	3.19	431091	4.15	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
12	28.489	1434937	3.46	547983	5.28	Methyl palmitate	74.05
13	29.187	1993826	4.80	584468	5.63	Hexadecanoic acid	73.05
14	29.975	533320	1.28	152787	1.47	(2-Methyl-3-nitrophenyl)methanol	210.05
15	31.721	2425595	5.84	924364	8.91	9,12-Octadecadienoic acid, methyl ester	67.05
16	31.849	2898795	6.98	925052	8.91	Alpha-linolenic acid methyl ester	79.10
17	32.053	2711506	6.53	811623	7.82	Phytol	71.05
18	32.196	678031	1.63	184333	1.78	9,10-Anthracenedione, 2-methyl-	165.10
19	32.270	590360	1.42	187948	1.81	Methyl stearate	74.05
20	32.422	990987	2.39	187814	1.81	Methyl linolelaidate	67.05
21	32.550	1060935	2.56	297817	2.87	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
22	38.270	511366	1.23	129656	1.25	Diethylene glycol dibenzoate	105.00
23	38.699	501171	1.21	188607	1.82	Glycerol 1-palmitate	57.05
24	40.878	1718027	4.14	295057	2.84	4,4-Dimethylandrost-5-en-17-ol	131.10
25	40.983	705283	1.70	164975	1.59	Docosahexaenoic acid, 1,2,3-propanetriyl ester	131.10
26	41.421	1926262	4.64	480803	4.63	Silicone oil	73.05
27	41.549	717314	1.73	165562	1.60	1-Linolensaeure-sn-glycerylester-2,3-diacetat	79.10
28	47.841	669038	1.61	170345	1.64	(-)-Beta-sitosterol	147.05
		41521652	100.00	10376668	100.00		

Appendix 42. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Penicillium multicolor* x *Athelia rolfsii* in Curative treatments

Peak #	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.655	1163149	0.91	339739	1.02	Thymine	126.10
2	9.548	961935	0.75	277288	0.84	1,5-Anhydro-6-deoxyhexo-2,3-diulose	144.10
3	11.340	11100403	8.69	2724102	8.21	2-Methylindoline	118.10
4	11.900	1736973	1.36	396958	1.20	Propyl butyrate	71.05
5	11.997	8457206	6.62	1619440	4.88	5-Hydroxymethylfurfural	97.05
6	12.282	7392709	5.79	1318441	3.97	Dimethyl 2-methoxysuccinate	75.05
7	14.425	1196371	0.94	196163	0.59	2-Methoxy-4-vinylphenol	150.10
8	16.199	5711776	4.47	1353746	4.08	L-Proline, 5-oxo-, methyl ester	84.10
9	18.616	1181423	0.93	409172	1.23	Benzoic acid, 2,6-dimethyl-	150.10
10	20.864	1503218	1.18	281497	0.85	3-Hydroxy-4-methoxybenzoic acid	168.10
11	22.301	10123199	7.93	634992	1.91	Chinasaure	60.00
12	24.809	789920	0.62	272504	0.82	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
13	28.500	6655195	5.21	2752190	8.30	Methylpalmitate	74.05
14	29.226	7247512	5.67	2079686	6.27	Hexadecanoic acid	73.05
15	31.731	10818051	8.47	4209865	12.69	Methyl octadeca-9,12-dienoate	67.05
16	31.860	12552800	9.83	3717419	11.21	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	79.05
17	31.929	906715	0.71	378283	1.14	Methyl oleate	55.05
18	32.056	3539221	2.77	1127651	3.40	Isophytol, acetate	71.05
19	32.199	2869090	2.25	935808	2.82	Beta-methylanthraquinone	165.10
20	32.284	3352883	2.63	1322380	3.99	Methyl stearate	74.05
21	32.456	4618483	3.62	1250439	3.77	9,12-Octadecadienoic acid, methyl ester, (E,E)-	67.05
22	32.590	7446990	5.83	1498659	4.52	alpha.-Linolenic acid	79.10
23	32.922	1299079	1.02	435940	1.31	Octadecanoic acid	73.05
24	35.755	774543	0.61	304151	0.92	Methyl 18-methylnonadecanoate	74.05
25	35.899	1411953	1.11	489060	1.47	Methyl podocarpate	288.20
26	37.442	1359815	1.06	349358	1.05	Anthraquinone-1-carboxylic acid	235.05
27	38.711	1623426	1.27	552271	1.67	Glycerol .beta.-palmitate	57.05
28	40.888	1705211	1.34	304916	0.92	.beta.-Sitosterol	55.10
29	41.433	3839446	3.01	833330	2.51	beta.-Monolinolein	67.05
30	41.563	2002595	1.57	543796	1.64	Butyl 9,12,15-octadecatrienoate	79.10
31	49.257	2369352	1.86	259374	0.78	Friedelan-3-one	69.05
		127710642	100.00	33168618	100.00		

Appendix 43. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Penicillium multicolor* x *Fusarium oxysporum* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.743	3456412	2.62	622181	2.67	Thymine	126.10
2	9.572	2319076	1.76	724279	3.11	Pyranone	144.10
3	11.356	9973368	7.56	2391284	10.27	2-Methylindoline	118.15
4	12.041	17171556	13.01	2203637	9.46	5-Hydroxymethylfurfural	97.10
5	12.275	5806902	4.40	1110726	4.77	Butanedioic acid, methoxy-, dimethyl ester	75.05
6	12.477	3988692	3.02	767097	3.29	13-Tetradece-11-yn-1-ol	67.10
7	17.826	3000729	2.27	822202	3.53	2,6-Cresotaldehyde	136.15
8	18.632	932522	0.71	314457	1.35	Benzoic acid, 2,3-dimethyl-	150.15
9	22.776	44604241	33.81	1427566	6.13	Chinasaure	60.05
10	24.829	2090318	1.58	679005	2.91	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
11	27.875	3058141	2.32	1139759	4.89	alpha.-Copaene-11-ol	59.05
12	28.501	2108801	1.60	884446	3.80	Methyl palmitate	74.05
13	29.222	5375416	4.07	1511003	6.49	Hexadecanoic acid	73.05
14	31.731	3939293	2.99	1558872	6.69	Methyl octadeca-9,12-dienoate	67.10
15	31.861	5358549	4.06	1804208	7.75	Linolenic acid methyl ester	79.10
16	32.063	4911924	3.72	1598610	6.86	Phytol	71.10
17	32.449	2533051	1.92	705421	3.03	Oxacycloheptadec-8-en-2-one	67.05
18	32.590	5950114	4.51	1561387	6.70	alpha.-Linolenic acid	79.10
19	38.712	1272176	0.96	461664	1.98	Glycerol .beta.-palmitate	57.10
20	41.429	2692820	2.04	610556	2.62	beta.-Monolinolein	67.10
21	41.564	1394255	1.06	395238	1.70	Methyl (Z)-5,11,14,17-eicosatetraenoate	79.10
		131938356	100.00	23293598	100.00		

Appendix 44. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Penicillium multicolor* x *Fusarium oxysporum* in Curative treatments

Peak #	R.Time	Area	Area %	Height	Height %	Name	Base m/z
1	7.666	1973872	3.28	428820	2.81	Thymine	126.05
2	9.549	907656	1.51	275248	1.80	Pyranone	144.05
3	11.339	4538359	7.55	1227021	8.03	2-Methylindoline	118.10
4	11.986	2446552	4.07	492284	3.22	5-Hydroxymethylfurfural	97.05
5	12.165	2240045	3.73	526820	3.45	4,4-Dimethoxy-2-butanone	75.05
6	13.476	800614	1.33	169908	1.11	Adipic dihydroxamic acid monohydrate	55.00
7	16.654	917206	1.53	233983	1.53	3-[N'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine	142.05
8	17.803	831426	1.38	254105	1.66	2,6-Cresotaldehyde	136.10
9	18.607	475554	0.79	160209	1.05	Benzoic acid, 2,3-dimethyl-	150.05
10	22.336	10730646	17.85	794127	5.20	Chinasaure	60.00
11	24.800	1216910	2.02	387057	2.53	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
12	28.495	2873916	4.78	1174800	7.69	Methylpalmitate	74.05
13	29.210	4216315	7.01	1218529	7.98	Hexadecanoic acid	73.05
14	31.724	3682495	6.13	1479300	9.69	Methyl octadeca-9,12-dienoate	67.05
15	31.853	4939947	8.22	1506975	9.87	Linolenic acid, methyl ester	79.05
16	32.057	3413110	5.68	1120248	7.33	Phytol	71.05
17	32.192	868610	1.44	233401	1.53	9,10-Anthracenedione, 2-methyl-	165.10
18	32.279	1220558	2.03	444086	2.91	Methyl stearate	74.05
19	32.433	1665016	2.77	455349	2.98	9-Octadecynoic acid	67.05
20	32.572	3487536	5.80	813126	5.32	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
21	32.913	523997	0.87	193908	1.27	Octadecanoic acid	73.05
22	37.439	637060	1.06	185491	1.21	Methyl (1R,2R,8aS)-2-(methoxycarbonyl)-2-hydroxy-5,5,8a-trimethyl-trans-decalin-1-acetate	235.00
23	38.702	829045	1.38	315093	2.06	Glycerol .beta.-palmitate	57.05
24	41.427	2180694	3.63	495781	3.25	1,E-11,Z-13-Octadecatriene	73.05
25	41.551	1080221	1.80	273091	1.79	Methyl (Z)-5,11,14,17-eicosatetraenoate	79.05
26	43.354	702622	1.17	220429	1.44	Silicone oil	73.05
27	47.847	713797	1.19	193695	1.27	3-Bromocholest-5-ene #	147.10
		60113779	100.00	15272884	100.00		

Appendix 45. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Purpureocillium lilacinum* x *Athelia rolfsii* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height %	Name	Base m/z
1	7.660	2755524	5.48	605216	4.33	Thymine	126.00
2	9.555	1212579	2.41	345950	2.48	Pyranone	144.00
3	11.345	5946323	11.83	1649551	11.81	2-Methylindoline	118.10
4	11.997	1258196	2.50	315128	2.26	5-Hydroxymethylfurfural	97.05
5	12.478	805721	1.60	246889	1.77	Cyclopentaneacetic acid, 2-(hydroxymethyl)-3-methyl-, .delta.-lactone	67.05
6	13.433	625168	1.24	155896	1.12	Heptanoic acid, 6-oxo-	55.00
7	16.651	810286	1.61	251012	1.80	Quinoline, 8-hydrazino-	142.00
8	22.102	3687588	7.34	472015	3.38	Chinasaure	60.00
9	24.803	881884	1.75	288094	2.06	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
10	27.862	693528	1.38	250195	1.79	alpha.-Copaene-11-ol	59.05
11	28.501	3074680	6.12	1271995	9.10	Methylpalmitate	74.05
12	29.200	2654390	5.28	851819	6.10	Hexadecanoic acid	73.00
13	31.728	4149052	8.25	1614601	11.56	9,12-Octadecadienoic acid, methyl ester	67.05
14	31.855	5396240	10.74	1565984	11.21	Alpha-linolenic acid methyl ester	79.05
15	32.061	3271309	6.51	1112923	7.97	Phytol	71.05
16	32.284	1226537	2.44	495365	3.55	Methyl stearate	74.05
17	32.436	1209358	2.41	342898	2.45	2-Chloroethyl linoleate	67.05
18	32.571	2793479	5.56	677565	4.85	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
19	38.710	852244	1.70	325233	2.33	Glycerol .beta.-palmitate	57.05
20	41.428	2083149	4.14	416320	2.98	beta.-Monolinolein	67.05
21	41.563	1143110	2.27	273407	1.96	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	79.05
22	42.320	1677944	3.34	213315	1.53	.beta.-Amyrin	218.15
23	44.056	2057077	4.09	229403	1.64	Methyl commate A	218.15
		50265366	100.00	13970774	100.00		

Appendix 46. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Purpureocillium lilacinum* x *Athelia rolfsii* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.705	2024219	3.58	377110	2.82	Thymine	126.10
2	9.545	1000670	1.77	292611	2.19	Pyranone	144.10
3	11.345	3024152	5.35	744966	5.57	2-Methylindoline	118.15
4	11.667	1076497	1.91	110324	0.83	Indoline, 1-nitroso-	59.05
5	12.001	8205002	14.52	1115462	8.34	5-Hydroxymethylfurfural	97.10
6	12.247	3293425	5.83	587608	4.39	4,4-Dimethoxy-2-butanone	75.05
7	17.794	988019	1.75	271652	2.03	2,6-Cresotaldehyde	136.15
8	22.358	3960904	7.01	318971	2.39	Chinasaure	60.05
9	24.796	553831	0.98	171973	1.29	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	91.10
10	28.486	2989509	5.29	1215578	9.09	Methyl palmitate	74.05
11	29.197	2530480	4.48	751673	5.62	Hexadecanoic acid	73.05
12	31.717	3686195	6.52	1478920	11.06	Linolelaidic acid, methyl ester	67.10
13	31.848	6018128	10.65	1885678	14.10	Methyl 8,11,14-heptadecatrienoate	79.10
14	32.047	2617953	4.63	855344	6.40	Phytol, acetate	71.10
15	32.184	894733	1.58	299422	2.24	2-Methylanthraquinone	165.15
16	32.273	1210690	2.14	458154	3.43	Methyl stearate	74.05
17	32.425	1182761	2.09	341628	2.56	Methyl linolelaidate	67.05
18	32.565	2780068	4.92	672693	5.03	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.10
19	37.428	420102	0.74	141424	1.06	Anthraquinone-1-carboxylic acid	235.10
20	38.699	673881	1.19	206257	1.54	Glycerol .beta.-palmitate	57.05
21	38.787	646198	1.14	156832	1.17	2-(3-Hydroxyphenyl)-1H-indene-1,3(2H)-dione #	238.10
22	40.892	1800592	3.19	246120	1.84	.gamma.-Sitosterol	55.05
23	41.412	1065220	1.89	220845	1.65	Lineoleoyl chloride	67.05
24	49.262	3856287	6.83	449093	3.36	Friedelan-3-one	69.10
		56499516	100.00	13370338	100.00		

Appendix 47. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Purpureocillium lilacinum* x *Fusarium oxysporum* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.648	1334279	7.23	267926	6.45	Thymine	126.10
2	9.554	434767	2.36	136318	3.28	Pyranone	144.10
3	11.365	3202398	17.35	848540	20.44	2-Methylindoline	118.15
4	12.052	1162759	6.30	203032	4.89	1-Ethyl-2-hydroxymethylimidazole	97.10
5	12.155	1381925	7.49	296902	7.15	4,4-Dimethoxy-2-butanone	75.10
6	12.382	587226	3.18	112159	2.70	1,2,3-Propanetriol, 1-acetate	103.10
7	12.478	1146852	6.21	277361	6.68	7A-Methylhexahydro-1-benzofuran-2(3H)-ONE #	67.10
8	13.909	214656	1.16	75873	1.83	1H-indole	117.15
9	16.657	326837	1.77	109494	2.64	p-Chlorophenyl methyl ether	142.10
10	17.818	461721	2.50	130982	3.15	2,6-Cresotaldehyde	136.15
11	18.626	271357	1.47	92081	2.22	4-Chromanol	150.10
12	22.165	579988	3.14	111851	2.69	Chinasaure	60.05
13	24.809	490144	2.66	162735	3.92	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
14	29.067	683019	3.70	106450	2.56	Hexadecanoic acid	60.05
15	29.185	1174559	6.36	316275	7.62	Pentadecanoic acid	73.10
16	31.727	273829	1.48	117193	2.82	9,12-Octadecadienoic acid, methyl ester	67.10
17	31.853	461755	2.50	140042	3.37	Alpha-linolenic acid methyl ester	79.10
18	32.417	999519	5.42	127566	3.07	Oxacycloheptadec-8-en-2-one	67.10
19	32.543	536424	2.91	182864	4.40	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.10
20	40.875	2731080	14.80	336439	8.10	.beta.-Sitosterol	81.15
		18455094	100.00	4152083	100.00		

Appendix 48. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Purpureocillium lilacinum* x *Fusarium oxysporum* in Curative treatments

