

**STUDIES ON ENDOPHYTIC MICROBIAL
DIVERSITY AND ANTAGONISTIC
EFFECT ON RHIZOME ROT PATHOGENS
IN TURMERIC (*Curcuma longa* L.)**

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B.Sc. (Hons.) Horticulture

**MASTER OF SCIENCE IN HORTICULTURE
(HORTICULTURAL PLANT PATHOLOGY)**



**DEPARTMENT OF HORTICULTURAL PLANT PATHOLOGY
COLLEGE OF HORTICULTURE
ANANTHARAJUPETA-516 105, Y.S.R. DISTRICT, ANDHRA PRADESH
Dr. Y.S.R. HORTICULTURAL UNIVERSITY**

JULY, 2017

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TURMERIC (*Curcuma longa* L.)**

BY

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B.Sc. (Hons.) Horticulture

THESIS SUBMITTED TO

**Dr.Y.S.R. HORTICULTURAL UNIVERSITY IN PARTIAL
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THE DEGREE OF**

**MASTER OF SCIENCE IN HORTICULTURE
(HORTICULTURAL PLANT PATHOLOGY)**



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JULY, 2017

CERTIFICATE

Ms. M. LAKSHMI NAGA NANDINI has satisfactorily prosecuted the course of research and that the thesis entitled “**STUDIES ON ENDOPHYTIC MICROBIAL DIVERSITY AND ANTAGONISTIC EFFECT ON RHIZOME ROT PATHOGENS IN TURMERIC (*Curcuma longa* L.)**” submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination.

I certify that neither the thesis nor its part there of has been previously submitted by her for a degree of any University.

Place: ANANTHARAJUPETA

(Dr. CH. RUTH)

Date:

Chairman

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LIST OF CONTENTS

Chapter No.	Title	Page No.
I	INTRODUCTION	
II	REVIEW OF LITERATURE	
III	MATERIAL AND METHODS	
IV	RESULTS AND DISCUSSION	
V	SUMMARY AND CONCLUSIONS	
	LITERATURE CITED	
	APPENDICES	

LIST OF TABLES

Table No.	Title	Page No.
3.1	Locations of collection of endophytic microflora of turmeric	
3.2	Dilution and media used for isolation and enumeration of endophytic microflora	
3.3	Source of germplasm collections	
3.4	Disease scale used for screening	
4.1	Details of locations and per cent disease incidence (PDI) of turmeric in Andhra Pradesh during 2016-17	
4.2	Sample wise isolation of organisms from infected rhizomes	
4.3	Morphological characteristics of fungi associated with rhizome rot of turmeric	
4.4	Population of endophytes of turmeric at different locations	
4.5	<i>In vitro</i> evaluation of turmeric endophytic fungal isolates against pathogens of turmeric	
4.6	<i>In vitro</i> evaluation of turmeric endophytic bacterial isolates against pathogens of turmeric	
4.7	<i>In vitro</i> evaluation of turmeric endophytic fluorescent pseudomonads isolates against pathogens of turmeric	
4.8	<i>In vitro</i> inhibition of pathogens of turmeric by non-volatile metabolites of endophytic fungi	

Table No.	Title	Page No.
4.9	Mechanism of action of selected bacterial antagonists	
4.10	Compatibility of selected fungal antagonist with fungicides	
4.11	Compatibility of selected fungal antagonist with insecticides	
4.12	Compatibility of selected bacterial antagonist with fungicides	
4.13	Compatibility of selected bacterial antagonist with insecticides	
4.14	Screening of turmeric germplasm against the rhizome rot disease under field conditions	
4.15	Rhizome rot reaction and yield parameter in natural field condition of turmeric germplasm in Andhra Pradesh	
4.16	Screening of turmeric germplasm against rhizome rot under pot culture conditions	
4.17	Cultural and biochemical characters of the bacterial isolates	

LIST OF ILLUSTRATIONS

Fig. No.	Title	Page No.
3.1	Layout of the experimental plot	
4.1	Screening of endophytic fungal isolates against pathogens of turmeric	
4.2	Screening of endophytic bacterial isolates against pathogens of turmeric	
4.3	Screening of endophytic fluorescent pseudomonads isolates against pathogens of turmeric	
4.4	Effect of non-volatile metabolites of endophytic fungi on growth of turmeric pathogens	
4.5	Compatibility of <i>Trichoderma harzianum</i> (Tc ed f 4) with fungicides	
4.6	Compatibility of <i>Trichoderma harzianum</i> (Tc ed f 4) with insecticides	
4.7	Compatibilty of <i>Pseudomonas</i> sp. (Tc ed b 3) with fungicides	
4.8	Compatibilty of <i>Pseudomonas</i> sp. (Tc ed b 3) with insecticides	

LIST OF PLATES

Plate No.	Title	Page No.
3.1	Media used for isolation of endophytes	
3.2	Mass multiplication of rhizome rot pathogens	
3.3	Field view of the experimental plot	
3.4	Field view of the pot culture experiment	
4.1	District mean per cent disease incidence of turmeric rhizome rot disease during 2016-17 in Andhra Pradesh	
4.2	View of rhizome rot infected farmer's field during the survey in Guntur district	
4.3	Healthy and rhizome rot infected turmeric plant along with rhizome	
4.4	Proving pathogenicity (rhizome rot) by <i>Pythium ultimum</i> and <i>Fusarium solani</i>	
4.5	Microscopic (400 X) and morphological characters of <i>Pythium ultimum</i>	
4.6	Microscopic (400 X) and morphological characters of <i>Fusarium oxysporum</i>	
4.7	Inhibitory effect of fungal endophytes on mycelial growth of turmeric pathogens in dual culture test	
4.8	Inhibitory effect of bacterial endophytes on mycelial growth of turmeric pathogens in dual culture test	
4.9	Inhibitory effect of different fluorescent pseudomonads isolates on mycelial growth of turmeric pathogens in dual culture test	