Peak #	R.Time	Area	Area%	Height	Height %	Name	Base m/z
1	7.690	3780199	5.97	741610	5.11	Thymine	126.05
2	9.552	2000584	3.16	523898	3.61	Pyranone	144.00
3	11.337	7768704	12.26	2180490	15.03	2-Methylindoline	118.10
4	11.988	4207245	6.64	834042	5.75	5-Hydroxymethylfurfural	97.05
5	12.125	1389373	2.19	264318	1.82	Butanedioic acid, methoxy-, dimethyl ester	75.05
6	12.361	958141	1.51	231182	1.59	3-Acetoxy-3-hydroxy-propionic acid methyl ester	103.05
7	12.473	834950	1.32	268696	1.85	Cyclopentaneacetic acid, 2-(hydroxymethyl)-3-methyl-, .delta.-lactone	67.05
8	14.420	756988	1.19	178211	1.23	2-Methoxy-4-vinylphenol	150.10
9	16.648	1998913	3.16	339259	2.34	2-Butene-1,4-diol, bis(trimethylsilyl) ether	142.05
10	17.806	1091425	1.72	315478	2.17	2,6-Cresotaldehyde	136.10
11	20.851	915383	1.44	182258	1.26	Isovanillic acid	168.05
12	22.309	8009760	12.64	628745	4.33	Chinasaure	60.00
13	24.803	1350156	2.13	434032	2.99	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
14	28.493	627589	0.99	256702	1.77	Methylpalmitate	74.05
15	29.205	4216029	6.66	1288064	8.88	Hexadecanoic acid	73.05
16	31.723	1618122	2.55	663631	4.57	Methyl octadeca-9,12-dienoate	67.05
17	31.853	2430058	3.84	820061	5.65	Linolenic acid, methyl ester	79.05
18	32.053	2052065	3.24	613048	4.23	Phytol	71.05
19	32.191	592645	0.94	179081	1.23	9,10-Anthracenedione, 2-methyl-	165.10
20	32.436	2099874	3.31	583999	4.03	Oxacycloheptadec-8-en-2-one	67.05
21	32.577	4550695	7.18	1123793	7.75	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
22	37.437	549377	0.87	157825	1.09	Ethyl geranyl acetate	235.00
23	38.703	857147	1.35	296900	2.05	Glycerol .beta.-palmitate	57.05
24	40.898	2973606	4.69	393681	2.71	.beta.-Sitosterol	55.05
25	41.419	1524126	2.41	364457	2.51	beta.-Monolinolein	67.05
26	41.550	1053173	1.66	291913	2.01	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	79.00
27	49.281	3142418	4.96	353827	2.44	Friedelan-3-one	69.05
		63348745	100.00	14509201	100.00		

Appendix 49. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma harzianum* x *Athelia rolfsii* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.694	3814345	4.38	750036	3.45	Thymine	126.05
2	9.559	1795744	2.06	558301	2.57	pyranone	144.00
3	11.342	9610304	11.03	2754184	12.66	2-Methylindoline	118.10
4	12.007	13345535	15.32	2002407	9.21	3,4-Anhydro-d-galactosan	61.00
5	12.227	3629015	4.17	930195	4.28	Butanedioic acid, methoxy-, dimethyl ester	75.05
6	12.479	1706618	1.96	288851	1.33	5-Octen-1-ol, (Z)-	67.05
7	18.626	1224604	1.41	315763	1.45	Benzoic acid, 2,4-dimethyl-	150.05
8	24.816	1476167	1.69	491454	2.26	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
9	27.874	4472981	5.14	1538385	7.07	alpha.-Copaene-11-ol	59.05
10	28.503	4337691	4.98	1738912	8.00	Methylpalmitate	74.00
11	29.215	4426877	5.08	1387473	6.38	Hexadecanoic acid	73.05
12	31.733	4871368	5.59	1910557	8.78	9,12-Octadecadienoic acid, methyl ester	67.05
13	31.859	5302390	6.09	1543296	7.10	Linolenic acid methyl ester	79.05
14	32.062	5213084	5.99	1660163	7.63	Phytol	71.05
15	32.288	1907961	2.19	547012	2.52	Methyl stearate	74.05
16	32.442	2146561	2.46	638661	2.94	Methyl-11,14-eicosadienoate	67.05
17	32.571	3651216	4.19	811404	3.73	.alpha.-Linolenic acid	79.05
18	38.710	953133	1.09	369049	1.70	Glycerol .beta.-palmitate	57.05
19	40.028	1282270	1.47	402687	1.85	4-t-Butyl-2-[4-nitrophenyl]phenol	256.05
20	40.915	10544721	12.11	745109	3.43	.beta.-Sitosterol	55.05
21	41.425	1381542	1.59	364517	1.68	.Beta.-monolinolein	67.05
		87094127	100.00	21748416	100.00		

Appendix 50. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma harzianum* x *Athelia rolfsii* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.611	1010887	2.49	270944	2.72	Thymine	126.10
2	11.332	4152664	10.22	731930	7.34	2-Methylindoline	118.15
3	11.963	1426142	3.51	381753	3.83	5-Hydroxymethylfurfural	97.10
4	12.063	693887	1.71	176417	1.77	4,4-Dimethoxy-2-butanone	75.10
5	21.671	261251	0.64	44665	0.45	Ethyl N-(o-anisyl)formimidate	179.10
6	21.950	1200712	2.95	232400	2.33	Isochiapin B	57.00
7	22.017	2052530	5.05	297707	2.99	Chinasaure	60.05
8	28.482	2254702	5.55	911755	9.15	Methylpalmitate	74.05
9	29.189	2676302	6.59	759687	7.62	Hexadecanoic acid	73.05
10	31.709	2911718	7.16	1173373	11.77	Methyl octadeca-9,12-dienoate	67.10
11	31.838	4044226	9.95	1217920	12.22	Methyl 8,11,14-heptadecatrienoate	79.10
12	32.041	2120786	5.22	715615	7.18	Phytol, acetate	71.10
13	32.177	527955	1.30	158580	1.59	9,10-anthracenedione, 2-methyl-	165.15
14	32.266	880602	2.17	356954	3.58	Methyl stearate	74.05
15	32.419	1085648	2.67	325651	3.27	Oxacycloheptadec-8-en-2-one	67.05
16	32.557	2913335	7.17	675974	6.78	alpha.-Linolenic acid	79.10
17	32.898	365495	0.90	123198	1.24	Octadecanoic acid	73.05
18	38.687	545144	1.34	169711	1.70	Glycerol .beta.-palmitate	57.10
19	40.874	3147471	7.74	355099	3.56	.beta.-Sitosterol	55.10
20	41.400	810731	1.99	166564	1.67	9,12-Octadecadienoyl chloride, (Z,Z)-	67.10
21	41.539	596673	1.47	142271	1.43	Methyl 8,11,14-heptadecatrienoate	79.10
22	42.256	363427	0.89	56764	0.57	Methyl commate A	218.20
23	49.220	4597746	11.31	522781	5.24	Friedelan-3-one	69.10
		40640034	100.00	9967713	100.00		

Appendix 51. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma harzianum* x *Fusarium oxysporum* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.698	4870418	4.40	674731	2.67	Thymine	126.10
2	9.552	3098954	2.80	741287	2.94	Pyranone	144.05
3	11.336	11921642	10.76	3082456	12.21	2-Methylindoline	118.10
4	12.039	22923080	20.69	3072273	12.17	5-Hydroxymethylfurfural	97.05
5	12.252	5499806	4.96	1121569	4.44	Butanedioic acid, methoxy-, dimethyl ester	75.05
6	12.466	3182550	2.87	545313	2.16	5-Methyl-5-octen-1-ol	103.05
7	22.433	2229298	2.01	443446	1.76	3-(4-Methylphenyl)-4,5-Isoxazoledione dioxime	118.05
8	22.708	11126226	10.04	831747	3.30	Chinasaure	60.00
9	28.499	4912015	4.43	2079373	8.24	Methyl Palmitate	74.05
10	29.220	5894724	5.32	1773802	7.03	Hexadecanoic acid	73.05
11	31.730	5965996	5.38	2369878	9.39	Methyl octadeca-9,12-dienoate	67.10
12	31.859	6180673	5.58	1847977	7.32	Alpha-linolenic acid methyl ester	79.05
13	32.061	6657243	6.01	2293993	9.09	Phytol	71.10
14	32.283	1808310	1.63	549215	2.18	Octadecanoic acid, methyl ester	74.05
15	32.445	2789775	2.52	753388	2.99	Methyl linolelaidate	67.10
16	32.585	6271332	5.66	1565486	6.20	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.10
17	32.919	955792	0.86	323251	1.28	Octadecanoic acid	73.05
18	38.709	1138501	1.03	412900	1.64	Glycerol .beta.-palmitate	57.05
19	41.424	2223822	2.01	452420	1.79	Beta-monolinolein	67.10
20	41.559	1163851	1.05	301554	1.19	Ethyl 9,12,15-octadecatrienoate	79.05
		110814008	100.00	25236059	100.00		

Appendix 52. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma harzianum* x *Fusarium oxysporum* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.749	5692727	2.96	836372	1.80	Thymine	126.05
2	9.570	2504306	1.30	792554	1.70	Pyranone	144.00
3	11.349	11169630	5.81	3066820	6.60	2-Methylindoline	118.10
4	12.022	3911151	2.03	1010833	2.17	5-Hydroxymethylfurfural	97.05
5	17.825	2127930	1.11	635972	1.37	2,6-Cresotaldehyde	136.10
6	18.625	1511971	0.79	497118	1.07	4-Chromanol	150.10
7	22.958	32088861	16.68	1442051	3.10	Chinasaure	60.00
8	24.825	3020371	1.57	1030017	2.22	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
9	28.502	10102546	5.25	4150248	8.93	Methyl Palmitate	74.05
10	29.267	23292699	12.11	5228424	11.25	Hexadecanoic acid	73.05
11	31.735	13330897	6.93	5426829	11.67	Methyl octadeca-9,12-dienoate	67.05
12	31.862	15661906	8.14	4507659	9.70	Alpha-linolenic acid methyl ester	79.05
13	32.066	12918541	6.72	4711959	10.14	Phytol	71.05
14	32.288	4799412	2.49	1453080	3.13	Methyl stearate	74.05
15	32.480	10965387	5.70	2263121	4.87	Oxacycloheptadec-8-en-2-one	67.05
16	32.631	22095261	11.49	4475524	9.63	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.10
17	32.950	3968533	2.06	1383818	2.98	Octadecanoic acid	73.05
18	38.717	3516057	1.83	1292776	2.78	Glycerol .beta.-palmitate	57.05
19	41.433	6110448	3.18	1335146	2.87	beta.-Monolinolein	67.05
20	41.566	3588050	1.87	950848	2.05	Butyl 9,12,15-octadecatrienoate	79.05
		192376684	100.00	46491169	100.00		

Appendix 53. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma koningii* x *Athelia rolfsii* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.681	3992313	4.51	792969	3.16	Thymine	126.05
2	9.548	2200986	2.49	704608	2.81	Pyranone	144.00
3	11.332	10181800	11.51	3105013	12.37	2-Methylindoline	118.10
4	11.986	1075848	1.22	373192	1.49	5-Hydroxymethylfurfural	97.05
5	22.383	6886475	7.79	726834	2.90	Chinasaure	60.00
6	24.805	2924932	3.31	974771	3.88	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.05
7	27.864	2952348	3.34	1072809	4.27	alpha.-Copaene-11-ol	59.05
8	28.497	2166203	2.45	797002	3.17	Methyl palmitate	74.05
9	29.223	8025718	9.08	2308204	9.20	Hexadecanoic acid	73.05
10	31.726	4769529	5.39	1851881	7.38	9,12-Octadecadienoic acid, methyl ester	67.05
11	31.856	6836512	7.73	2232367	8.89	Methyl linolenate	79.05
12	32.057	6012979	6.80	2019801	8.05	Phytol	71.05
13	32.196	2556285	2.89	807690	3.22	9,10-Anthracenedione, 2-methyl-	165.10
14	32.453	4258672	4.82	1055133	4.20	Oxacycloheptadec-8-en-2-one	67.05
15	32.596	11168554	12.63	2526415	10.06	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
16	34.391	3463160	3.92	1273079	5.07	2-Hydroxy-3-[.beta.-iodo-.beta.-isopropylvinyl]-1,4-naphthoquinone	241.00
17	35.896	1029971	1.16	367455	1.46	N-(4-(Methylthio)-1-naphthyl)acetamide tbdms	288.10
18	38.709	1872242	2.12	607910	2.42	Glycerol .beta.-palmitate	57.05
19	38.809	1094301	1.24	304644	1.21	Indane-1,3-dione, 2-(3-hydroxyphenyl)-	238.00
20	41.427	3351147	3.79	735209	2.93	beta.-Monolinolein	67.05
21	41.559	1614475	1.83	465478	1.85	Butyl 9,12,15-octadecatrienoate	79.05
		88434450	100.00	25102464	100.00		

Appendix 54. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma koningii* x *Athelia rolfsii* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.570	208796	0.88	70777	1.09	Thymine	126.10
2	11.341	2294689	9.69	588268	9.07	2-Methylindoline	118.15
3	11.967	280377	1.18	81565	1.26	5-Hydroxymethylfurfural	97.05
4	12.074	739525	3.12	210410	3.24	4,4-Dimethoxy-2-butanone	75.05
5	13.905	2863209	12.09	1136888	17.53	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	141.10
6	21.847	3535077	14.93	391002	6.03	Chinasaure	60.05
7	28.493	1350424	5.70	557989	8.60	Methyl palmitate	74.05
8	29.184	1353408	5.71	425568	6.56	Hexadecanoic acid	73.05
9	31.722	1880626	7.94	707936	10.92	Linoleic acid, methyl ester	67.10
10	31.849	2693478	11.37	785733	12.11	Methyl 8,11,14-heptadecatrienoate	79.10
11	32.049	711556	3.00	235388	3.63	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	71.05
12	32.278	575762	2.43	229682	3.54	Methyl stearate	74.05
13	32.419	472677	2.00	143382	2.21	Linolelaidic acid, methyl ester	67.05
14	32.553	1239283	5.23	251658	3.88	alpha.-Linolenic acid	79.10
15	32.906	242553	1.02	75242	1.16	2-Aminoethanethiol hydrogen sulfate (ester)	73.10
16	38.698	430225	1.82	135330	2.09	Glycerol .beta.-palmitate	57.05
17	40.889	1759208	7.43	223613	3.45	.beta.-Sitosterol	55.05
18	41.413	486565	2.05	103790	1.60	Linoleoyl chloride	67.05
19	41.545	257542	1.09	71414	1.10	cis,cis,cis-7,10,13-Hexadecatrienal	79.05
20	46.142	309656	1.31	60156	0.93	.alpha.-Tocopheryl acetate	165.15
		23684636	100.00	6485791	100.00		

Appendix 55. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma koningii* x *Fusarium oxysporum* in Preventive treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.676	1960085	3.94	463661	2.97	Thymine	126.10
2	11.344	5629946	11.31	1887700	12.09	2-Methylindoline	118.10
3	12.002	2738863	5.50	355868	2.28	5-Hydroxymethylfurfural	97.05
4	22.265	4793761	9.63	498124	3.19	Chinasaure	60.00
5	27.887	18914141	38.01	6602234	42.28	Alpha.-copaene-11-ol	59.05
6	28.309	1232721	2.48	482484	3.09	Hedycaryol	59.10
7	29.197	1923656	3.87	690230	4.42	hexadecanoic acid	73.05
8	29.557	2505773	5.04	996052	6.38	Beta.-selinenol	59.05
9	31.727	1615049	3.25	653508	4.19	9,12-Octadecadienoic acid, methyl ester	67.10
10	31.857	2060048	4.14	716382	4.59	Linolenic acid methyl ester	79.10
11	32.059	1296203	2.60	501761	3.21	Isophytol, acetate	71.10
12	40.029	5092713	10.23	1765832	11.31	2,7-Dimethoxyphenazine 5-oxide	256.15
		49762959	100.00	15613836	100.00		

Appendix 56. Bio-chemical compounds in the methanol extract of teak seedlings treated with *Trichoderma koningii* x *Fusarium oxysporum* in Curative treatments

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	7.671	3009136	7.74	590382	8.64	Thymine	126.05
2	9.308	471629	1.21	59328	0.87	N-Methyl-N-nitrosoisopropylamine	102.05
3	9.551	1666942	4.29	381261	5.58	pyranone	144.05
4	11.340	6306523	16.23	1556777	22.79	2-Methylindoline	118.10
5	11.988	4838064	12.45	1034954	15.15	5-Hydroxymethylfurfural	97.00
6	12.117	1621546	4.17	281473	4.12	1-Dimethyl(isopropyl)silyloxypropane	75.05
7	12.349	640332	1.65	152360	2.23	Dimethyl dl-malate	103.05
8	12.474	505250	1.30	165618	2.43	13-Tetradece-11-yn-1-ol	67.05
9	16.647	1096680	2.82	344083	5.04	3-[N'-(3H-indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine	142.00
10	17.809	465255	1.20	143518	2.10	2,6-Cresotaldehyde	136.10
11	21.617	455993	1.17	104854	1.54	Methyl 6-O-[1-methylpropyl]-.beta.-d-galactopyranoside	60.00
12	22.329	14001420	36.03	867316	12.70	Chinasaure	60.00
13	24.805	691062	1.78	231180	3.39	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	137.10
14	25.531	484146	1.25	110379	1.62	2-Bornanone oxime	167.05
15	29.185	967690	2.49	309426	4.53	Hexadecanoic acid	73.05
16	32.048	660435	1.70	239184	3.50	Alpha-lapachone, dehydro-	225.00
17	32.548	473231	1.22	133197	1.95	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	79.05
18	41.431	502738	1.29	124189	1.82	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	73.05
		38858072	100.00	6829479	100.00		

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Bio-control potentiality of *Penicillium multicolor* Grig.-Man. and Porad., against important root pathogens

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Abstract

Penicillium multicolor a soil-borne fungus was evaluated for its activity against root pathogens *Fusarium oxysporum* and *Sclerotium rolfsii*. *In vitro* plate assay exhibited mycelia inhibition of the pathogens on PDA medium. Microscopic analysis revealed conidiophores and conidia deformations and mycelia piercing in the case of *F. oxysporum* where as mycelia coiling and cytoplasmic disintegration resulted for *S. rolfsii*. Rhizosphere competence of *P. multicolor* where sterile paddy straw segments placed at different centimetres up to 7cm depth to which 40ml of 2 x 10^6 conidial suspensions were poured and determined at 21 days of incubation exhibited mycelia growth up to 6 cm depth but observed very low colonisation frequency rate, 22%. *In vitro* root colonisation of *P. multicolor* and pathogens was studied for Teak and Mahogany seedlings. 2 x 10^5 and 2 x 10^6 conidial suspensions of pathogens and the bio-agent respectively at different concentrations v/v (1pathogen:1 biological agent and 1 pathogen: 5 biological agent) were tested and 1:5 ratio was found to be effective where complete suppression of pathogens were observed. The present study exerted on the further standardisation of biological agent for their success in field application.