Plate No.	Title	Page No.
4.10	Mechanism of action of fungal antagonists with <i>Pythium ultimum</i> of turmeric	
4.11	Mechanism of action of selected bacterial antagonists	
4.12	Compatibility of <i>Trichoderma harzianum</i> (Tc ed f 4) with fungicides in <i>in vitro</i> conditions	
4.13	Compatibility of <i>Trichoderma harzianum</i> (Tc ed f 4) with insecticides in <i>in vitro</i> conditions	
4.14	Screening of turmeric germplasm for resistance against rhizome rot disease under field condition	
4.15	Screening of turmeric germplasm for resistance against rhizome rot disease under pot culture condition	
4.16	Identification of fungal and bacterial antagonists	

LIST OF APPENDICES

Appendix No.	Title	Page No.
I	Results of mechanical and chemical analysis of soil of the experimental Field	
II	Weekly meteorological data recorded during the period of study (June, 2016 - March, 2017)	
III	Media composition (Ingredients per litre)	
IV	Phosphate buffer composition	

CERTIFICATE

This is to certify that the thesis entitled “**STUDIES ON ENDOPHYTIC MICROBIAL DIVERSITY AND ANTAGONISTIC EFFECT ON RHIZOME ROT PATHOGENS IN TURMERIC (*Curcuma longa* L.)**” submitted in partial fulfillment of the requirements for the degree **MASTER OF SCIENCE IN HORTICULTURE (Horticultural Plant Pathology)** of **Dr. Y.S.R. Horticultural University, Venkataramannagudem**, is a record of the bonafide research work carried out by **Ms. M. LAKSHMI NAGA NANDINI** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all the assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
&	:	and
/	:	Per
@	:	at the rate of
^o C	:	Degree centigrade
ABD	:	Augmented Block Design
ANOVA	:	Analysis of variances
CD (P=0.05%)	:	Critical difference
CFU	:	Colony Forming Unit
cm	:	Centimeter
COH	:	College of Horticulture
CRD	:	Complete Randomized Design
CV%	:	Percentages of Co-efficient of Variance
DAP	:	Days After Planting
EC	:	Emulsifiable concentrate
<i>et al.</i>	:	Et alii, and others
etc.	:	Etcetera, and so on; and other people / things
f. sp.	:	forma specialis
Fig.	:	Figure
g	:	Gram
h	:	hours
ha	:	Hectare
ha ⁻¹	:	Per hectare
HRS	:	Horticultural Research Station
<i>i.e.,</i>	:	that is
Kg	:	Kilogram
m	:	meter

mg	:	Milligram
min	:	Minutes
ml	:	Milliliter
Mm	:	micro molar
mm	:	millimeter
MSL	:	Mean sea level
NA	:	Nutrient agar
NCFT	:	National Centre for Fungal Taxonomy
NHB	:	National Horticulture Board database
No.	:	Number
PB	:	Potassium phosphate buffer
PDA	:	Potato Dextrose Agar media
PDI	:	Per cent Disease Incidence
p ^H	:	Puissance de hydrogen
Plant ⁻¹	:	Per plant
SC	:	Suspension concentrate
SEd	:	Standard Error of the Difference
SEm	:	Standard error mean
SL	:	Soluble (liquid) concentrate
sp.	:	Species
t	:	Tonne
U.V	:	Ultra Violet
<i>viz.</i> ,	:	Namely
w/v	:	weight by weight
WDP	:	Water Dispersible Powders
WG	:	Water Dispersible Granules
WP	:	Wettable Powders
µg	:	Micro gram
µl	:	Micro litre

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ABSTRACT

The present investigation entitled “**Studies on Endophytic microbial diversity and antagonistic effect on rhizome rot pathogens in turmeric (*Curcuma longa* L.)**” was carried out at College of Horticulture, Anantharajupeta during the year, 2016-2017. A roving survey was conducted to estimate rhizome rot incidence under field conditions in five districts of Andhra Pradesh. The mean maximum disease incidence was observed in Guntur district (16.12%) followed by West Godavari district (12.68%) and in Visakhapatnam district least mean disease incidence (2.18%) was noticed. The organisms causing rhizome rot disease of turmeric were isolated from infected rhizomes and their pathogenicity proved. Based on cultural and morphological characters, pathogens were identified as *Pythium ultimum* and *Fusarium solani*.

Endophytes were isolated from healthy rhizome samples collected from different locations of Andhra Pradesh. The population of endophytic microflora varied among the samples collected from different locations. Bacteria (10.40×10^6 cfu g⁻²) and fluorescent Pseudomonads (13.40×10^6 cfu g⁻²) population were more in number than fungi (5.42×10^6 cfu g⁻²). A total of 58 endophytes were isolated. Among these, 24 isolates (Fifteen fungi, five bacteria and four fluorescent Pseudomonads) were found to exert antagonism towards pathogens of turmeric. On further *in vitro* evaluation, seven isolates including four fungi and three bacteria of endophytic origin were selected as efficient antagonists.

For studying the mechanism of antagonism of the selected isolates, they were subjected to various tests like production of ammonia, siderophore, HCN,

IAA, volatile and non volatile metabolites. The bacterial isolates Tc ed b 2 and Tc ed b 3 produced more ammonia. All isolates were negative to HCN and siderophore production while others produced varying levels of IAA. The selected fungal isolates produced non-volatile metabolites inhibitory to the pathogens tested.

Further, the compatibility of selected antagonists with seven fungicides and five insecticides were studied. The fungal antagonist was incompatible with fungicides like Bordeaux mixture, Carbendazim (12%) + Mancozeb (64%) (Saaf) and insecticide Phosphamidon (40%) (Demecron), showed cent percent inhibition on the growth of the antagonist. Others showed varying levels of inhibition.

The bacterial antagonist was compatible with mancozeb (4%) + metalaxyl-M (64%) (Ridomil Gold MZ), carbendazim (12%) + mancozeb (64%) (Saaf), cyamoxanil (8%) + mancozeb (64%) (Curzate M8), mancozeb (Indofil M-45), lower concentration of fenamidone (10%) + mancozeb (50%) (Sectin) while it was incompatible with Bordeaux mixture, copper oxychloride (50%) (Blitox). All concentrations of thiomethoxam (25%) (Cruiser) and phosphamidon (40%) (Demecron) were compatible with antagonist while the reverse was with that of chlorpyrifos (20%) (Dursban), dimethoate (30%) (Rogar) and malathion (50%).