Key words – Bio-agent – Colonisation – *In vitro* assay – Root pathogens – Saprophytic ability

Introduction

Sustainable production of plants depends upon culture practices producing quality planting stocks, soil nutrient management and minimal chemical usage. Seedling health in nurseries determine the quality of the output which besides culture practices also influenced by biotic and abiotic factors. Biotic agents such as bacteria, fungi, nematodes, protozoans, viruses, insects, infest plants especially during seedling stage thereby hindering quality planting stocks. Forest nursery diseases- collar rots, damping-off, root rots, foliar diseases, blights, powdery mildews and rusts result either total damage to seedlings or weaken them to different degrees so that the nursery-initiated diseases are carried over to the main field resulting in disease spread and severe loss (Sharma et al. 1985). Collar rots, damping-off and root rots affect most of the forestry species in nurseries incited by fungi majorly- species of *Cylindrocladium, Fusarium, Pythium, Phytophthora, Rhizoctonia* and *Sclerotium* resulting in unpredictable damage and severely hampering the economy.

Kerala, popularly known as God's own Country, South western state of India is bestowed with nature's beauty and homes diverse wildlife with forest cover about 52.30% of the total geographical area as per Kerala forest statistics 2018. Forests contribute considerably to the

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government revenue and many tribes and locals depend upon forest products for their livelihood. Forests besides other natural produce majorly serves as a source of timber and considerable area has been allocated for various plantations. Teak (*Tectona grandis* Linn.) and Mahogany (*Swietenia macrophylla* King) are important timber yielding crops and have been widely cultivated since 19th century (Ball et al. 2000). As per Kerala forest statistics report (2018), 13.51% of total forest area is occupied by different plantations of which Teak about 49.61% and Mahogany about 0.33% of total plantation area together share for major timber plantations in Kerala. On-set of Teak and Mahogany seedlings are greatly hampered by root diseases of which species of *Fusraium, Sclerotium* and *Rhizoctonia* are most devastating (Sharma et al. 1985, Mohanan 2001).

Fusarium oxysporum Schltdl. a severe fungal pathogen distributed through-out the world and inhabit different soil types (Burgess 1981). This species is normally present in the rhizosphere communities of different plant groups and upon favourable conditions invade plant roots causing tracheomycosis resulting in wilting of plants or causing root-rots (Gordon & Martyn 1997, Olivain & Alabouvette 1997).

Sclerotium rolfsii Sacc. another important soil-borne fungal pathogen has wide host range resulting collar and root rots (Aycock 1966, Bhattacharrya et al. 1977). The species produce amber-coloured hardened structures called sclerotia which help them to strive in unfavourable conditions and upon the advent of suitable conditions reactivate resulting in severe infestation on different parts of the plants (Agrios 2005).

The present containment strategy mostly relies on chemical pesticides and unchecked application pushed for an alternative approach so as to manage the disease economically as well as sustainably (Eziashi et al. 2007). A number of bio-agents *Trichoderma* sp. Pers. (Shabir-U-Rehman et al. 2013), *Aspergillus* sp. Micheli. (Suárez-Estrella et al. 2007) and *Penicillium* sp. Link. (De Cal et al. 2009) found success *in vitro* but on field it proves to be a failure stresses on in-depth study of various aspects of biological activity. The present study deals to understand the activity of *Penicillium multicolor* against root pathogens- *F. oxysporum* and *S. rolfsii* of Teak and Mahogany seedlings *in vitro*.

Materials & Methods

Fungal cultures

Fungal pathogens namely *Fusarium oxysporum* and *Sclerotium rolfsii* were previously identified and obtained from Forest Pathology Department, Kerala Forest Research Institute (KFRI), Thrissur, Kerala. The isolates were stored as live cultures in Potato Dextrose Agar (PDA) slants at -10^{o}C and were then reactivated on antibiotic amended PDA medium by culturing mycelia discs at 25 ± 2^{o}C for seven days.

The bio-agent *Penicillium multicolor* was previously isolated from rhizosphere regions of grasses and was stored as live culture in PDA slants at -10^{o}C. The fungal culture was sub-cultured and purified as mentioned earlier.

Antagonistic ability

In vitro antagonism was analysed via dual culture method on PDA medium. Seven mm diameter agar plugs of *P. multicolor* and pathogens *F. oxysporum* and *S. rolfsii* were cut from actively growing edge of five-day-old cultures using a cork borer. The mycelia discs were paired leaving 3-4 cm gap in between. The control plates were inoculated with the pathogens and the antagonist separately. The plates were incubated for ten days at 25 ± 2^{o}C and observed for dual culture activity. The percent inhibition of radial growth of fungal plant pathogens was calculated using formula given by (Kucuk & Kivanc 2004).

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

Where,
R1 = Radial growth of fungal pathogens in control
R2 = Radial growth of fungal pathogen in dual culture

Microscopic analysis was done via slide culture method. Water agar (WA) medium 1% was poured and allowed to solidify and 1 cm square bits were cut and placed over sterile microscopic slides. To one corner, mycelia of *P. multicolor* was placed and diagonally mycelia of pathogens. A sterile cover slip was mounted and slides were incubated for 3-5 days at 25 ± 2ºC and regularly moistened so as to avoid the drying of agar discs. Slides were observed under Leica DM2000 LED microscope and photo-micrographs were taken using attached Leica DMC2900 camera on the microscope.

Rhizosphere competence of *P. multicolor*

Preparation of fungal inoculum

Penicillium multicolor was cultured in PDA medium for 7 days at 25 ± 2ºC. Freshly grown cultures were inoculated in Potato Dextrose Broth (PDB) were kept at incubator shaker for 14 days at 25 ± 2ºC. Mycelial mat was separated out by filtering through Whatsman No.1 filter paper, dried, grounded using mortar and pestle and centrifuged at 10000 rpm for 15 min to remove hyphal debris. Conidial suspensions thus obtained were then suspended in sterile distilled water and the concentration was adjusted to 2 x 10^6 conidia per ml.

Testing saprophytic competency

Saprophytic ability of *P. multicolor* was tested by the Cambridge method (Garret 1970). Freshly harvested paddy straw were cut in to 1-cm long segments and autoclaved. Sterile plastic cups procured from the market were perforated at the bottom and plugged with sterile cotton pads. These cups were filled with 200 g autoclaved potting medium up to 7-cm length of the cup and placed with paddy straw segments at 1cm apart from the bottom of the cup. Eighteen autoclaved straw pieces were placed in a radial fashion and sterile potting medium was over laid up to 8-cm length of the cup. Forty ml of conidial suspensions were poured over the potting medium separately and each set was placed in an individual plastic tray containing sterile distilled water. The cups were not watered from the top but the potting medium in the cup was allowed to imbibe water only through capillary action from the holes at the bottom of the cup. Colonization of paddy straw segments was determined after 21 days of incubation.

Isolation of *P. multicolor*

Paddy straw segments removed after regular intervals of incubation were washed in slow running tap water, then twice in sterile distilled water and placed on antibiotic amended PDA medium at 25 ± 2ºC for 14 days. The fungal colonies developing from these segments were identified and compared with the characteristics of the original colony culture. Percent colonization by *P. multicolor* at different depth levels at given time was determined.

$$\text{Colonisation frequency (CF)} = \frac{\text{Number of fungus isolated in each bits}}{\text{Total number of bits observed}} \times 100$$

***In vitro* Root colonisation**

P. multicolor and root pathogens *F. oxysporum* and *S. rolfsii* were studied for their interactions with roots. Mycelial discs were incubated in 1% sucrose solution for 14 days at 25 ± 2ºC and were separated out by filtering through Whatsman No.1 filter paper and centrifuged at 10000 rpm for 15 min to remove hyphal debris. Conidial suspensions thus obtained were then suspended in sterile distilled water and the concentration was adjusted to 2 x 10^5 and 2 x 10^6 conidia per ml for pathogens and the bio-agent via haemocytometer. 15 day old Teak and Mahogany

seedlings were subjected with different concentrations v/v (1pathogen:1 biological agent and 1 pathogen: 5 biological agent) and a separate set for control was also maintained. Seedlings were incubated for 21 days and were observed for possible interactions via Root clearing method. Root samples were treated with 10% KOH solution for 1 hour in a hot water bath at 60^{o}C and were washed with distilled water and treated with 2% HCl solution. Samples were stained with 0.05% Trypan blue in lactic acid and kept in a hot water bath for 10-15 min. Samples were de-stained with lactic acid and were observed under the microscope to observe the interaction. Slides were observed under Leica DM2000 LED microscope and photo-micrographs were taken using attached Leica DMC2900 camera on the microscope.

Results

Antagonistic ability

Current *In vitro* dual culture assay resulted in the inhibition of mycelia growth of the pathogens, *F. oxysporum* and *S. rolfsii* by *P. multicolor* (Table 1). Comparatively the activity of the bio-agent varied among pathogens exerting maximum inhibition on hyphal development of *F. oxysporum* 65.71%. Microscopic studies revealed conidiophores and conidia deformations and mycelia lysis thereby inhibiting conidial germination (Fig. 1A, B).

Sclerotium rolfsii exhibited resistance to the *P. multicolor* stress to about 51% but further elongation of the hyphae was restricted. Mycelia interactions resulted in coiling of the bio-agent hyphae and subsequently disintegrating cytoplasmic content thus restricting the vegetative propagation (Fig. 1C, D).

Table 1 Antagonistic activity of *P. multicolor* against root disease causing fungal pathogens

	F. oxysporum	*S. rolfsii*
P. multicolor	65.71±2.85[1]	48.89±2.22[1]

[1]standard deviation

Rhizosphere competence and colonisation frequency

Penicillium multicolor was subjected for saprophytic and colonizing ability. The present work was to analyse the competency of the bio-agent *in vitro*. Sterile paddy straw segments placed at 1 cm apart upto a depth of 7 cm and over flowed with 40 ml of 2 x 10^6 conidial suspensions per ml were incubated for 21 days. Paddy straw segments at each depth level on isolation for *P. multicolor* resulted upto a depth of 6 cm. Similarly colonization frequency was also checked at different levels of depths and exhibited 72% CF at 2 cm but with increasing depth level a decrease in CF was observed and only exhibited 22% at 6 cm depth (Fig. 2). The ability of microbes as a successful bio-control agent relies on rhizosphere compatibility and to cope up with the plant root-soil interface.

In vitro Root colonisation

To understand the nature of interaction, Teak and Mahogany seedlings were treated with the bio-agent and pathogens. Fifteen day old seedlings were treated with 2 x 10^5 and 2 x 10^6 conidial suspensions per ml of pathogens and the bio-agent at different concentrations v/v (1pathogen:1 biological agent and 1 pathogen: 5 biological agent). Roots were then observed for possible colonisation and interactions by the fungal agents.

Penicillium multicolor was found to attach to the surface of root segments as was evidenced for Teak seedlings (Fig. 3A). But for treatments it was observed that 1:1 concentration was not sufficient from preventing the attack of the pathogen. In case of *F. oxysporum* the hyphae colonized root epidermal tissues and resulted in the damage of root cells and on the contradictory 1:5 concentrations inhibited hyphae elongation and disintegrating mycelia (Fig. 3B, C). For *S. rolfsii* on the other hand for 1:1 concentration, heavy mycelia tissues were observed on the surface of root nd

damaging root cells but for 1:5 concentrations, *S. rolfsii* though were found to present over the root surface but cytoplasmic disintegrations were observed thereby weakening mycelia activity (Fig. 3D, E).

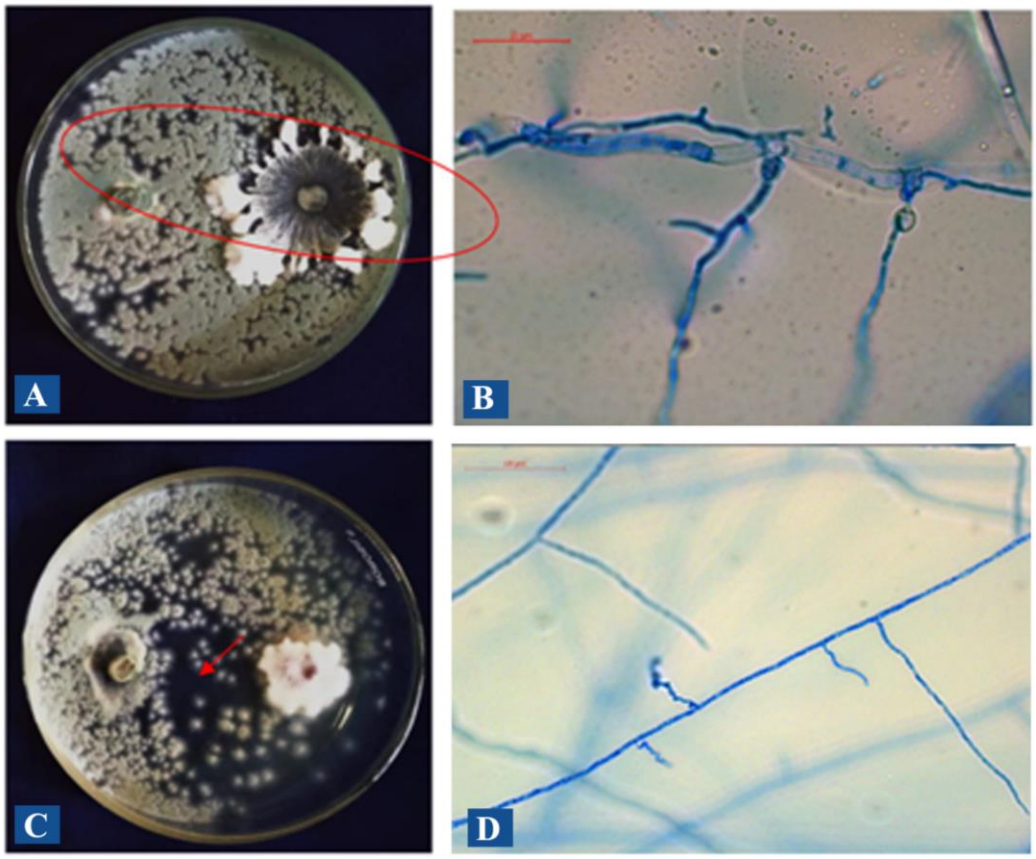

Fig. 1 – A-D Dual culture assay and Microscopic observations. *P. multicolor* and *S. rolfsii* exhibiting mycoparasitic interactions, hyphal coiling and cytoplasmic disintegration (A, B). *P. multicolor* and *F. oxysporum* exhibiting mycoparasitic interactions, conidiophore deformations (C, D). Photomicrographs taken at 100X.

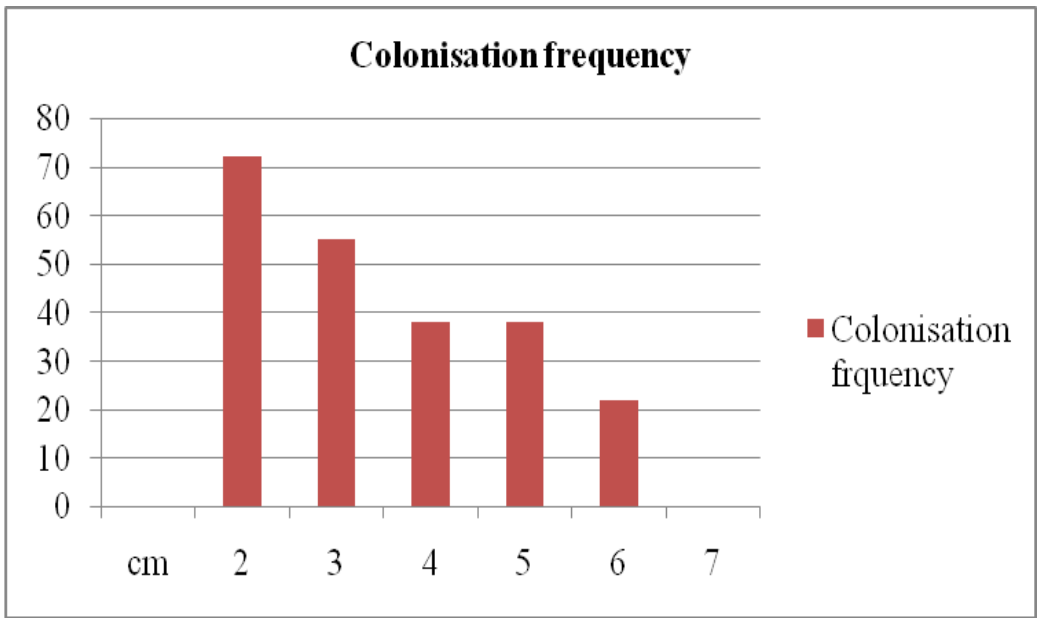

Fig. 2 – Colonization frequency of *P. multicolor* 21 days of incubat

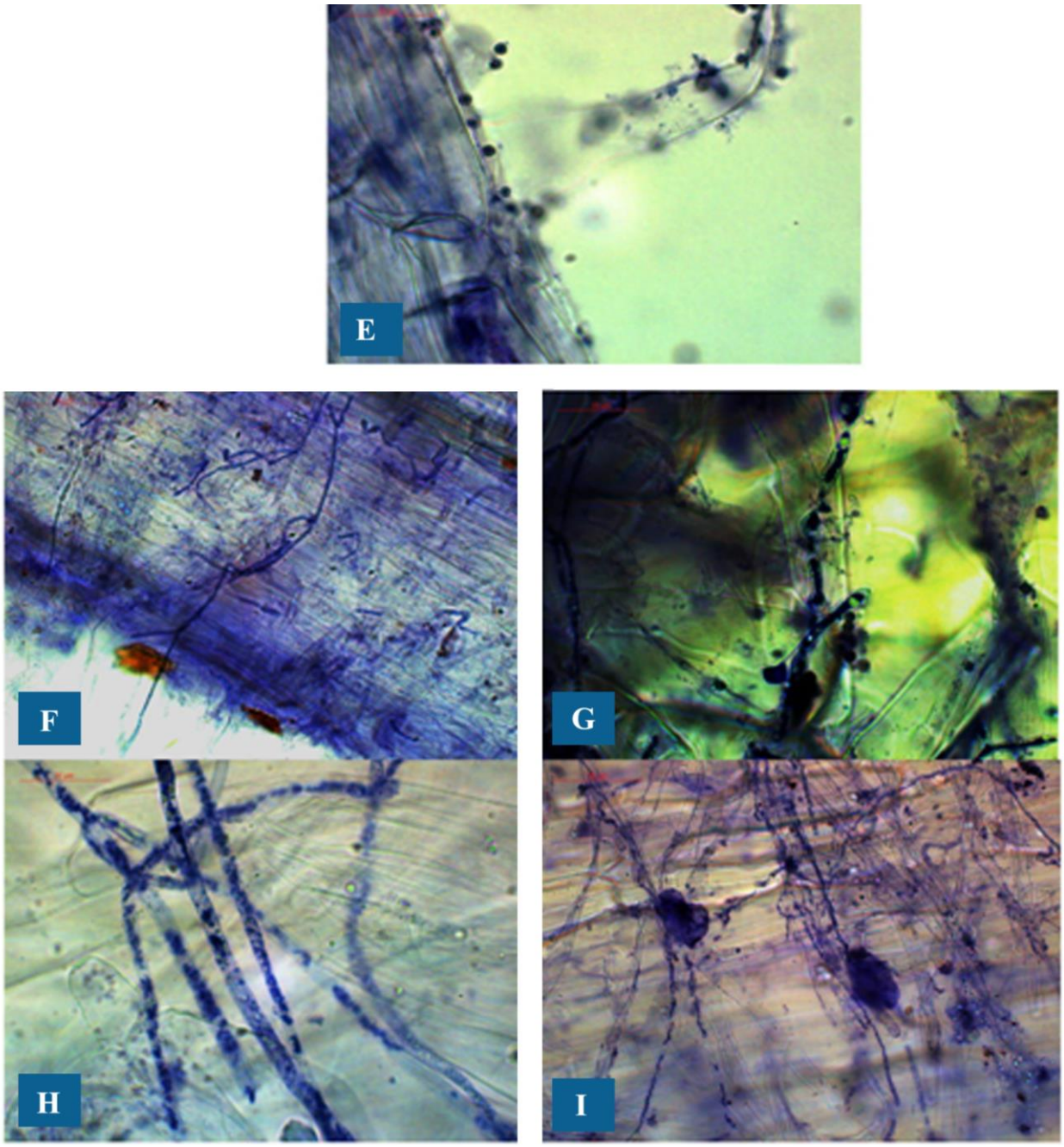

Fig. 3 – E-I Root colonisation of fungi in Teak seedlings. *Penicillium multicolor* colonising over root surface (E). Interactions of *F. oxysporum* and *P. multicolor* at 1:1 and 1:5 conidial concentrations (F, G). Interactions of *S. rolfsii* and *P. multicolor* at 1:1 and 1:5 conidial concentrations (H, I). Photomicrographs taken at 100X.

In the case of Mahogany seedlings heavy conidia concentration and hyphal strands of *P. multicolor* were seen on the root surface and no signs of any attachments clearly indicating the bio-agent acting as a shield over the surface of the root (Fig. 4A). 1:1 concentrations of pathogens and bio-agent were found ineffective in the management of pathogens. *Fusarium oxysporum* hyphae invaded root tissues and subsequently damaging root cells. When 1:5 concentration was applied heavy conidial mass were seen over the root surface appeared to be acting as a shield (Fig. 3B, C). Again for *S. rolfsii* when 1:1 concentration was analysed root surface were found to be colonized by *Sclerotium* mycelia on the other hand when subjected to 1:5 concentration, numerous *P. multicolor* conidia mass was observed and *Sclerotium* hyphae with cytoplasmic disintegrations were observed (Fig. 4D, E

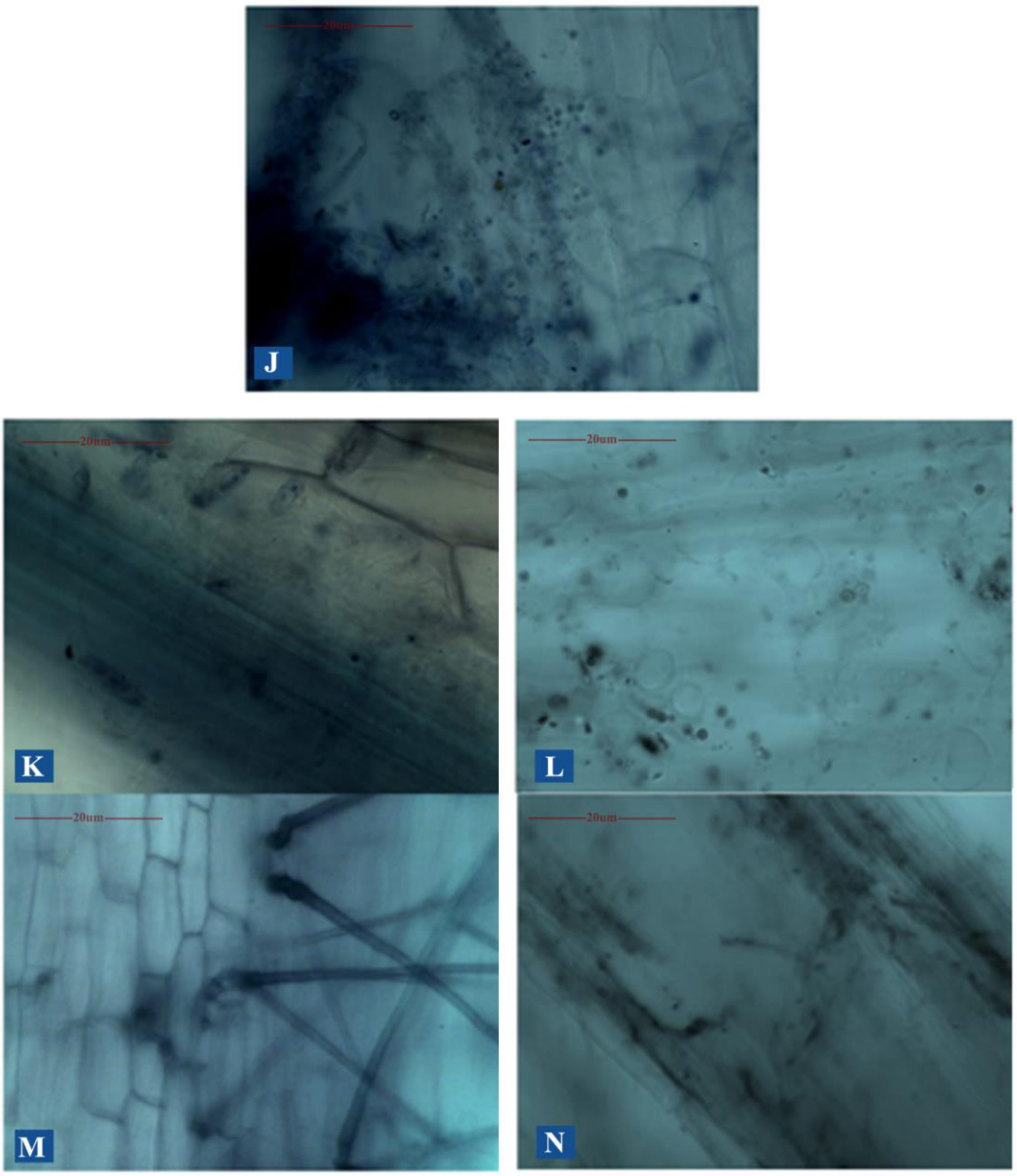

Fig. 4 – J-N Root colonisation of fungi in Mahogany seedlings. *P. multicolor* colonising over root surface (J). Interactions of *F. oxysporum* and *P. multicolor* at 1:1 and 1:5 conidial concentrations (K, L). Interactions of *S. rolfsii* and *P. multicolor* at 1:1 and 1:5 conidial concentrations (M, N). Photomicrographs taken at 100X.

Discussions

Penicillium multicolor against root pathogens *Fusarium oxysporum* and *Sclerotium rolfsii* exhibited parasitic activity inhibiting mycelium growth by lysing and coiling of hyphae and disintegrating cytoplasmic contents. Many bio-control agents against *Fusarium* wilt such as *T. harzianum*, *Penicillium oxalicum* Currie & Thom, and non-pathogenic *F. oxysporum* having ability to inhibit conidia production over 90% have been demonstrated (El-Sheshtawi et al. 2014). Ability of *P. oxalicum* inhibiting *F. oxysporum* mycelium and subsequent reduction in wilt incidence in Tomato plants are also reported by various researchers (Duijff et al. 1998, Larena et al. 2003, Shishido et al. 2005). There are limited studies on the activity of *Penicillium* sp. over *S. rolfsii*. Hadar & Gorodecki (1991) observed a 90% reduction in the production of sclerotia by *Sclerotium rolfsii* within seven days of mixing of *Penicillium* sp. in peat thus effecting the germination of sclerotia and further mycelia progression. Further evaluation of the bio-agent for rhizosphere competency revealed good saprophytic ability but a low colonising frequency. Numerous studies

on microbial density and rhizosphere competence with respect to plant species have been evaluated by various researchers (Papavizas 1967, Wells et al. 1972, Newman & Bowen 1974, Chao et al. 1986). A low percent of colonisation by *P. multicolor* with successive depth levels highlighting the importance in field success stresses on application modifications so as to efficiently establish in the natural conditions.

The present work gave an overview on the interactions and rhizosphere compatibility of *P. multicolor*. Hossain et al. (2007) studied root colonising ability of PGPF *Penicillium simplicissimum* GP17-2 and observed resistance induction in host plants. In tomato plants infested with *Fusarium oxysporum* f. sp. *lycopersici* on prior treatment with *Penicillium oxalicum* observed to induce resistance against the pathogen (Sabuquillo et al. 2005, 2006). Shishido et al. (2005) for non-pathogenic strains of *Fusarium oxysporum* reported the bio-agent was more effective when applied under sterile seedbeds, compared with non-sterile soil, as a decrease in soil microbes competition enhanced bio-agent activity. Furthermore, the bio-control agents were more effective when antagonist propagules exceeded that of pathogens. Similar observations were observed in the present analysis when the magnitude of the bio-agent was increased five times that of pathogen suppressed pathogen activity near the root surface.

Antagonistic affectivity of members of *Penicillium* genus against several plant pathogens have been reported (Ma et al. 2008, Sabuquillo et al. 2010, Sempere & Santamarina 2010). The activity of *Penicillium* members performs different actions against various pathogens which involve hyphal interactions, production of various metabolites and in some cases inducing resistance on host plants (Samson 2004, Samson et al. 2009, Houbraken et al. 2010, Kim et al. 2012). However, proper identification of *Penicillium* sp. and management strategies need to standardised for its effectiveness in field conditions (Peterson et al. 2011, Varga et al. 2011). Hence, understanding antagonism mechanism and modifying development protocols need to be aimed before application in natural ecosystems (Heydari & Pessarakali 2010, Oliveira et al. 2015).

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In vitro evaluation of *Trichoderma* species for antagonistic activity, fungicide tolerance and competitive saprophytic ability

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Abstract

Soil microbe interactions directly or indirectly affect plant health and soil quality. Plant growth-promoting and bio-control microorganisms have emerged as safe alternatives to chemical pesticides. *Trichoderma* species are known to exhibit antagonistic activity against a number of plant pathogenic organisms. The present study aims to understand antagonistic, pesticide tolerance and rhizosphere competence of three *Trichoderma* species namely *Trichoderma harzianum*, *T. koningii*and *T. pseudokoningiiin vitro*. *Trichoderma* sp. were subjected against root wilt and rot causing pathogens namely *Fusarium oxysporum* and *Sclerotiumrolfsii*via dual culture and exhibited effective antagonistic activity. Dithane M-45 75% W. P. fungicide at different concentrations viz. 50,100,150,200,250,300 ppm was tested by poison food method and *Trichoderma harzianum* and *T. koningii*exhibited tolerance to fungicide at 300 ppm. Fungal cultures, 2 x 10^6spore suspensions were analysed for saprophytic ability. 40 ml of *Trichoderma* sp. conidial suspensions and colonization of paddy straw segments at 1cm apart from the bottom of the sterile plastic cup filled with 200 g of autoclaved potting medium up to 7-cm length was determined at 10 and 21 days of incubation. *Trichoderma harzianum* and *T. koningii*exhibited good saprophytic and colonization ability. Both *T.harzianum* and *T. koningii* was isolated at 7cm depth with colonization frequency of 100% and 88% respectively whereas *T. pseudokoningii* colonized till 4cm depth with isolation frequency of 38% at 21 days of incubation. Most of the *Trichoderma* species show effective *in vitro* antagonistic ability but success in field depends on colonization efficiency. Thus present study details on applicability and necessary modifications for field triumphs of biological agents.

Key words – Bio – control – Colonization – Fungicide – Saprophytic ability

Introduction

Trichoderma sp. are habitual in soil and root ecosystems and have been demonstrated as parasites of several soil borne phytopathogens, plant growth enhancers and also inducers of defense responses (Chang et al. 1986, Windham et al. 1986, Lorito et al. 1994, Harman et al. 2004, Vinale et al. 2009, Ha 2010). A number of commercially available compounds against numerous fungal pathogens involve in the use of *Trichoderma* spp. (Jash 2006). Most of the *Trichoderma* sp. interact with phytopathogens includes competition, myco-parasitism (Papavizas 1985) and antibiosis i.e. production of cell wall degrading enzymes and secondary metabolites (Sivan et al.1984).

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The potentiality of *Trichoderma* sp.to parasitize destructive plant pathogens have beckoned attention of agricultural scientists, farmers, policy makers worldwide and a vast information on biological control of plant pathogens by *Trichoderma* have accumulated in the recent past (Weindling 1932, Bliss 1951, Rifai 1969, Samuel 1996, Mukherjee et al. 2013, Jaklitsch 2014, Bissettet al. 2015).

Increase in world population demands for high productivity and this necessitated indiscriminate application of chemical fertilizers and in effect has led in the development of resistance of pathogens and pests. Their persistent use affected environment and lead in quest for an alternative approach for eco-friendly management for sustainable production (Denholm & Rowland 1992, Leroux et al. 2002).

Prospects of biological control by the genus *Trichoderma* as a promising bio-control agent, has been described (Morsy et al. 2009, Sabalpara et al. 2009). However the effectiveness of different isolates of *Trichoderma* showed considerable *in vitro* and *in vivo* variations stressing on the selection of successful isolates against particular pathogens (Biswas & Das 1999, Ramezani 2008).

Trichoderma sp. growth and functional attributes have been vastly studied on artificial culture media but their correlation between various growth responses on agar and in soil is vague (Cook & Baker 1983). The lack in the understanding of the ecology of these bio-control agents and also the variability and complexity in environment and field soil might have limited *Trichoderma* sp. as successful bio-control agents in field conditions (Lewis &Papavizas 1991). The present objective was to understand the saprophytic competency of *Trichoderma* spp. namely *T.harzianum*, *T.koningii* and *T.pseudokoningii in vitro*.

Materials & Methods

Isolation and Purification of *Trichoderma* sp.

Trichoderma sp. namely *T. harzianum*, *T. koningii* and *T. pseudokoningii*were obtained from Forest Pathology Department, Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala. *Trichoderma* cultures were purified on antibiotic amended Potato Dextrose Agar medium (PDA) and incubated for 5-7 days at 25±2^{o}C.The cultures were identified morphologically referring standard manuals (Barnett 1972, Gams &Bisset 1998).