The fungal antagonists were identified as *Aspergillus oryzae* (Tc ed f 1), *Penicillium chrysogenum* (Tc ed f 2), *Trichoderma viride* (Tc ed f 3) and *Trichoderma harzianum* (Tc ed f 4). Three of the bacterial isolates (Tc ed b 1 and Tc ed b 3) were tentatively identified as *Pseudomonas* sp. while the other one (Tc ed b 2) as *Bacillus* sp.

Under field conditions, out of 120 turmeric lines screened, thirty lines (14 short duration, 5 medium duration, 11 long duration) were found resistant to rhizome rot disease. IC-319341, Tenali Kasturi, VK-23, GS, IC-420606, IC-033007, IC-211641, PTS-8, Vikici, Dhindigam, ACC-48, Sonia, NB-60, Kasturi in short duration group, Prathibha, Thodupuzha, KTS-9, Prasangali, ACC-79 in medium duration group and NH-1, Ranga, Salem, Salem-2, Wagon, PTS-12, CL-8, CL-9, CL-10, CL-3, CL-4 in long duration group were resistant to rhizome rot showed 0.0% infection. In case of rhizome yield, medium duration turmeric line RH-50 (1.69 kg/plant) was significantly superior over all other genotypes evaluated.

Under pot culture conditions, out of 30 turmeric lines screened, Salem and Prathibha were found resistant to rhizome rot with 0.0% infection.

Chapter I

INTRODUCTION

Among many commercial crops sown in India, spices occupy an important place. They have been famous for their fine quality and flavour and the demand for these items has gone up considerably in the international market. The total output of Indian spices has shown a positive increase in recent years.

Turmeric (*Curcuma longa* L), the “golden spice of life” is one of the most essential spice use as an important ingredient in culinary all over the world. It is the herbaceous perennial belongs to the family Zingiberaceae and a native of South Asia particularly India and cultivated for its underground rhizome (Chirangi *et al.*, 2004). Indian subcontinent is considered as the “land of spices” and enjoys from time immemorial a unique position in the production and export of turmeric because of its high curcumin content. It is an ancient and sacred spice of India known as “Indian saffron”, the “poor man’s saffron”, “haridra”, or “haldi”. The rhizome of the turmeric contains a colouring pigment known as “curcumin” and volatile oil “tumerol”.

The rhizome is valued for its medicinal property and its usefulness as dyeing agent to cotton, silk, etc. (Appaji Rao and Sarmal, 1962). The anti-oxidant attributes of this spice protect against the high energy free radical damage to organic cells (Maheshwari *et al.*, 2006). The antibacterial, antiviral, antifungal and antiinflammatory properties of this herb appear as an effective cure for several diseases (Negi *et al.*, 1999; Kim *et al.*, 2009; Chen *et al.*, 2010; Koosirirat *et al.*, 2010). It is also reported to detoxify the liver, balance cholesterol levels, fight allergies, stimulate digestion, boost immunity and enhance the complexion (Patel and Srinivasan, 1996; Ram *et al.*, 2003; Arafa, 2005).

Turmeric is an important commercial and remunerative spice crop which occupies a distinct position in Indian spices market as well as in the international market. It occupies about 6% of the total area under spices and condiments in India. Our country is the world’s largest producer and exporter of turmeric which

accounts for 80 per cent of the international trade followed by China, Myanmar and Bangladesh. (Satishkumar, 2005).

The total area under cultivation and production of turmeric has recorded an increasing trend in the country. In India, the total area under cultivation is 233 thousand hectares with a production of 1190 thousand tonnes during 2014-15 (National Horticultural Board, 2014-15). Andhra Pradesh ranks first in production of turmeric in India. In A.P, the total area under cultivation is 17.82 thousand ha with a production of 151.90 thousand tonnes during 2014-15 (Spice Board, 2015). It was intensively cultivated in the districts of Kurnool, Guntur, Kadapa, West Godavari, Visakhapatnam, Krishna, East Godavari, Karimnagar, Nizamabad, Warangal, Adilabad and parts of Khammam in Andhra Pradesh (AP) and Telengana in India.

Turmeric is vulnerable to a number of fungal diseases of both soil and air borne nature. The important diseases affecting the crop are rhizome rot, leaf spot, leaf blotch and leaf blight (Ravindran *et al.*, 2007). Among these diseases, rhizome rot is the most destructive one causing enormous economic damage mainly causes severe yield reduction and reduces the quality of rhizome (Rathiah, 1982). Rhizome rot caused by *Pythium* sp. is a major constraint in all turmeric growing areas of India (Rathiah, 1987, Nageshwar Rao, 1994, Ramarethinam and Rajagopal, 1999). Crop loss can be as much as 50 per cent, as reported in the Telengana region of A.P. in India (Rao and Rao, 1988), but can vary in extent from region to region. *Fusarium solani* was also reported to be pathogenic and cause rhizome rot disease in turmeric (Dohroo, 1988a).

To combat the rhizome rot problem, farmers used chemical fungicides. Often use of fungicides, may not give desired effect and will lead to many ecological problems as well as the development of resistant strains of pathogens. Therefore, it is imperative to develop sustainable disease management strategy with emphasis on reducing the use of chemicals and to search for antagonistic microbes residing in and outside the plants. It is well known that plants growing in humid tropical conditions are rich source of endophytic microbes that can directly or indirectly offer beneficial effect. Since turmeric prefers humid

condition, there is every possibility of innate association of beneficial microflora. Associations of endophytic microbes with nutmeg and cinnamon have been documented by Gary *et al.* (2001) and Soapalun (2003). Further, the potential of bacterial endophytic microflora of turmeric, another crop of humid tropics for the management of *Fusarium solani* have also been established (Ajay *et al.*, 2016).