In vitro antagonism by dual culture technique

In vitro antagonism of *Trichoderma* sp. were studied against root wilt and rot causing pathogens namely *Fusarium oxysporum* and *Sclerotium rolfsii* via dual culture method (Gopalakrishnan et al. 2011) on PDA medium.Fungal cultures were obtained from Forest Pathology Department, Kerala Forest Research Institute (KFRI), sub-cultured on antibiotic amended PDA medium and characterized morphologically referring standard manuals (Barnett 1972, Leslie &Summerell 2006). Seven mm diameter discs of selected fungal pathogens and *Trichoderma* sp.were taken from the actively growing edge of five-day-old cultures using a cork borer. Antagonistic activity was evaluated by inoculating the pathogen at one side of the Petri plate and *Trichoderma* sp.at opposite side of the same plate by leaving 3-4 cm gap. The control plates were inoculated with the pathogens and the antagonist separately. The plates were incubated for ten days and observed for dual culture activity. The percentage inhibition of radial growth of fungal plant pathogens was calculated using formula given by Vincent (1947).

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

Where,
R1= Radial growth of fungal pathogens in control

R2= Radial growth of fungal pathogen in dual culture

In vitro fungicide resistance of *Trichoderma* sp. by Poisoned Food Method

Poison food method was used to test the *in vitro* efficacy of *Trichoderma* sp. against Dithane M-45 (a. i. Mancozeb [dithiocarbamates group of compounds] 75% W. P.) fungicide at different concentrations viz. 50,100,150,200,250,300 ppm (Nene &Thapliyal 1993). Different concentrations of fungicides were supplemented in a conical flask containing 100 ml molten PDA medium. The flasks containing poisoned medium were well shaken to get uniform mixture of fungicide and 10-15 ml of medium were poured in each sterilized petri-dishes. Seven mm discs of fungal mycelium were inoculated at the center of fungicide amended PDA plates and incubated at 25 ± 2°C for 5- 7 days. The plates without the fungicide served as control. After 7 days of incubation the radial growth of the mycelium was measured. The percent growth inhibition of the pathogens over control was calculated using formula given by Vincent (1947).

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

Where,
R1= Radial growth of fungal pathogens in control
R2= Radial growth of fungal pathogen in dual culture

In vitro Competitive Saprophytic Ability

Preparation of fungal inoculum

Trichoderma sp. were cultured in PDA medium for 7 days at 25±2^{o}C. Freshly grown cultures were inoculated in Potato Dextrose Broth (PDB) were kept at incubator shaker for 14 days at 25±2^{o}C. Mycelial mat was separated out by filtering through Whatsman No.1 filter paper, dried, grounded using mortar and pestle and centrifuged at 10000 rpm for 15 min to remove hyphal debris. Conidial suspensions thus obtained were then suspended in sterile distilled water and the concentration was adjusted to 2×10^6.

Testing saprophytic competency

Saprophytic ability of the *Trichoderma* sp. was tested by the Cambridge method (Garret 1970). Freshly harvested paddy straws were cut in to 1-cm long segments and autoclaved. Sterile plastic cups procured from the market were perforated at the bottom and plugged with sterile cotton pads. These cups were filled with 200 g autoclaved potting medium up to 7-cm length of the cup and placed with paddy straw segments at 1cm apart from the bottom of the cup. Eighteen autoclaved straw pieces were placed in a radial fashion and sterile potting medium was over laid up to 8-cm length of the cup. 40 ml of conidial suspensions were poured over the potting medium separately and each set was placed in an individual plastic tray containing sterile distilled water. The cups were not watered from the top but the potting medium in the cup was allowed to imbibe water only through capillary action from the holes at the bottom of the cup. Colonization of paddy straw segments by *Trichoderma* sp. was determined at 10 and 21 days of incubation.

Isolation of *Trichoderma* sp.

Paddy straw segments removed after regular intervals of incubation were washed in slow running tap water, then twice in sterile distilled water and placed on antibiotic amended PDA medium at 25±2^{o}C for 14 days. The fungal colonies developing from these segments were identified and compared with the characteristics of the original colony culture. Percent colonization by *Trichoderma* sp. at different depth levels at given time was determined.

Results

Antagonistic activity

Dual culture analysis of *Trichoderma* sp. against the pathogens: *Fusariumoxysporum* and *Sclerotiumrolfsii* resulted good antagonistic activity though the percent of inhibition varied among the species. *T. harzianum* exhibited greater inhibitory activity (>66%) against both the pathogens followed by *T. koningii* (>64%) and *T. pseudokoningii* (>61%) (Table 1).

Table 1 Antagonistic activity of *Trchoderma* sp. against root disease causing fungal pathogens

Sl. No.	***Trichoderma* sp.**	**Percent growth inhibition of fungal species against root wilt and rot pathogens**	
		Fusarium oxysporum	***Sclerotiumrolfsii***
1	*T. harzianum*	66.63±0.64[1]	67.14±0.28[1]
2	*T. koningii*	64.56±0.44[1]	65.18±0.68[1]
3	*T. pseudokoningii*	61.88±0.66[1]	61.28±0.18[1]

[1] Percent inhibition mean value and standard deviation

Fungicide tolerance

Trichoderma sp. were subjected for their efficacy to tolerate fungicide Dithane M-45 at different concentrations (50,100,150,200,250,300ppm) respectively. Many *Trichoderma* species has an innate resistance to many fungicides but resistance levels vary with the fungicide. The study showed variable tolerance to different fungicide concentrations by *Trichoderma* sp. where *T. harzianum* exhibited 100% tolerance followed by *T. koningii* 88% at 300 ppm on the other hand *T. pseudokoningii* exhibited 28% tolerance at 250 ppm and 0% tolerance at 300 ppm (Fig. 1).

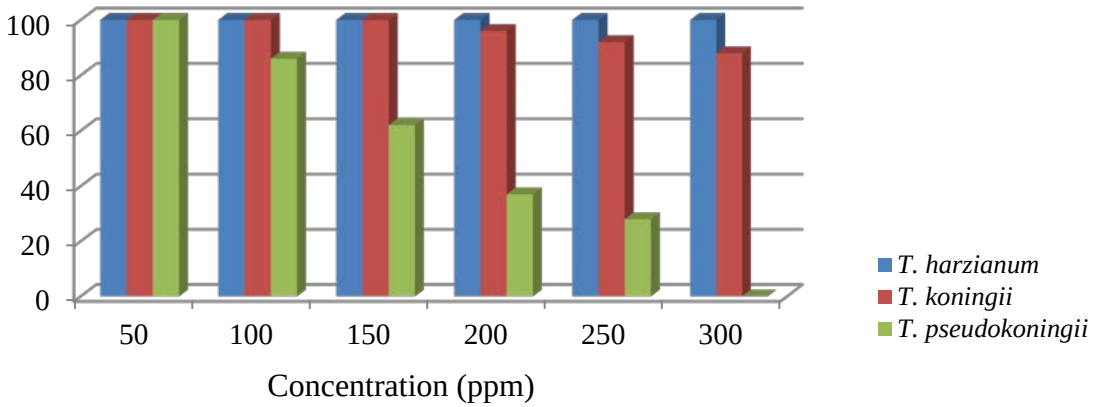

Fig. 1 – Effect of Fungicide on *In vitro* Growth of *Trichoderma* sp.

Competitive saprophytic ability

Paddy straw segments infested with *Trichoderma* sp. were subjected for saprophytic and colonizing ability at various levels of depths after 10 and 21 days of incubation (Fig. 2). Isolation of fungi from dead plant materials inhumed in soil provides information on the ability of fungi recovered as potential saprophytes (Garret 1970). *Trichoderma harzianum* and *T. koningii*were found to exhibit good saprophytic and colonizing ability.

Saprophytic and Colonization ability

Saprophytic ability of *Trichoderma* sp. was analysed after 10 and 21 days of incubation. The current research effort was to assess the competency of potential rhizosphere inhabitant *in vitro*. The study demanded certain criteria viz. quantitative analysis of *Trichoderma* sp. densities at each depth level, use of raw soil to maintain ecological conditions, no watering in order to prevent the

possible leaching out of spores. The results showed *T. harzianum*and *T. koningii*colonised at 6 and 5 cm depth respectively where as*T. pseudokoningii*was isolated at a depth of 3cm after 10 days of incubation. At 21 days of incubation, *T. harzianum*and *T. koningii*was isolated at 7 cm depth where as*T. pseudokoningii*was able to colonise only up to 4cm depth (Fig. 3).

Fig. 2 – Competitive saprophytic and colonizing ability of *Trichoderma* sp.

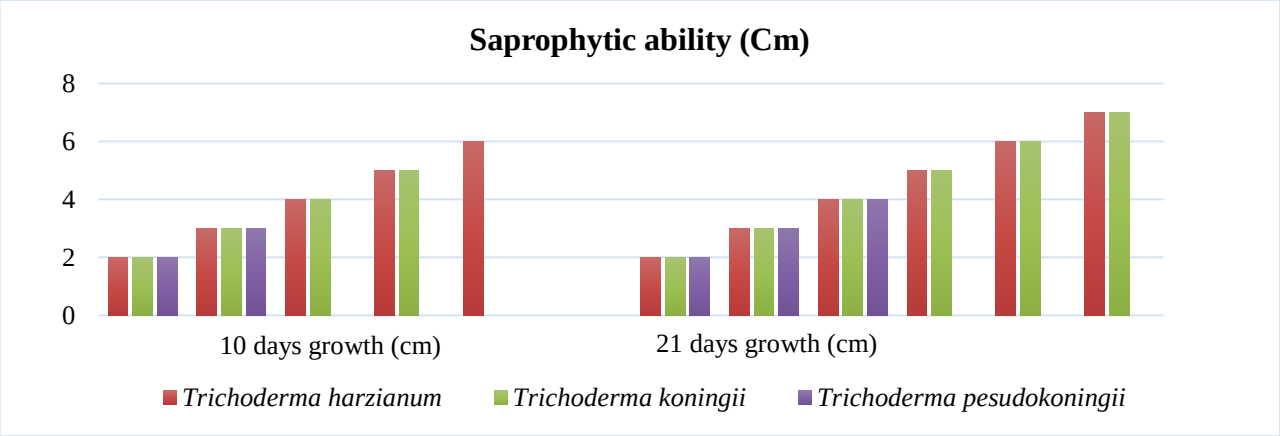

Fig. 3 – Saprophytic ability of *Trichoderma* sp. at various levels of depth at 10 and 21 days of incubation

Similarly colonization frequency of paddy straw segments by *Trichoderma* sp. was also enumerated at various levels of depths. *T. harzianum* exhibited resulted in 100% and 88% colonization frequency for *T. harzianum* and *T. koningii* respectively at a depth level of 7cm whereas *T. Pseudokoningii* showed 38% colonization frequency at 4 cm depth after 21 days of incubation (Fig. 4). *Trichoderma* sp. tested in this experiment showed variation in the competency among the species and also the time and magnitude of their colonization. *T. harzianum* followed by *T. koningii* exhibited better saprophytic activity even at 10 days of incubation whereas *T. pseudokoningii* failed to colonize beyond 4 cm depth even after 21 days of incubation. The

colonization frequency of the species in the given days also showed variations for *T. harzianum*followed by *T. koningii*as efficient colonizer their by stressing detailed analyses for field triumphs. Thus competitive saprophytic ability is relevant in determining the success of biocontrol agents which are affected by a number of biotic and abiotic factors.

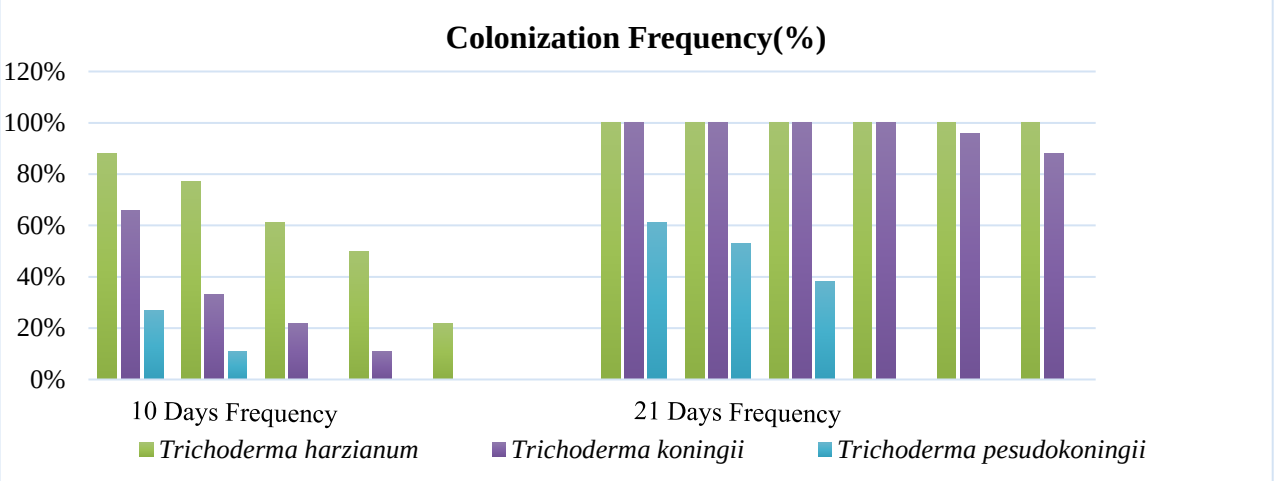

Fig. 4 – Colonization frequency of *Trichoderma* sp. at 10 and 21 days of incubation

Discussion

Trichoderma sp. is widely isolated soil fungi and has gained great significance as bio-control and plant growth enhancer (Papavizas 1985, Sreenivasaprasad&Manibhushanrao 1990, Ha 2010). A number of species of *Trichoderma* in the management of various phyto-pathogenic fungi *in vitro* have been reported by various workers (Chet et al. 1997, Dubey 2002, Dubey 2003, Poddar et al. 2004). Nonetheless, *in vitro* successes of *Trichoderma* sp. are not always positively antagonistic *in vivo* (Campanile et al. 2007) stresses on the ability to survive and colonize the applied soil conditions influenced by nutrient requirements and temperature has been reported by various researchers (Danielson & Davey 1973, Tronsmo& Dennis 1977). Success of *Trichoderma* sp. as a bio-control agent implies the ability to cope up with the biotic and abiotic conditions and thereby minimising the excess application of chemical pesticides. Integrated approach asks for the possible compatibility of *Trichoderma* strains to the chemicals (Kredicset al. 2003) as their combined application has attracted much consideration in order of synergistic approach in the management of soil-borne pathogens (Locke et al. 1985).

Present study, in dual culture resulted *Trichoderma* sp. active against root pathogens *Fusarium oxysporum* and *Sclerotiumrolfsii*. The ability of *Trichoderma* sp. to inhibit mycelial growth of various soil borne pathogens namely *Fusarium* sp., *Rhizoctonia* sp. *Sclerotium* sp., *Phytophthora* sp., *Macrophominaphaseolina* etc. have been reported by various authors (Kirik&Steblyuk 1974, Henis et al. 1983, Patale&Mukadam 2011, Kakde&Chavan 2011). Also, the evaluation to tolerate the fungicide Dithane M-45 resulted in variant tolerance capability of three *Trichoderma* sp. Dithane M-45 was found safe to incorporate with *T. harzianum* (Parabet al. 2009, Saxena et al. 2014) and *T. koningii* at prescribed concentrations but was found to be negatively correlated with *T. pseudokoningii*. Studies with fungicides Benomyl, Topsin-M and Carbendazim for *T. pseudokoningii* suppressed growth of the fungi but for *T. harzianum*, *T.longibrachiatum*and *T. viride* prescribed concentrations of the fungicides found to be tolerating (Khan&Shahzad 2007). Fungicide composition and dosage is critical in determining compatibility of bio-agent in field application (Monte 2001).

In order to minimise chemical pollution an integrated strategy whereby fungicide compatible bio-agent proves to be effective in the management of pathogens can be practised (De Cal et al. 1994) again delimited by rhizosphere survivability. A number of preliminary studies based on population densities of microbes in rhizosphere and non-rhizosphere have been done to evaluate the

rhizosphere competence of microbes with respect to plant species (Papavizas 1967, Wells et al. 1972, Newman & Bowen 1974, Chao et al. 1986). *T. harzianum* and *T. koningii*exhibited higher colonization frequency indicating potentiality of these species at rhizopshere regions. The ability of *Trichoderma* sp. can be attributed to the enzymatic degradation of cellulose on or near the root surface but has not been justified (Garret 1970, Foster et al. 1983). Although bio-control agents exhibit good antagonistic activity but lacks rhizosphere and rhizoplane competency which are also influenced by biotic factors (Papavizas 1967). The current work highlighted on the fact that different antagonistic evaluating strategies need to be carried out thereby an integrated approach with minimal chemical application and higher rhizosphere competent strains can be selected as potential biological agents.