Hence, search on the endophytic microbiota in turmeric will throw light on the diversity and possibility of harnessing their biocontrol potential against rhizome rot disease. Further, studies on the mechanism of antagonism and tolerance to plant protection chemicals will help to evolve compatible and sustainable biocontrol agents for large scale use. Further, pot culture (soil inoculation), field screening trials were conducted to identify the resistant cultures in the available germplasm lines against the rhizome rot disease. Hence, the present investigation was undertaken to understand the rhizome rot disease affecting turmeric in A.P. and to elucidate the potential of endophytic microbes against the rhizome rot disease of turmeric crop, so as to develop a sustainable and eco-friendly management practice.

Further, the management of rhizome rot has become a hercules task for the farmer in turmeric fields. Hence, an attempt was made to make a study with the following objectives.

Objectives of investigation

1. To survey major turmeric growing areas in A.P. for the occurrence of rhizome rot disease.
2. To isolate endophytic microbes in turmeric crop.
3. To study antagonistic activity of endophytes against rhizome rot pathogens *in vitro*.
4. To study compatibility of selected antagonists with recommended plant protection chemicals *in vitro*.
5. To screen turmeric germplasm for resistance to rhizome rot disease under pot culture and field conditions.

Chapter II

REVIEW OF LITERATURE

Turmeric is one among the major spices in A.P. after chillies, cumin, coriander and garlic which occupy an area of 233,000 ha in India and 17,820 ha in Andhra Pradesh. It tops in production of turmeric in India and it is called as “Turmeric bowl of India” as it has highest share of 38% in total India’s turmeric area followed by Orissa, Tamil Nadu, Assam and West Bengal. Recent utility of turmeric by the pharmaceutical industries as a source of antiseptic, antipyretic hepatoprotectant, antimicrobial in addition to its use in cardiovascular and gastrointestinal disorders has categorized it as a major industrially important crop of high demand. (Srimal, 1997, Aggarwal *et al.*, 2007, Aggarwal and Sung, 2009). The International Trade Centre, Geneva, has estimated an annual growth rate of 10% in the world demand for turmeric. However, turmeric yield is not adequate as per the demand in the recent times. They act as one of the major source of foreign exchange in India. Among several factors, diseases are the most important cause associated with low productivity and heavy mortality of this crop. Dohroo *et al.* (1988a) reported crop losses up to 60% has been realized in the recent times mainly due to the infection by a necrotrophic oomycetic fungus *Pythium aphanidermatum* causing the rhizome rot disease in turmeric. Anoop and Suseela Bhai (2014) conducted systematic studies on rhizome rot disease of turmeric spice. To manage this disease, farmers, nowadays adopt indiscriminate use of plant protection chemicals which leads to major health hazards. Hence, there is a need to search an alternate tool for the management of this disease in an eco-friendly manner.

The literature available on rhizome rot pathogens in turmeric pertaining to the present investigation was reviewed and presented here under.

2.1 DISEASE OCCURRENCE

A total of 288 diseased samples were collected from 195 ginger fields spread all over Kerala during 1984 and 1985. Of them, samples yielded *Pythium*

aphanidermatum, *P. myriotylum* (19.79%), *Fusarium oxysporum* f.sp. *zingiberi*, *F. Solani* (6.6%) and *Pseudomonas solanacearum* (26.71%) (Dake and Edison, 1989).

During the year 1991, the survey report of different ginger growing areas in four districts of Himachal Pradesh *i.e.*, Sirmour, Solan, Shimla and Bilaspur revealed the maximum disease incidence of 87 per cent in Solan. Fifty per cent losses were reported in storage by rhizome rot of ginger caused by *Fusarium oxysporum* f.sp. *zingiberi* (Dohroo, 1995).

Kulkarni *et al.* (2004) conducted a roving survey during *kharif* 2003-04 to estimate rhizome rot incidence in major ginger growing areas in Karnataka. During *kharif* 2003-04, the per cent disease incidence was noticed in all the locations surveyed, with a range of 5.50% to 45.60%. In Uttara Kannada district, maximum per cent disease incidence was observed in Manchikeri (14.40%). In Bidar district, Maximum per cent disease incidence was observed in Honnikeri (28.70%) and lowest incidence was in Islampur (5.50%).

Kulkarni *et al.* (2006) conducted an intensive survey during *kharif* 2004-05, the per cent disease incidence was present throughout the Karnataka state, wherever ginger was grown and incidence ranged from 2.00 to 47.28 per cent. Maximum disease incidence was observed in Korlakatta of Uttara Kannada district (47.28%) and least incidence in Markoppa of Haveri district (2.00%). Among the districts, the maximum disease incidence of 23.70 per cent was recorded in Shimoga district followed by Kodagu (22.90%), Uttara Kannada (20.24%), Chickmagalur (18.99%) and Bidar (13.00%). Therefore, these places can be considered as 'hot spots' of rhizome rot of ginger.

Anon (2007) conducted a detailed study of the etiology of the rhizome rot was undertaken at IISR, Calicut, Kerala state, India during 2005-2009. Systematic field survey was conducted in different turmeric growing regions of the country, in the four southern states, namely, Andhra Pradesh, Tamil Nadu, Karnataka, Kerala and Karnataka. During the survey, 118 samples were collected from 38 locations. It became clear that *Pythium* was the principal causative

organism to cause rhizome rot, from this study as well. This was to the extent of 72.9%. This trend was followed by the causative fungus *Rhizoctonia* sp. which accounted for 30.5% infestation. *Fusarium* sp. caused the least infestation at 27.1%. Pathogenic tests revealed that 75% of the pathogenic isolates were *P. aphanidermatum*.

Sagar *et al.* (2008) to know the prevalence of ginger rhizome rot in Karnataka, a survey was conducted during *Kharif* 2003-05 in major ginger growing areas of Karnataka. Rhizome rot incidence was noticed in all the locations surveyed with a range from 5.50 to 45.60 per cent. The maximum disease incidence of 23.70 per cent was recorded in Shimoga district followed by Kodagu (22.90%), Uttara Kannada (20.24%), Chickmagalur (18.99%) and Bidar (13.00%). Therefore, these places can be considered as hot spots of rhizome rot disease of ginger. *Pythium aphanidermatum* was isolated from all the locations collected and proved to be most predominant pathogen among the rhizome rot pathogens. Next predominant pathogen was *Fusarium solani*, *Ralstonia solanacearum* was detected only from the southern parts of Karnataka.

Sharma *et al.* (2010) reported the Ginger production in major growing areas of Darjeeling hills, West Bengal, India during recent years suffered tremendously. Rhizome rot disease caused by fungi (*Pythium* sp. and *Fusarium oxysporium*) and bacteria [*Pseudomonas* (*Ralstonia*) *solanacearum*] are the major problems all over ginger growing areas of India. The main reason being the occurrence of disease having complex etiology and so far a very little work has been done in this regards. A survey was undertaken during the year 2005 and 2006 in ginger growing area to gather the basic information as well as to collect plant and soil samples from ginger fields for further study. About 80-84% of the plots suffered from the disease severely during both 2005 and 2006 ginger growing season. From the survey, it was observed that June-July was the most vulnerable period for initiation and spread of the disease and the less incidence of the disease were noticed if the disease initiates after the second fortnight of July.

Anoop *et al.* (2014) made detailed survey on rhizome rot disease of turmeric (*Curcuma longa* L.) in the major turmeric growing tracts of South India.

A total of 37 different locations of A.P, Karnataka, Kerala and Tamil Nadu were covered during the survey. The highest disease incidence was noted in Guntur district of A.P. (32.22 per cent) followed by Salem district of Tamil Nadu (20.67 per cent). Among the four states surveyed, three locations in Kerala and Andhra Pradesh recorded no disease incidence. According to the information collected, the highest disease incidence and spread of the disease often coincided with the monsoon season of the respective locations. Differences were noted on the system of cultivation, practices of crop rotation and crop protection strategies among the different locations during the survey. The analysis of rhizome rot samples collected during the survey revealed that *Pythium* is the predominant organism actively involved in the rhizome rot disease. *Rhizoctonia* and *Fusarium* are the other organisms found frequently associated with the infected samples. Out of 118 samples, 74.5 per cent yielded *Pythium* sp., 30.51 per cent yielded *Fusarium* and 28.8 per cent yielded *Rhizoctonia* sp. Some samples yielded a combination of these three organisms. The pathogenicity studies of these isolates may prove the role of these organisms for the exact cause of rhizome rot disease in turmeric in the region.

Sarathi *et al.* (2014) collected the samples of disease infected rhizomes of turmeric were collected from 5 different agricultural fields in Pudukkottai District. Samples were screened for isolation and identification of rhizome rot causing fungal pathogen. The isolate was found in *Pythium aphanidermatum* belonging to Phycomycetes is a well-known devastating pathogen of many vegetables, fruits, rhizomes, grasses and ornamental crops in several parts of the world with a wide host range.

Rekha *et al.* (2015) conducted the survey during *kharif* seasons of 2011 and 2012 in major ginger growing areas of Malnad districts *viz.*, Shimoga, Coorg, Chikkamagalore, Hassan and Uttara Kannada to find out the prevalence of rhizome rot. Average disease incidence was more during *kharif* of 2011 (24.68%) than *kharif* of 2012 (20.80%). Average rhizome rot incidence in Malnad districts during 2011 and 2012 was 22.74%.

2.2 SYMPTOMATOLOGY OF TURMERIC RHIZOME ROT DISEASE

Turmeric is affected by various diseases of fungal origin. Of these the leaf blotch, leaf spot and rhizome rot are the major diseases. Leaf blotch caused by *Taphrina maculans*, leaf spot by *Clletotrichum capsici* and rhizome rot caused by *Pythium graminicolum* result in considerable crop losses (Butler, 1918). Rhizome rot, which accounts for the loss of collections in the farm, is caused by *Pythium graminicolum* L. Subram. (Chattopadhyay, 1967). Rhizome rot of turmeric incited by *Pythium aphanidermatum* was first reported in Sri Lanka by Park (1934), later it was reported as *P. graminicoloum* from the Krishna district of Andhra Pradesh by Ramakrishnan and Sowmini (1954) and *P. myriotylum* from Assam by Rathaiah (1982). The pathogen is soil and seed borne. *Fusarium* sp. was also pathogenic and caused rhizome rot disease in turmeric (Sharma and Jain, 1977). Dubey *et al.* (1976) noticed the attack of maggots of Dipteran flies (*Mimegralla* species) in infected rhizomes.

2.2.1 Pathogens associated with rhizome rot

Butler and Bisby (1931) reported turmeric rhizome rot induced by various fungal pathogens *i.e.*, *Pythium myriotylum* Drech., *Sclerotium* sp. and *Fusarium* sp. The rhizome borne fungi are responsible for low germinability to reduce yield and deteriorate quality of rhizome. Different species of *Pythium* were recorded as pathogens involved in rhizome rot.

In ginger rhizome rot the isolated fungi were reported as species of *Pythium viz.*, *Pythium aphanidermatum*, *P. deliense*, *P. myriotylum*, *P. pleroticum*, *P. vexans* and *P. ultimum* and *Fusarium* (Waterhouse, 1967 and Booth, 1971). The symptoms of the disease were reported to be developed in sixteen days with *Fusarium oxysporum* f.sp. *zingiberi* (Dake and Edison, 1989).