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RESEARCH ARTICLE

RHIZOPLANE AND RHIZOSPHERE MYCOFLORA OF *CYNODON DACTYLON* (L.) Pers. GRASS AND THEIR ANTAGONISTIC ACTIVITY AGAINST FUNGAL DISEASES OF MAHOGANY SEEDLINGS

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ABSTRACT

Microorganisms found associated with plants reside in rhizosphere, rhizoplane, phylloplane and inside plant tissues. These microbes play an important role in various stages of plant growth and development. They also find application as biocontrol agent against various pathogens of plants and can be used as an alternative to chemical fungicides. Grasses and their roots are major source for microbial interaction and get rejuvenated therefore the chances of colonization of microbe is high. In view of this, the present work has been carried out to study the antagonistic activity of fungal organisms associated with rhizosphere and rhizoplane regions of grass *Cynodon dactylon* from Northern Kerala parts of Western Ghats in different seasons against fungal diseases of Mahogany seedlings of Central Nurseries of Kerala *in vitro*. Rhizosphere and rhizoplane fungi included *Aspergillus niger*, *Curvularia* sp. *Fusarium oxysporum*, *Fusarium* sp. *Mucor* sp. *Penicillium* sp. *Rhizopus* sp. *Trichoderma harzianum* and NSF. Antagonistic activity was conducted against the fungal species associated with the root rot diseases of mahogany seedlings caused by *Fusarium oxysporum* and *Fusarium monoliforme,* damping off by *Sclerotium rolfsii* and foliar diseases caused by *Colletotrichum gloeosporiodes, Alternaria alternata, Curvularia lunata,Cladosporium cladosporioides* and *Pestalotiopsis* sp. *Trichoderma harzianum* was found to be effective controlling agent *in vitro*.

Key words: *Cynodon dactylon,*Rhizosphere, Rhizoplane, *Trichoderma harzianum*, Antagonism

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INTRODUCTION

Mahogany (*Swietenia macrophylla* King) belongs to family Meliaceae known for its timber. The out-planting success greatly depends upon their seedling health which are hindered majorly by fungal pathogens. Forest nursery diseases- collar rots, damping-off, root rots, foliar diseases and blights cause great damage to seedlings. Fungicides due to their assured results are being practised but indiscriminate use led to the development of fungicidal resistance inpathogens and toxicity to non – target organisms (Tjamos *et al.*, 1992). This led into the search for an alternative, risk free strategy for the management of diseases employing the use of biocontrol micro-organisms. The role of soil fungi is complex, helping in nutrient cycling, plant growth and development (Thorn 1997, Bridge and Spooner 2001, Martin *et al.* 2001). Rhizosphere and Rhizoplane inhabiting micro-organisms competitiveness for water, nutrients and space plays an important role in the growth and ecological fitness of their host (Hartmann *et al.* 2009). Grasses which forms an important part of ecosystem keeps rejuvenating in every growing season, homes a number of diverse microorganisms. Fungal communities of anamorphic and teleomorphic ascomycetes, zygomycetes and certain non-sporulating fungi resides in rhizosphere and rhizoplane regions (Vasanthakumari *et al.*, 2007). These microorganisms can be used for their antagonistic potential. The present work has been carried to characterize the rhizosphere and rhizoplane mycoflora associated with grass *Cynodon dactylon* and study their antagonistic activity against fungal pathogens *in vitro.*

MATERIALS AND METHODS

Isolation of pathogenic fungi: Disease survey was carried out in Central nurseries(Chettikulam, Kannavam, Kulathupuzha and Nilambur) of Kerala. Infected Mahogany seedling samples were collected, washed thoroughly in running tap water, surface disinfected with $HgCl_2$ (0.0001%), blotted and were inoculated on streptomycin amended PDA medium. Petridishes were incubated under 12/12 hr alternate light regime at 25±2° C. Fungal colonies were isolated and identified on the basis of colony morphology, mycelium, fruiting-body, spore shape and size by referring standard manuals (Arx, 1981; Ellis and Ellis, 2001; Gilman, 1994; Ramarao and Manoharachary, 1990; Subramanian, 1983).

Isolation of rhizosphere andrhizoplane fungi: Rhizosphere and rhizoplane samples of *Cynodon dactylon* were collected from Northern Kerala parts of Western Ghats. Root samples were washed thoroughly in slow running tap water, surface disinfected with $HgCl_2$ (0.0001%), blotted and were cut into 1 cm segments.

Table 1. Disease symptomology and fungal pathogens associated with diseased parts of Mahogany seedlings from central nurseries of Kerala

Sl. No.	Fungal pathogens	Disease symptoms and description	Central Nurseries of Kerala			
			KNM	NBR	CKM	KPZ
1	*Alternaria alternata*	Leaf blight: greyish spot which coalesced to form necrotic lesions	+	-	+	-
2	*Cladosporium cladosporioides*	Leaf spot: brown to black colour spot	+	-	-	+
3	*Colletotrichum gloeosporioides*	Leaf spot and blight: irregular spot, light to dark brown in colour, with a pale margin	+	+	+	+
4	*Curvularia lunata*	Leaf spot: olivaceous brown spot	+	+	+	+
5	*Fusarium monoliforme*	Root rot and Leaf spot	+	+	-	-
6	*Fusarium oxysporum*	Root rot	+	+	+	+
7	*Pestalotiopsis* sp.	Leaf spot: brown to dark brown spots, occasional fructifications developed	+	+	+	+
8	*Sclerotium rolfsii*	Damping off	-	+	-	+

KNM – Kannavam, NBR – Nilambur, CKM – Chattikulam, KPZ – Kulathu[puzha; '+'presence, '-'absence

Table 2. Fungal species from rhizosphere and rhizoplane regions of *Cynodon dactylon* grass

Sl. No.	Fungal Organisms	*Cynodon dactylon* grass	
		Rhizoplane region	Rhizosphere region
1	*Aspergillus niger*	+	+
2	*Curvularia* lunata	+	-
3	*Fusarium oxysporum*	+	+
4	*Fusarium monoliforme*	-	+
5	*Fusarium* sp. (3)	+	-
6	*Mucor* sp.	+	+
7	*Penicilliumchrysogenum*	-	+
8	*Penicilliumoxalicum*	+	+
9	*Rhizopus* sp.	+	+
10	*Trichoderma harzianum*	+	-
11	NSF-1	+	-
12	NSF-2	-	+

'+'presence, '-'absence

Table 3. Rhizosphere and rhizoplane fungi of *Cynodon dactylon* showing antagonism to fungal pathogens isolated from Mahogany seedlings *in vitro*

Sl. NO.	Fungal organisms isolated from *Cynodon dactylon*	Antagonistic activity of fungal isolates against fungal pathogens isolated from mahogany seedlings (%)[1]							
		F.o	*F. m*	*S. c*	*A. a*	*C. g*	*C. c*	*C. l*	*P.* sp.
1	*Aspergillus niger*	30.54±0.5[2]f[3]	33.23±0.7c	35.87±0.9e	36.76±0.5f	40.42±0.1g	37.14±0.2h	32.56±0.9f	39.87±0.1f
2	*Curvularialunata*	20.59±0.8b	32.56±0.8b	33.24±0.3c	17.98±0.1b	16.67±0.1b	11.98±0.0e	23.57±0.4c	22.70±0.2b
3	*Fusarium oxysporum*	23.53±1.1c	51.16±0.6f	32.45±0.4c	24.51±1.2e	29.87±1.1e	12.50±0.4e	21.43±0.2b	29.50±0.8d
4	*Fusarium monoliforme*	32.35±0.5g	39.53±1.0de	34.53±0.2d	24.65±0.2e	19.97±0.0c	6.00±0.08b	29.91±0.8e	25.00±0.4c
5	*Fusarium* sp. (3)	17.65±0.3a	41.86±0.5e	42.01±0.3f	19.87±0.0c	23.54±1.1d	8.17±0.06d	24.58±0.4d	34.09±0.5e
6	*Penicilliumchrysogenum*	27.86±1.2d	32.43±0.5b	35.67±0.9de	39.76±0.2h	33.56±0.0f	28.76±0.3g	36.98±0.2h	43.64±0.7h
7	*Penicilliumoxalicum*	29.14±0.4e	37.32±0.1cd	33.29±0.6c	38.74±0.2g	34.42±0.6f	25.44±0.0f	33.57±0.6g	41.77±0.5g
8	*Trichoderma harzianum*	73.20±0.2h	77.50±0.9g	72.09±0.1g	70.96±0.4i	82.14±0.2h	72.50±0.3i	71.16±0.5i	71.00±0.3i
9	NSF-1	17.65±0.7a	23.26±0.2a	6.78±1.16a	13.21±0.9a	12.87±0.2a	7.14±0.87 c	16.73±0.4 a	13.64±0.6 a
10	NSF-2	17.63±0.7a	32.56±0.4b	27.89±0.8b	21.86±0.1d	19.87±1.1c	5.04±0.77a	21.32±0.4b	29.55±0.3d

[1]Data is an average of three replicates, [2] Standard deviationand [3] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference. Among the fungal isolates tested against various pathogens, *Trichoderma harzianum* exhibited higher antagonistic activity.
F.o- Fusarium oxysporum, F. m- Fusarium monoliforme, S. c- Sclerotium rolfsii, C. g- Colletotrichum gloeosporioides, C. c- Cladosporium cladosporioides, C. l- Curvuaria lunata, P. sp.- *Pestalotiopsis* sp.

Root segments were inoculated on streptomycin amended PDA medium maintaining equal distance. Fungal colonies were identified as described earlier. Rhizosphere samples were subjected for dilution plate technique. The samples of desired dilution 10^{-3} and 10^{-4} were inoculated on PDA medium and were incubated for 5-7 days. Fungal colonies were isolated and identified as described earlier.

***In vitro* antagonism by dual culture technique:** Pathogenic fungi isolated from mahogany seedlings, and the test fungi from rhizosphere and rhizoplane of *Cynodon dactylon* grass were cultured on their respective medium under 12/12 hr light and dark cycle at 25±2°C for five days. Five mm diameter disc of selected fungi from grass and test pathogenwere taken from the growing edge of a five-day-old pure culture using a cork borer. The control plates wereinoculated with the pathogen and antagonists separately. Petri-plates were incubated at 25±2°C and daily growth measurements of fungal colonies were recorded for seven days.

The percentage inhibition of radial growth of the pathogen was calculated using formula (Vincent, 1947).

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 – Test organism in Control
R_2 – Test organism in Dual culture

Statistical analysis: Antagonistic ability of fungal isolates were statistically analysed and compared by Duncan's Multiple Range Test (DMRT) using SPSS (ver. 21) software developed by IBM Corporation.

RESULTS AND DISCUSSION

Disease survey of mahogany seedlings conducted in Central nurseries of Kerala yielded seven genera and eight species of fungi and showed the disease symptoms were root rot, damping off and foliar infections (Table 1).

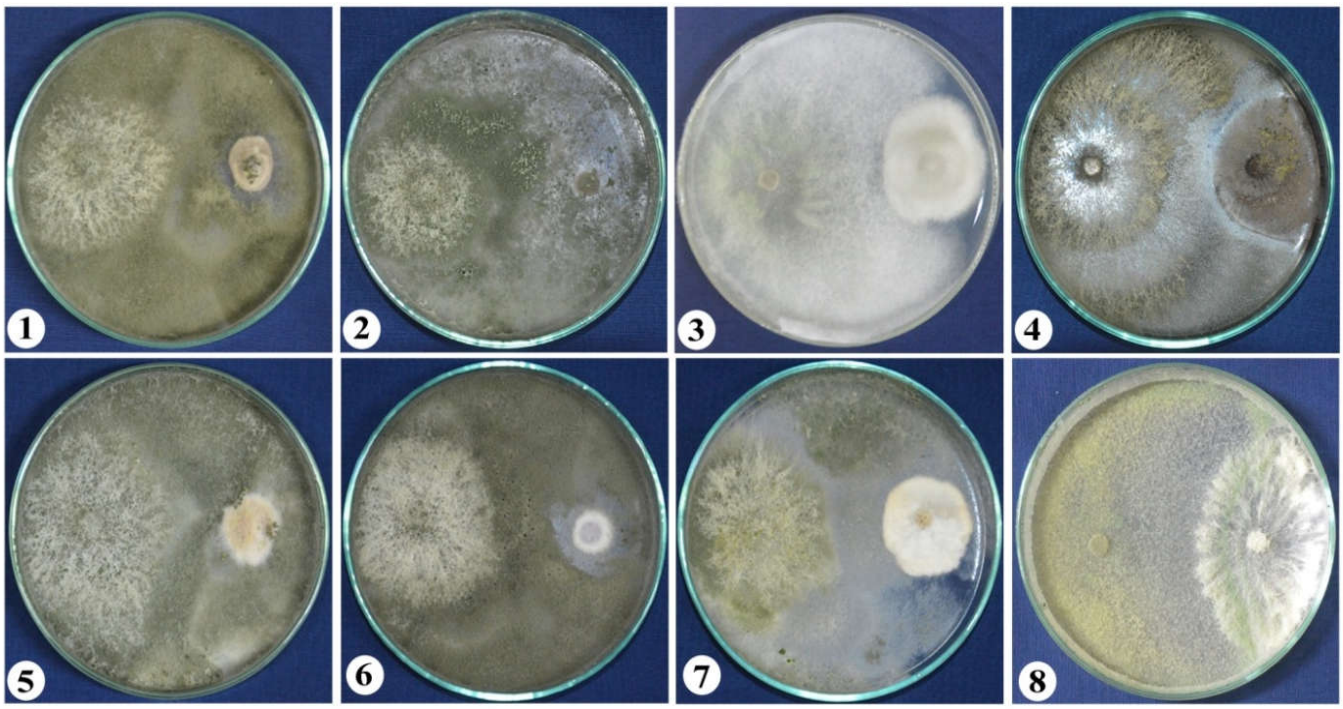

Fig 1-8. Antagonistic activity of *Trichoderma harzianum* against fungal pathogens (1) *Alternaria alternata*, (2)*Cladosporiumcladosporioides*, (3) *Colletotrichum gloeosporioides*, (4) *Curvularia lunata*, (5) *Fusarium monoliforme*, (6) *Fusraium oxysporum*, (7) *Pestalotiopsis* sp. *and* (8) *Sclerotium rolfsii*

Root rot caused by *Fusarium oxysporum* and foliar diseases caused by *Alternaria alternata*, *Cladosporium cladosporioides*, *Colletotrichum gloeosporioides, Curvularaia lunata*, *Pestalotiopsis* sp., damping-off by *Sclerotium rolfsii* and root rot and leaf spot caused by *F. monoliforme*were found to be major disease causing fungal pathogens in Central nurseries of Kerala. Various diseases in forest crops, Teak (Mohanan, 2001, 2011; Sharma *et al.*, 1985), *Acacia* (Sharma and Florence, 1996), *Albizia* (Sharma and Sankaran, 1987) have been reported. Various bio-control approaches have been practised in forest nurseries. Mohanan (2007) reported that *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* were effective against damping-off pathogens *Rhizoctonia solani* and *Cylindrocladium quinqueseptatum*. Rhizosphere and rhizoplane regions of *Cynodon dactylon* were associated with diverse mycoflora (Table 2). The antagonistic interaction of *Trichoderma harzianum* (Fig 1- 8) among the fungal isolates showed maximum inhibition activity (70 – 83%), other isolates tested were shown to be moderate to low activity against the pathogens (Table 3). Species of *Trichoderma* namely *T. koningii*, *T. harzianum* and *T. viride*, respectively have been studied for their antagonistic activity *in vitro* (Mathew and Gupta, 1998; Prasad *et al.*, 1999; Bunker and Mathur, 2001; Pandey *et al.*, 2005; Grosch *et al.*, 2007). The present work showed the potentiality of rhizosphere and rhizoplane mycoflora against various forest plant pathogens and can be used as an alternative to chemical fungicides.

Conclusion

Upsurge in the use of chemical agents and its potential threat to the ecosystem has led to foresee an alternate and eco-friendly strategy. Biological control has been practised with such an aim. Rhizosphere and rhizoplane regions of grasses homes diverse fungal organisms and can be used as biological weapons against various plant pathogens.

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Colletotrichum Diseases of Forest Nurseries and their Biological Management in vitro using Rhizoplane Mycoflora of Grasses

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Abstract: ***Microbes are the promising source for the biological management of many plant pathogenic organisms. Biocontrol agents are the viable alternative to chemical fungicides and also reduces developing fungicidal resistance in pathogens. Grasses and their roots are liable sources for microbial activity. The aim of the study was to isolate and identify major saprophytic rhizoplane mycoflora of selected perennial grass species Alloteropsis cimicina, Ischeamum indicum, Opplismenus compositus, Panicum repens and Perotis indica. Fungal species were isolated from rhizoplane regions by agar plate method in different seasons. Total 55 fungal isolates belongs to six genera, nine species of fungi and two were non-sporulating fungal isolates. The fungal isolates were identified and tested for their hypersensitive activity in sensitive plant species. Thirty six isolates out of 55, exhibited in vitro antagonism to phytopathogenic fungal species – Colletotrichum gleoesporioides. The fungus C. gleoesporioides is the major foliar pathogen cause anthracnose, leaf spot and blight are the major disease symptoms observed in nurseries. Among fungi tested Trichoderma harzianum was found to be more effective antagonistic activity against the pathogen in vitro.***
Key words: Perrenial grasses, Rhizoplane fungi, Colletotrichum gloeosporioides, Biological control, In vitro

I. INTRODUCTION

Forest nurseries established with the aim of providing healthy plant stocks is hindered by a number of factors. Diseases pose a major threat of which fungi forms a major pathogen for the successful production of seedlings and their by hindering planting programmes [1], [2]. Rots, Wilts, damping off and various foliar diseases are the major disease symptoms found in seedlings. Foliar diseases in plants are caused by a number of fungal pathogens among which *Colletotrichum* sp. are the major ones. *Colletotrichum gloeosporioides* Penz. causes anthracnose, leaf spot and leaf blight diseases on a wide variety of plants. *Colletotrichum gloeosporioides* is a common pathogenic fungi found more abundantly distributed in tropical and subtropical regions of the world than in temperate regions. A number of fungicides are available and indiscriminate usage resulting in environmental pollution has led in search of an alternative and eco-friendly methods.