Sharma and Jain (1977) observed that out of the 29 fungi associated with ginger externally or internally, four fungi *viz.*, *Fusarium oxysporum*, *Pythium deliense*, *P. myriotylum* and *Pseudopapulaspora kendrickii* were present internally in rhizomes and were responsible for deterioration of the rhizomes in storage. *Fusarium solani* and *Fusarium moniliforme* were associated with

rhizome rot of ginger (Rath *et al.* 1978). *Curvularia lunata* was found associated with ginger rhizomes (Sinha *et al.* 1987). The storage rot affected rhizomes yielded only fungal pathogens *Pythium pleroticum*, *P. apanidermatum*, *Fusarium equiseti* and *Fusarium solani*. *Pythium* induced rhizome rot of ginger (Rajan and Agrihotri, 1989) and other saprophytic fungi such as *Geotrichum candidum* caused storage rot (Mishra and Rath, 1989). *Aspergillus flavus* and spp. of *Rhizopus*, *Mucor*, *Penicillium*, *Trichothecium* and *Fusarium* were found on seed surface of ginger during storage (Geeta and Reddy, 1990).

Dohroo and Sharma (1992) reported *Cladosporium tenuissium* Bainier (Solan), *Graphium album* (Corda) Sacc. (Shillai, Sirmaur), *Mucor racemosus* Fres (Deona, Sirmaur), *Stachybotrys sansevieriae* (Malothi, Bilaspur), *Thanetophorus cucumeris* (Frank) Donk (Shamoga, Sirmaur) and *Verticillium chlamyosporium* Goddard associated with seed ginger in Himachal Pradesh.

Okigbo (2005) reported four fungal species *viz.*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopus* sp. to be consistently associated with stored ginger samples. *F. oxysporum* showed water soaked lesions on infected ginger samples and rhizomes were completely disintegrated and covered with white cottony mycelium with pinkish mass of fungal spores. *A. niger* formed black sporulation and the diseased parts did not exude water freely.

2.2.2 Symptomatology

Root infection in rhizome rot is also important and has been recognized *Pythium* sp. often infects immature and undifferentiated parts of host plant. In turmeric both pre-emergence and post-emergence rhizome rots are noticed. Pre-emergence rots result when infected seed rhizomes are sown or through bud infection and subsequent rotting of infected buds. New buds arising from the rhizomes also contract infection leading to total crop loss. Post-emergence rot can occur at any time after sprouting. The pathogen may penetrate the sprout through the roots or through the collar region finally reaching the rhizome. Initial symptoms appear as water soaked patches at the collar region of the pseudostem.

These patches enlarge and the tissues of the collar region become soft, watery and rot. Sprouts turn pale and collapse (Sarma, 1994).

In well differentiated clumps, the infection starts at the collar region of the pseudostem as water soaked area which generally spreads both upwards and downwards. Foliar yellowing is seen clearly from the margins of the lower most leaves (oldest) and progresses upwards. As the infection spreads gradually to the inner most part of the pseudostem, the intensity of the foliar yellowing increases. Later the infection spreads to the rhizome. The affected rhizomes rot, emit a foul smell and the pseudostems come off with a gentle pull. In the early stages, the root infection often reaches the germinating sprouts leading to the rhizome rot. (Anandaraj and Sarma, 1993; Sarma, 1994).

When the role of root infection in the soft rot of ginger was studied, it was found that pseudostem infection was more when the inoculum was placed at the surface and decreased with the depth of the inoculum source (Anandaraj and Sarma, 1993).

In a majority of diseased turmeric rhizomes examined, active maggots of *Mimegrella coerulifrons* and *Eumerus* sp. were found. This fly was found to be the primary causal agent of rhizome rot in Maharashtra State (Ajiri *et al.*, 1982). However in Kerala, it was found to be associated with rotten rhizomes only and does not play a significant role in causing the disease (Koya, 1988; Premkumar *et al.*, 1982). At Rudrur (Nizamabad District, Andhra Pradesh) the fly infestation was preceded by rhizome rot incidence (Sankaraiah *et al.*, 1991).

2.3 CHARACTERS OF RHIZOME ROT PATHOGENS

2.3.1 *Pythium aphanidermatum*

The studies on the cultural and morphological characters of the isolated pathogen showed its close identity with *P. aphanidermatum* which was described by earlier workers (Lucas, 1975; Mehrotra and Aggrawal, 2004; Rangaswami and Mahadevan, 2005 and Gaur and Chauhan, 2007). *Pythium* sp. has been identified on morphological features, particularly those of the antheridia, oogonia

and associated oospores, supplemented by the structures producing zoospore-containing sporangia (Hashem, 2010). Prabhukarthikeyan (2015) reported that the *Pythium* mycelium is hyaline, ramified, non septate and hyphae grew on the plate very fast, forming white colonies with loose and aerial mycelia.

2.3.2 *Fusarium solani*

Dohroo (1988b) noticed that the *F. solani* produces sparse to abundant, white cream mycelium on PDA medium. Macroconidia have three to four septa on average, are slightly curved, are rather wide and thick walled, and may have a slightly blunted apical end. Microconidia are abundant, oval to kidney shaped, and formed in false heads on very long monophialides. Colonies are fast growing, variable in color and texture, often granular or fluffy, rose-red, purple, but may start out as white, cottony colonies that darken with maturity.

2.4 ENDOPHYTES

Many authors have defined it and some of the relevant definitions are included Anton de Bary coined the term endophyte in 1886 to describe microorganisms that colonize internal tissues of stems and leaves. Endophyte is derived from the Greek word 'endon' (within) and 'phyte' (plant). The term endophyte refers to interior colonization of plants by bacterial or fungal microorganisms. Petrini (1991) first defined endophyte as microorganism living in the plant organization for a certain stage of its life and would not cause disease.

Perotti (1926); Hallmann *et al.* (1997) and Azevedo *et al.* (2000) reported that bacteria on roots and in the rhizosphere benefit from root exudates, but some bacteria and fungi are capable of entering the plant as endophytes that do not cause harm and could establish a mutualistic association. Wagenaar and Clardy (2001) identified endophytes as microorganisms growing in the intercellular spaces of higher plants and they are recognized as one of the most chemically promising groups of microorganisms in terms of diversity and pharmaceutical potential.