Plant-microbe interactions especially between roots and microbes provide a wide array of opportunities to explore the complexities in association as well as their interaction in the growth and development. Rhizosphere and Rhizoplane regions supports a large number of microorganisms. The role of soil microbes is very complex, they help in nutrient cycling, provide nutrients to plants and stimulate plant growth [3], [4], [5].

Grasses form an important component of ecosystem which keeps rejuvenating in every growing season. They produce fibrous roots which homes abundance of diverse microbes. Rhizosphere and Rhizoplane regions supported populations of fungal communities of anamorphic ascomycetes, teleomorphic ascomycetes, zygomycetes and certain non-sporulating fungi [6]. A great diversity of rhizosphere and rhizoplane microorganisms have been described and also in many cases been used as bio-controlling agents. The present work has been carried out to study the antagonistic activity of rhizoplane fungi against *C. gloeosporioides* for their effectiveness *in vitro*.

II. MATERIALS AND METHODS

A. Isolation of pathogenic fungi

Disease survey was carried out in Central nurseries of Kerala located at Chettikulam, Kannavam, Kulathupuzha and Nilambur. Infected seedling samples were collected and were brought to the laboratory. The samples were washed thoroughly, blotted and were inoculated on antibiotic amended PDA medium. The associated fungal species were isolated and identified by referring standard manuals [7], [8], [9], [10], [11].

B. Isolation Of Rhizoplane Fungi

Rhizoplane fungi were isolated from roots of grass species *Alloteropsis cimicina, Ischeamum indicum, Opplismenus compositus, Panicum repens* and *Perotis indica*. Root samples were washed thoroughly in slow running tap water, blotted and were fragmented into 1 cm long segments. Root segments were inoculated on antibiotic amended PDA medium. Fungal colonies were isolated and identified by referring to standard manuals as described earlier.

C. Dual Inoculation Of Colletotrichum Gloeosporioides And Potential Antagonist On Pda

Dual inoculation of pathogen and an antagonist was set up. A 5 mm disc of pathogen with similar size of each potential antagonist was taken from the growing edge of five day-old pure culture using a cork borer. The control plates were inoculated for pathogen and antagonists separately. Three replications per treatment were set up for each pathogen and antagonist combinations. Inoculated petri-dishes were incubated at room temperature. Daily growth measurements of fungal colonies were taken for 7 days. The percentage inhibition of radial growth of pathogen was calculated using formula [12].

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 – Test organism in Control
R_2 – Test organism in Dual culture

D. Statistical Analysis

Antagonistic ability of fungal isolates were statistically analysed and compared by Duncan's Multiple Range Test (DMRT) using SPSS (ver. 21) software developed by IBM Corporation.

III.RESULTS AND DISCUSSION

Disease survey conducted in the Central Nurseries of Kerala resulted in the observation of seedling diseases, leaf spots and blights. A number of fungal species were isolated and among the isolates Colletotrichum gloeosporioides (Fig 1 & 2) was found to be a major pathogen producing symptoms appeared as irregular spot, light to dark brown in colour surrounded by necrotic margin (Table 1) (Fig 3 - 8). Rhizoplane fungi isolated from grasses - Alloteropsis cimicina, Ischeamum indicum, Opplismenus compositus, Panicum repens and Perotis indica resulted a total of 55 fungal isolates belongs to six genera, nine species of fungi and two were non-sporulating fungal isolates. The fungal isolates were identified and tested for their hypersensitive activity in sensitive plant species (Chilli, Tomato and Tobacco). Thirty six isolates out of 55, exhibited in vitro antagonism to phytopathogenic fungal species – Colletotrichum gleoesporioides. The fungal organisms namely Gliocladium, Chaetomium and Trichoderma have been known for their antagonistic activity [13], [14], [15]. The result showed the inhibition percentage of Trichoderma harzianum (Fig 3) to be highest (73%-78%). Other tested isolates were seen to be showing moderate antagonistic activity against the pathogen (Table 2) (Fig 9). Trichoderma sp. have been known for their antagonism interaction and various species of Trichoderma namely T. koningii, T. harzianum and T. viride, respectively have been studied for their antagonistic activity in-vitro [16] [17]. Colletotrichum sp. are known to cause various foliar diseases and their management with natural agents have been practised [18], [19], [20], [21], [22]. Biological agents against forest nursery diseases are being practised to some extent. [23] reported that Trichoderma viride, T. harzianum and Pseudomonas fluorescens were effective against damping-off pathogens Rhizoctonia solani and Cylindrocladium quinqueseptatum.

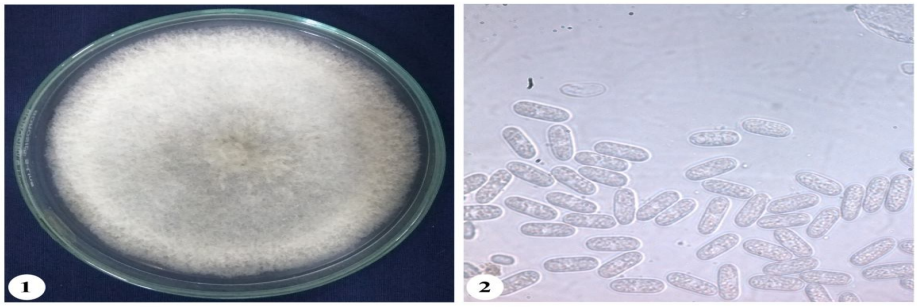

Fig 1 & 2. 1. Pure culture of Colletotrichum gloeosporioides & 2. Conidia of C. gloeosporioides

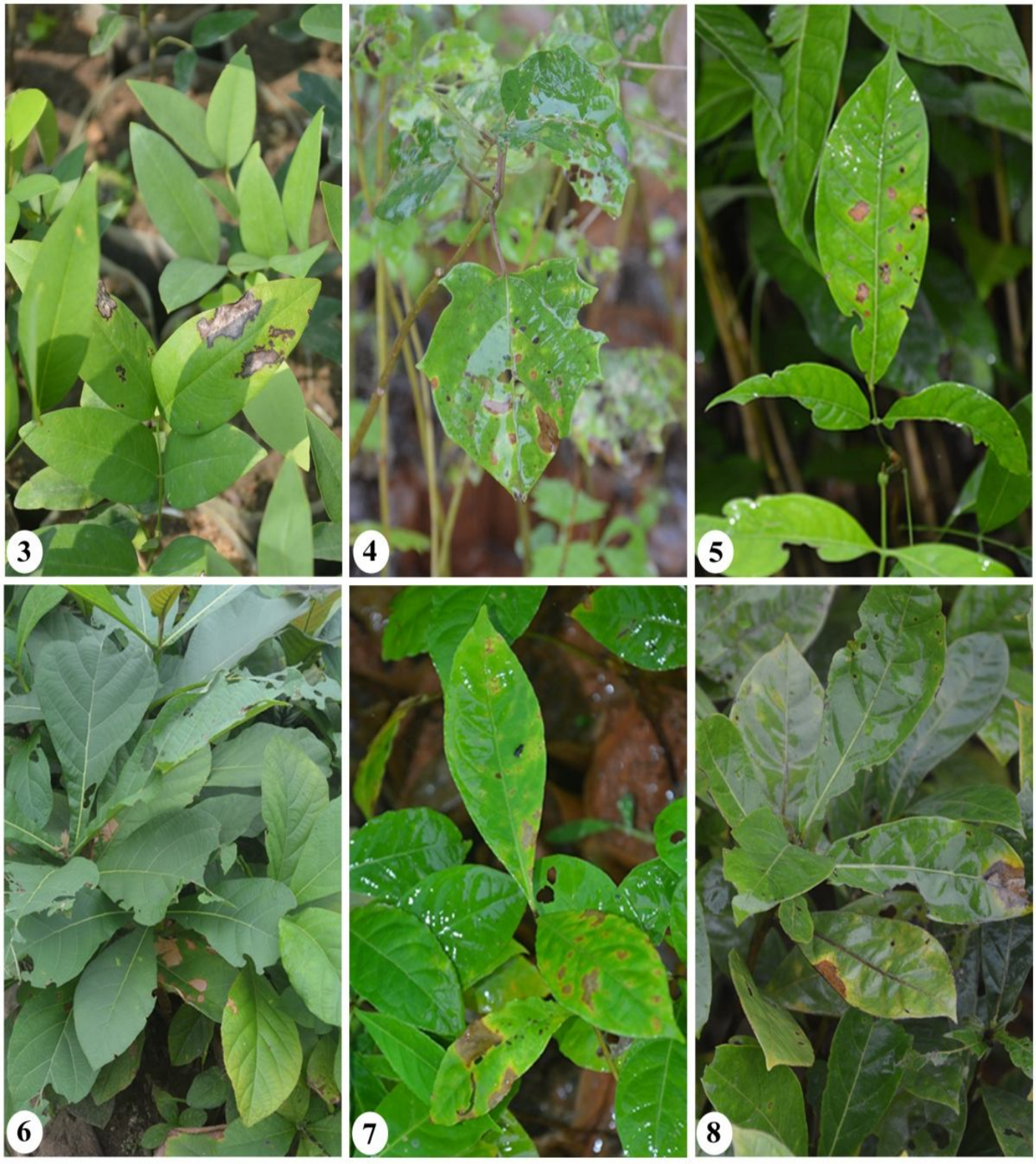

Fig 3-8. Leaf spot blight diseases of Colletotrichum gloeosporioides of 3. Cassia fistula, 4. Gmelina arborea, 5. Swetinia macrophylla, 6. Tectona grandis, 7. Terminalia bellirica and 8. T. arjuna.

Table 1. Colletotrichum gloeosporioides and associated fungi isolated from various seedlings from Central Nurseries of Kerala

Sl. No.	Tree species	Fungal diseases	Fungal organisms	Central nurseries
1	Acacia auriculiformis	Leaf spot / blight	Cladosporium Cladosporioides Colletotrichum gloeosporioides* Curvularia lunata Myrothecium roridum Pestalotiopsis sp.	KPZ
2	Acacia mangium	Leaf spot / blight	Cladosporium cladosporioides Colletotrichum gloeosporioides* Curvularia lunata Myrothecium roridum Pestalotiopsis sp.	KPZ
3	Aegle marmelos	Leaf spot	Alternaria alternata Colletotrichum gloeosporioides* Phoma sp.	CKM
4	Cassia fistula	Leaf spot	Colletotrichum gloeosporioides* Curvularia lunata Pestalotiopsis sp.	NBR
5	Gmelina arborea	Leaf spot	Colletotrichum gloeosporioides* Curvularia lunata	KNM, NBR, KPZ
6	Pongamia pinnata	Leaf spot	Bipolaris sp. Colletotrichum gloeosporioides*	KNM, NBR
7	Saraca asoka	Leaf spot	Colletotrichum gloeosporioides*	CKM
8	Swetinia macrophylla	Leaf spot / blight	Alternaria alternata Cladosporium cladosporioide s Colletotrichum gloeosporioides* Curvularia lunata Pestalotiopsis sp.	KNM, CKM
9	Tectona grandis	Leaf spot / blight	Alternaria alternata Cladosporium cladosporioide Colletotrichum gloeosporioides* Curvularia lunata Pestalotiopsis sp.	KNM, NBR, CKM
10	Terminalia arjuna	Leaf spot	Cladosporium cladosporioide s Colletotrichum gloeosporioides*	CKM
11	Terminalia bellirica	Leaf spot	Colletotrichum gloeosporioides* Pestalotiopsis sp.	KNM, KPZ
12	Thespesia populinea	Leaf spot	Colletotrichum gloeosporioides* Fusarium oxysporum	KNM

*Dominating Fungus associated with naturally infected plant materials
CKM- Chettikulam, KNM- Kannavam, KPZ- Kulathupuzha, NBR- Nilambur

Table2. Antagonistic activity of fungal species from rhizoplane regions of grasses against Colletotrichum gloeosporioides in vitro

Sl. No.	Fungal organisms	Antagonistic activity of fungal species (%)[1]				
		A. c.	I. i.	O. c.	P. r.	P. i.
1	Aspergillus flavus	0.00±0.00[2] a[3]	0.00±0.00 a	0.00±0.00 a	33.45±0.33 e	0.00±0.00 a
2	Aspergillus niger	0.00±0.00 a	30.67±0.56 d	0.00±0.00 a	35.77±0.24 f	39.67±0.34 f
3	Curvularia lunata	26.67±0.45 b	0.00±0.00 a	16.73±0.08 b	0.00±0.00 a	0.00±0.00 a
4	Fusarium oxysporum	0.00±0.00 a	21.43±0.11 c	40.48±0.75 g	21.43±0.14 b	42.67±0.61 g
5	Fusarium sp.	0.00±0.00 a	0.00±0.00 a	26.91±0.08 c	23.81±0.35 c	31.43±0.54 c
6	Penicillium chrysogenum	0.00±0.00 a	32.36±0.77 e	0.00±0.00 a	0.00±0.00 a	0.00±0.00 a
7	Penicillium sp.	29.43±0.74 cd	31.42±1.44 de	38.76±0.38 f	40.06±1.72 g	37.16±1.08 e
8	Phomopsis sp.	30.33±0.73 d	0.00±0.00 a	30.38±1.59 e	40.44±1.23 g	35.76±0.87 d
9	Trichoderma harzianum	73.30±1.35 e	75.78±0.85 f	75.77±0.77 h	77.87±1.30 h	74.76±1.05 h
10	NSF-1	30.13±1.09 d	19.05±1.06 b	28.75±0.40 d	29.76±0.91 d	25.68±1.10 b
11	NSF-2	28.57±1.05 c	19.05±0.63 b	27.75±0.39 cd	0.00±0.00 a	0.00±0.00 a

[1]Data is an average of three replicates, [2] Standard deviation and [3]Experiment was conducted in a factorial design. Means carrying same letters in a row are not significantly different (DMRT, $P_{0.05}$)

A. c. - Alloteropsis cimicina, I. i.- Ischaemum indicum, O. c.- Opplismenus compositus, P. r.- Panicum repens,
P. i. - Perotis indica

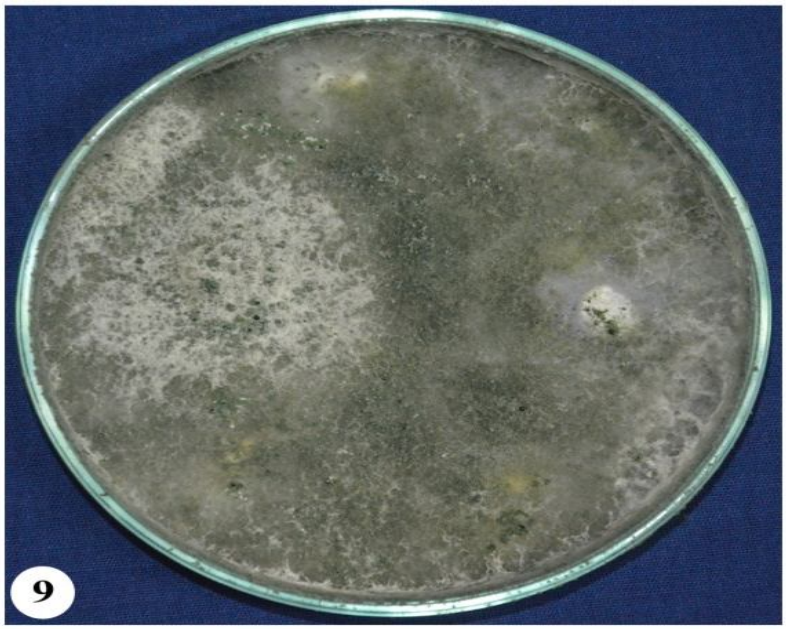

Fig 9. Antagonistic activity of Trichoderma harzianum against Colletotrichum gloeosporioides

IV. CONCLUSION

Shallow fibrous roots of grasses harbours a variety of microbes which are important for plants growth and development and can be possibly applied as an alternative for the management of plant diseases. The desirable demand of plant stock has led to the over dependence on chemicals for time bound supply of materials but the negative effects that they imply has not been stressed so far. The alternative strategy for environmental friendly applications now a days need more serious attention for sustainable management of natural resources. Natural biocontrol agents give an insight into the eco-friendly management of diseases thereby helping in growth and development. Among various natural agents, the fungus Trichoderma harzianum proves their antagonistic potential for their effective application as an alternative to the chemical control against a wide set of fungal plant pathogens [24]. Thus, the potentialities of such agents can also applied not only in agricultural field but in forestry sector also.

V. ACKNOWLEDGEMENTS

The authors are grateful to the Director, KFRI and to the field staffs of Kerala Forest Department at various Central Nurseries in the State.