James and Olivares (1997) stated that all bacteria that colonize the interior of plants, including active and latent pathogens, can be considered to be as endophytes. Kado (1992) and Quispel (1992) suggested that, endophytic bacteria establish endosymbiosis with the plant, whereby the plant receives an ecological benefit from the presence of the symbiont. It is now commonly accepted that each of the nearly 3,00,000 existing plant species hosts, at least one or even several hundred strains of endophytes. (Strobel and Daisy, 2003). Sopalan *et al.* 2003 isolated the endophytic fungus *Muscudor albus* residing in the cambium tissue of nutmeg tree in Thailand. Gary *et al.* (2001) also reported that the occurrence of *Muscudor albus* in small limbs of *Cinnamomum zeyalanicum*. Zhang *et al.* (2006) and Aly *et al.* (2010) studied that the great potential of endophytes as a major source of biologically active compounds with promising medicinal or agricultural applications. Kurian (2011) reported that the antagonistic nature of bacterial endophytes isolated from cocoa against the management of *Phytophthora palmivora*.

Hatem *et al.* (2013) reported the antagonistic nature *Acremonium* sp as endophytic bioagent in date palm against *Fusarium* wilt.

2.4.1 Mode of action of endophytes

Cook and Baker (1983) suggested different mechanisms by which the endophytic microbes controlled *Fusarium* wilt of different crops. These mechanisms include production of antifungal compounds, siderophore production, nutrient competition, niche exclusion and induction of systemic resistance. It is possible that several of these mechanisms play role in biological control exhibited by these organisms. According to Backman *et al.* (1997), the effectiveness of endophytes as biocontrol agents is dependent on many factors. These factors include: host specificity, population dynamics and pattern of host colonization, the ability to move within the host tissues, and the ability to induce systemic resistance. Production of antimicrobial compounds and mycoparasitism, the feeding on a fungus by another organism are the mechanisms where by *Trichoderma* sp. provides protection to plants against plant pathogens (Chet *et*

al., 1998; Howell, 2003; Harman *et al.*, 2004). A perusal of the literature revealed no reports of endophytes on turmeric spice.

Production of volatile inhibitory substances by endophytes was studied by (Nejad and Johnson, 2000). They found that, most of the endophytic isolates from oilseed rape were HCN negative but the isolates produced volatile metabolites which had fungal inhibitory action. Hence they concluded that the endophytes are producing antifungal volatiles other than HCN.

Volatile substances such as 2, 3- butanediol and acetone produced by the bacteria have been reported to be responsible for plant growth promotion (Ryu *et al.*, 2003).

Maclas-Rubalcava *et al.* (2008) first reported production of allelochemicals with antifungal activity by the newly discovered endophytic fungus *Edenia gomezpompae* on cocoa. They observed antagonism by the endophyte towards *Phytophthora capsici* and *Phytophthora parasitica* against fungi *Fusarium oxysporum* and *Alternaria solani*.

2.4.1.1 Siderophore production

Findings by Cao *et al.* (2005) indicated the potential of siderophore producing *Streptomyces* endophytes for the biological control of *Fusarium* wilt disease of banana whereas, among the endophytic bacteria from sunflower none of the strains produced siderophores (Forchetti *et al.* 2007). A total of 29 endophytes strains were isolated from the halophyte *Prosopis stombulifera* grown under extremely saline conditions (Sgroy *et al.*, 2009). However, only one was able to produce siderophores and none of them solubilised phosphate. It was observed that, *in vitro* growth of *Xylella fastidiosa sub sp. pauca* was stimulated by the presence of supernatant siderophores of endophytic *Methylobacterium mesophilicum* (Lacava *et al.*, 2008).

Kajula *et al.* (2010) reported siderophore production by endophytic fungi. The siderophore produced *in vitro* was ferricrocin, quantities ranging between 7.9 to 17.6 mg lit⁻¹. Only the fungi with antibacterial activity produced

ferricrocin and any well known siderophores were not detected in the broths of antioxidant-producing fungi.

2.4.2 Isolation of endophytes

Several workers studied the isolation procedures of endophytes. Gardener *et al.* (1982) and Gagne *et al.* (1987) elucidated the isolation procedures of endophytes which are mainly of two types. According to them in vacuum extraction method the sap is extracted using the vacuum extraction apparatus and spread plate method is used for plating where as homogenization or trituration methods invariably involves surface sterilization followed by homogenization under aseptic condition. The triturate is then serially diluted and placed. Similarly Bell *et al.* (1995) isolated xylem inhabiting bacteria from grape vine by vacuum extraction and homogenization method. They compared the efficiency of two methods. The size of the population varied with the method of extraction: 2.65×10^2 to 3.46×10^3 / ml xylem sap with vacuum method of extraction, 3.83×10^3 to 1.31×10^4 /g xylem tissue with homogenization. Thus trituration or homogenization technique is considered as ideal method for isolation of endophytes because it yields higher number of endophytic bacteria (Hallman *et al.*, 1997; Uppala, 2007; Balan, 2009; Kurian, 2011). According to Wilson and Carroll (1994) collection of rhizome samples from different locations helped in the isolation of diverse group of organisms in sufficiently large numbers.

Kado (1992) isolated and characterized the endophytes from the papaya fruits and reported fermentative potential of strains. James (2015) isolated the endophytic bacteria and reported their antagonistic property against bacterial wilt of tomato caused by *Ralstonia solanacearum*.

2.5. MANAGEMENT OF RHIZOME ROT DISEASE

Several workers studied the effect of plant protection chemicals against the rhizome rot pathogens. Efforts to control the rhizome rot pathogens should be started from very early stage of disease, immediately after the expression of symptom. Nowadays the effect of plant protection chemicals, botanicals and antagonists in the management of diseases has practical importance.

2.5.1 Chemical control

Seed treatment and soil drenching with Bordeaux mixture (2:2:50) (Bhagawat, 1960) were found to effectively control rhizome rot. Pre sowing soil drenching and subsequent treatments every week with Bordeaux mixture (4:4:50) or Perenox (0.35%) and Dithane D-78 reduced the rhizome rot caused by *P. aphanidermatum* and *P. myriotylum* (Shahare and Astahna, 1962). Rathaiah (1987) observed that a mixture of Ridomil and Captafol controlled the disease and increased the yield. Metalaxyl was recommended for the seed treatment and soil drenching based on its performance in disease control after prolonged on farm evaluations of the fungicides at the Indian Institute of Spices Research, Calicut (Sarma, 1994; Koshy *et al.*, 1988a). Similarly increased germination, reduced disease incidence of rhizome rot and better yield was obtained with captafol when used for seed treatment (Koshy *et al.*, 1988b; Mathur *et al.*, 1984).