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In vitro efficacy of *Trichoderma harzianum* against major fungal pathogens of Teak and Mahogony seedlings

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ABSTRACT

Trichoderma an asexually reproducing filamentous fungi commonly found in varied soil types in all climatic zones. Their ability to grow and multiply rapidly in various substrates makes it a good biocontrol agent. Six isolates of *Trichoderma harzianum* from rhizoplane regions of grasses *Cynodon dactylon* (CD-01, CD-02 and CD-03) and *Paspalum conjugatum* (PC-01, PC-02 and PC-03) were tested for their antagonistic activity against major fungal pathogens - *Fusarium oxysporum* and *F. solani* causing root rot and wilt, *Sclerotium rolfsii* causing damping off and *Colletotrichum gloeosporiodes, Curvularia lunata* and *Pestalotiopsis macqulens* causing foliar diseases of Teak and Mahogany seedlings from Central Nurseries of Kerala by dual culture method. All the isolates showed antagonistic activity against *Fusarium oxysporum* (53-70%), *F. solani* (60-72%), *Sclerotium rolfsii* (51-62%), *Colletotrichum gloeosporiodes* (52-75%), *Curvularia lunata* (47-70%) and *Pestalotiopsis macqulens* (61-67%). Among the *T. harzianum* isolates tested PC-03 was found to exhibit minimal antagonistic activity. However, *Trichoderma* – Pathogen interaction showed variations indicating the activity of *Trichoderma* isolates varied to a greater or lesser extent depending upon the pathogenic species.

Keywords: Fungal diseases, *Trichoderma harzianum*, Rhizoplane and Antagonism

INTRODUCTION

Teak (*Tectona grandis* L.) and Mahogony (*Swietenia macrophylla* King) are mainly known for their timber and a number of nurseries have been established for producing healthy plant stocks. A number of factors affect their successful out plantings. Among seedling diseases are the major ones of which fungi being the primary pathogenic agent (Bakshi, 1976; Bloomberg, 1985). Rots, Wilts, damping off and various foliar

diseases are the major diseases found in seedlings. Chemical agents have been practised for their assured results but indiscriminate use has resulted in environmental pollution. Alternative strategy has been in search there by minimise pollution and other hazards caused by chemicals.

Biological control is being applied as an alternative and a number of microbes have been found to show potentiality as bio-controlling agents. Microbes can be found on the leaves, roots, soil adjacent to roots and even inside plant tissues as endophytes and their interactions provide a wide array of opportunities to explore the complexities in association as well as their interaction in the growth and development. Rhizosphere and Rhizoplane inhabiting micro-organisms competitiveness for water, nutrients and space plays an important role in the growth and ecological fitness of their host (Hartmann *et al*. 2009).

Trichoderma a filamentous fungi have been extensively studied for its potentiality as an antagonistic agent (Henis and Chet, 1975; Hadar *et al.,* 1979 and Elad *et al.,* 1980). Their ability to successively thrive in diverse environment and easy to isolate the species makes it an important biocontrol agent. Besides antagonising, their role in plant growth promotion and inducing defence mechanism have also been reported (Harman *et al.,* 2004 and Vinale *et al.,* 2009).

Grasses form an important component of ecosystem which keeps rejuvenating with each growing season. They produce fibrous roots which homes abundance of diverse microbes. A great diversity of rhizosphere and rhizoplane microorganisms have been described and also in many cases been used as bio-control agents. The present work has been carried out to study the antagonistic activity of rhizoplane fungi *Trichoderma* against Fungal pathogens of Teak and Mahogany seedling diseases from Central Nurseries of Kerala *in vitro*

MATERIALS AND METHODS

Isolation of pathogenic fungi

Disease survey have been carried out in Central nurseries of Kerala located at Cheruvanchery, Valluvassery, Chettikulam and Kulathupuzha. Infected samples were collected and were taken to the laboratory. The samples were washed thoroughly, blotted and were inoculated on antibiotic amended PDA medium. The pathogen was isolated and identified by referring to standard manuals (Arx, 1981; Barnett and Hunter, 1972; Domesch and Gams, 1972; Ellis and Ellis, 2001 and Gilman, 1994).

Isolation of *Trichoderma* sp. from rhizoplane region

Trichoderma sp. were isolated from roots of grass species *Cynodon dactylon* and *Paspalum conjugatum.* Root samples were collected from Northern Kerala parts of Western Ghats, washed in slow running tap water, blotted and were fragmented into 1 cm long segments. Root segments were inoculated on antibiotic amended PDA medium. Fungal colonies were isolated and identified by referring to standard manuals as described earlier.

***In vitro* antagonism by dual culture technique**

Pathogenic fungi isolated from Teak and Mahogany seedlings and test rhizoplane fungi from grasses *Cynodon dactylon* and *Paspalum conjugatum* were cultured on their respective medium under 12/12 hr light and dark cycle at 23±2^{o}C for five days. Five mm diameter disc of selected fungi from grass and test pathogen were taken from the growing edge of a five-day-old pure culture using a cork borer. The control plates were inoculated with the pathogen and antagonists separately. Petri-dishes were incubated at 23±2^{o}C and daily growth measurements of fungal colonies were recorded for seven days. The percentage inhibition of radial growth of the pathogen was calculated using a formula by Vincent (1947).

$$\text{Percentage of Inhibition} = \frac{R_1 - R_2}{R_1} \times 100$$

R_1 – Test organism in Control

R_2 – Test organism in Dual culture

Statistical analysis

Antagonistic ability of *Trichoderma* isolates were statistically analysed and compared by Duncan's Multiple Range Test (DMRT) using SPSS (ver. 21) software developed by IBM Corporation.

Evaluation of Antagonism

The evaluation of antagonism between the *Trichoderma* and the test pathogen was scored 1-5 (Bell *et al*., 1982). The cultures were observed after seven days of incubation. The given isolate of *Trichoderma* was considered to be antagonist if the score was ≤ 2 and not highly antagonist if the score was ≥ 3.

Colony Interaction	Type of Antagonism
Complete overgrowth of the antagonist over the pathogen	1
75% overgrowth of the antagonist over the pathogen	2
Both the antagonist and the pathogen grow 50% and neither organism dominate	3
75% overgrowth of the pathogen and withstand antagonism	4
Complete overgrowth of the pathogen	5

RESULTS AND DISCUSSION

Disease survey conducted in the Central Nurseries and incubation of samples for the associated pathogens resulted in the isolation of *Fusarium oxysporum* and *F. solani* causing root rot and wilt, *Sclerotium rolfsii* causing damping off, *Colletotrichum gloeosporiodes* causing leaf spots and blights, *Curvularia lunata* causing leaf spots and *Pestalotiopsis macqulens* causing leaf spots to be major symptoms associated with Teak and Mahogany seedlings. *Trichoderma harzianum* isolated from grasses *Cynodon dactylon* (CD-01, CD-02 and CD-03) and *Paspalum conjugatum* (PC-01, PC-02 and PC-03) (Table-1) (Fig-1) were tested for their antagonistic activity against Teak and Mahogany fungal pathogens (Table 2) (Fig-2). The isolates showed inhibition against *Fusarium oxysporum* (53-70%), *F. solani* (60-72%), *Sclerotium rolfsii* (51-62%), *Colletotrichum gloeosporiodes* (52-75%), *Curvularia lunata* (47-70%) and *Pestalotiopsis macqulens* (61-67%). Among the isolates PC-03 was found to exhibit minimal inhibitory activity.

Table 1. Morphological characteristics of *Trichoderma harzianum* isolates isolated from rhizoplane regions of grasses

SL. no.	Grass species and *T. harzianum* Isolate No.	Culture characteristic
1	*Cynodon dactylon* CD-01	Colony initially white with 11mm growth per day later turning into yellow and finally to green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
2	*Cynodon dactylon* CD-02	Colony initially white with 10mm growth per day later turning into green. Reverse light coloured. Phialides (8-11 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
3	*Cynodon dactylon* CD-03	Colony initially white with 14mm growth per day later turning into green and finally to dark green. Reverse light coloured. Phialides (5-8 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
4	*Paspalum conjugatum* PC-01	Colony initially white with 10mm growth per day later turning into yellow and finally to light green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
5	*Paspalum conjugatum* PC-02	Colony initially white with 11mm growth per day later turning into green and finally to dark green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)
6	*Paspalum conjugatum* PC-03	Colony initially white with 11mm growth per day later turning into light green. Reverse light coloured. Phialides (5-9 x 1-3 µm), spores globose to oval (2-5 x 1-3µm)

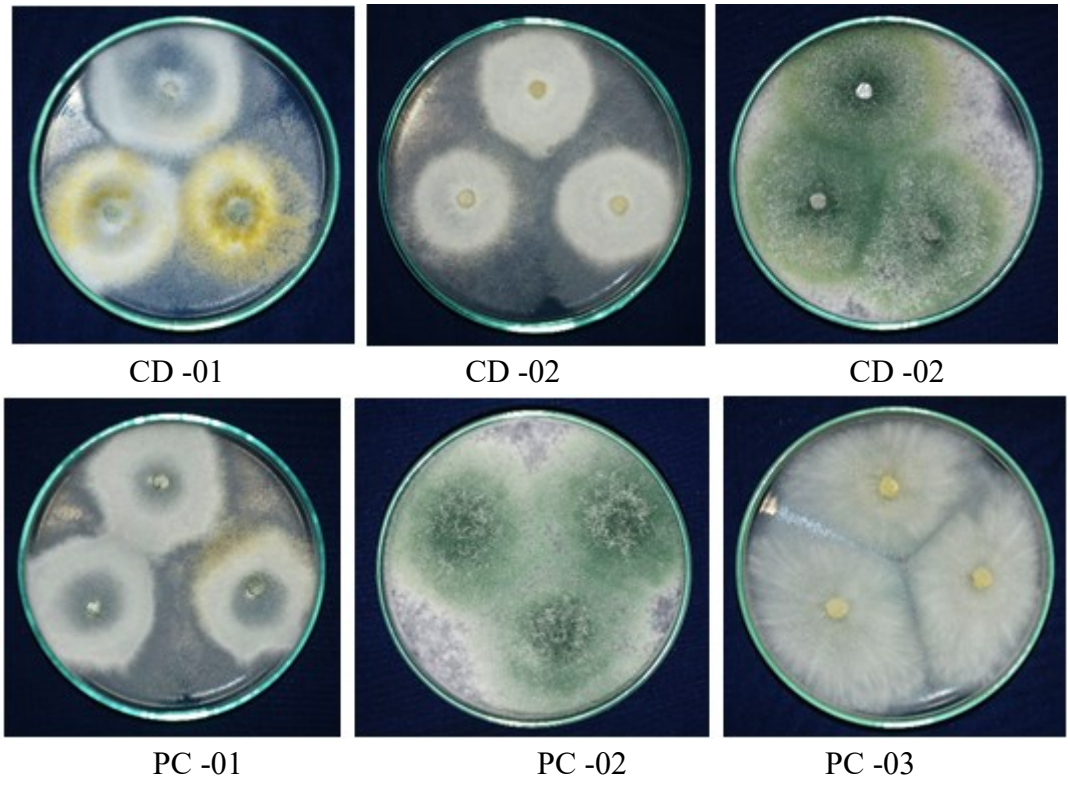

Fig 1. ***Trichoderma harzianum*** **isolates at Five days of incubation**

Table 2. Antagonistic activity of ***Trichoderma*** **isolates and reaction types against fungal pathogens**

Trichoderma harzianum isolates	Percent inhibition and colony interaction types against fungal pathogens											
	F O		*F S*		*S R*		*C G*		*C L*		*P M*	
	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT	Percent Inhibition	*RT
CD-01	69.23 ± 0.48[1] e[2]	-	68.57 ± 0.78[1] b[2]	1	60.00 ± 0.23[1] c[2]	3	75.00 ± 0.54[1] c[2]	1	58.82 ± 0.78[1] b[2]	1	61.90 ± 0.36[1] a[2]	1
CD-02	57.69 ± 0.56[1] b[2]	-	60.00 ± 0.86[1] a[2]	1	60.00 ± 0.36[1] c[2]	3	75.00 ± 0.16[1] c[2]	2	64.70 ± 0.54[1] d[2]	1	66.60 ± 0.42[1] a b[2]	1
CD-03	65.38 ± 0.96[1] d[2]	-	68.57 ± 0.18[1] b[2]	1	62.20 ± 0.45[1] d[2]	3	75.00 ± 0.77[1] c[2]	1	70.50 ± 0.49[1] e[2]	1	64.28 ± 0.13[1] a b[2]	1
PC-01	61.53 ± 0.98[1] c[2]	-	71.43 ± 0.73[1] c[2]	1	62.20 ± 0.95[1] d[2]	3	72.50 ± 1.10[1] b[2]	1	64.70 ± 0.76[1] d[2]	1	64.28 ± 0.47[1] a b[2]	1
PC-02	57.69 ± 0.53[1] b[2]	-	71.43 ± 0.73[1] c[2]	1	51.10 ± 0.57[1] b[2]	3	72.50 ± 0.87[1] b[2]	1	61.76 ± 0.83[1] c[2]	1	64.28 ± 0.49[1] a b[2]	2
PC-03	53.84 ± 0.69[1] a[2]	-	60.00 ± 1.13[1] a[2]	2	-	5	52.50 ± 0.69[1] a[2]	2	47.05 ± 0.62[1] a[2]	3	64.28 ± 0.77[1] b[2]	3

Data is an average of three replicates

*RT - Reaction Type [1] Standard deviation

[2] DMRT ≤ 0.05 Data set with same alphabets were found to show no significant difference

F O- Fusraium oxysporum, F S- Fusarium solani, S R- Sclerotium rolfsii, C G- Colletotrichum gloeosporioides, C L- Curvularia lunata, P M- Pestalotiopsis macqulens

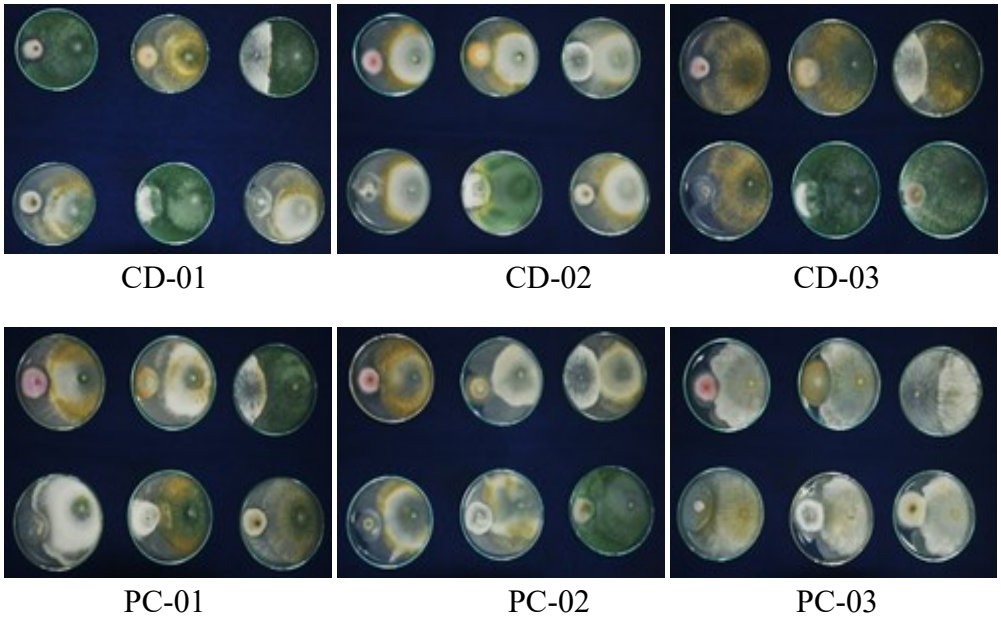

Fig 2. Antagonistic activity of *Trichoderma harzianum* isolates against fungal pathogens *Fusraium oxysporum, Fusarium solani, Sclerotium rolfsii, Colletotrichum gloeosporioides, Pestalotiopsis macqulens and Curvularia lunata*

Various species of *Trichoderma* namely *T. koningii*, *T. harzianum* and *T. viride*, respectively have been studied for their antagonistic activity *in vitro* (Mathew and Gupta, 1998; Prasad *et a*l., 1999; Bunker and Mathur, 2001; Pandey *et al*., 2005; Grosch *et al*., 2007). *Trichoderma* isolates also varied in their reaction types, this was evident in the case of *Sclerotium rolfsii*. Among the root pathogens *F. solani* was more susceptible to the antagonist and this was also evident with the interaction type as the antagonists were able to completely overgrow the pathogen. *Sclerotium rolfsii* exhibited an interaction type where both the pathogen and the antagonist grew 50% and neither dominated on each other except for PC-03 where the pathogen was able to over grow the antagonist. In case of *F. oxysporum* zone of inhibition was observed. In case of foliar pathogens all the species were susceptible to the antagonist. This was also evident with the reaction type as all the antagonists were able to completely overgrow the pathogens. A vast variety of microbes have the ability to be potentially used as biocontrol agent but the selection of an appropriate isolate forms an important aspect for its success in field application. The present work showed the potentiality of rhizoplane mycoflora and its efficacy against various forest plant pathogens and can be further analysed for its use as an alternative to chemical fungicides.

CONCLUSION

Trichoderma harzianum exerted good antagonistic activity against all the pathogens studied and makes this species as a biocontrol agent which can be used as an alternative to chemicals. The variations among the isolates stressed on the selection of effective isolate and needs a series of steps in their appropriate application for their infield success (Ravensberg, 2011). Rhizoplane regions of grasses homes diverse fungal organisms and can be used as biological weapons against various plant pathogens.

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