When six non systemic fungicides and four systemic fungicides were tested against *P. aphanidermatum*, Metalaxyl, Captafol, Ziride and Captan and metalaxyl formulations reduced rhizome rot incidence and increased germination and yield (Thakore *et al.*, 1988; Manmohandas *et al.*, 1990). Fungicides though effective they are prone for leaching off during heavy monsoon period and also not cost effective (Sarma, 1994).

2.5.2 Biological control

Biological control, using microorganisms to suppress plant disease, offers a powerful alternative to the use of synthetic chemicals. Numerous studies have shown that biological control offers an environmental friendly alternative to protect plants from soil-borne pathogens (Emmert and Handelsman, 1999; Whipps, 2001; Weller *et al.*, 2002). Mechanisms of biological control are diverse. One effective mechanism the use of antagonist microorganisms such as bacteria, yeast and fungi to control plant disease. Continuous use of chemicals in controlling the diseases is not advisable due to the cost and adverse environmental hazards, besides development of resistance against pathogens. So

utilization of fungal/bacterial antagonists was effective to overcome such problems.

2.5.2.1 Fungal antagonists

Though several antagonistic fungi were reported as efficient in the management of plant diseases, *Trichoderma* occupies as pride place. Liu and Baker (1980) reported the genus *Trichoderma* as a potential biocontrol agent against fungal pathogens. The efficacy of *T. harzianum* in reducing PPR has been reported by Galindo (1992) and it was suggested as potential biocontrol agent to include in the integrated disease management of *Phytophthora* pod rot of cocoa.

Gary *et al.* (2001) reported *Muscodor albus* as an endophytic fungus from small limbs of *Cinnamomum zeylanicum* showing volatile antimicrobial properties. Similarly Sopalun *et al.* (2003) also reported *Muscodor albus* an endophyte residing in cambium of *Myristica fragrans* a nutmeg tree in Thailand. According to Adedeji, *et al.* (2010) *Trichoderma* spp as biocontrol agents were successfully combined with fungicides there by reducing the frequency of fungicide application from four to one with significant pod rot infection in the field. Hatem *et al.* (2013) reported the antagonistic nature of endophytic fungus *Acremonium kilense* against the *Fusarium* wilt of date palm caused by *Fusarium oxysporum* f. sp. *albedenis*.

Twenty two isolates of *Trichoderma* sp. isolated from the turmeric rhizosphere soil collected from major turmeric growing tracts of South India were evaluated for their antagonistic potential against *Pythium aphanidermatum* (Edson) causing rhizome rot of turmeric. The isolates were evaluated based on the dual plating, growth rate and volatile and non-volatile metabolites production *in vitro* reported by Anoop and Suseela Bhai (2014).

2.5.2.2 Bacterial antagonists

Endophytic microflora comprises resident and transient microorganisms. The main components of microflora were bacteria, yeast and filamentous fungi (Baker and Cook, 1974). Galindo (1992) noticed that endophytic population was

the highest during periods of precipitation and high relative humidity and the lowest during dry periods.

The inhibitory effect of *Pseudomonas aeruginosa* against *Phytophthora palmivora* under *in vitro* conditions was reported by Attafuah (1965). Galindo (1992) reported that endophytic microflora like *P. fluorescens* and *P. aeruginosa*, *Serratia marscens*, *Burkholderia cepacia* and five isolates of *Bacillus* sp. against *P. palmivora* and *P. nicotianae* from cocoa rhizosphere based on *in vitro* screening.

Anandaraj *et al.* (2005) recently described the Fluorescent pseudomonads were selectively isolated from black pepper (*Piper nigrum*) roots and screened for volatile and non-volatile metabolite production and inhibition in growth of *Phytophthora capsici*, the causal organism of foot rot disease. Among the isolates tested, the inhibition of *P. capsici* varied from 36.3% to 70.0% by non-volatile metabolites and from 2% to 23% by volatile-metabolites.

Fourteen endophytic bacterial isolates were isolated from the rhizome of *Curcuma longa* L. were identified as six strains namely *Bacillus cereus* (ECL1), *Bacillus thuringiensis* (ECL2), *Bacillus* sp. (ECL3), *Bacillus pumilis* (ECL4), *Pseudomonas putida* (ECL5) and *Clavibacter michiganensis* (ECL6). All the strains produced IAA and solubilized phosphate and only two strains produced siderophore (ECL3 and ECL5) during plant growth promoting trait analysis. The endophytic strains effectively inhibit the growth of *Escherichia coli*, *Klebsiella pneumoniae* and some of the fungal strain like *Fusarium solani* and *Alternaria alternate* reported by Ajay *et al.* (2016).

Toh *et al.* (2016) reported the endophytic bacteria were isolated from six *Piper nigrum* roots and screened for *in vitro* antagonistic activity against *P. capsici* through dual culture, mycelial growth, spore germination and double plate assay. The antagonism testing involved the secretion of volatile and diffusible bioactive compounds by the endophytic bacteria. Out of 19 isolates tested, two isolates DB(2)7 and SB(2)6 produced volatile bioactive compounds and these two isolates showed highest antagonism against *P. capsici* mycelia

with the percentage of inhibition up to 47.63% and 43.33%, respectively. Diffusible compounds from isolates DB(2)7, DB(2)9 and SB(2)6 produced clear zones in spore germination test with radii measurements of 10.0-17.0 mm.

2.5.2.3 Endophytes

Endophytic organisms are ubiquitous in most plant species and influence the host fitness, disease suppression, contaminant degradation and plant growth promotion. They colonize the plant interior, interact more closely with less competition for carbon sources and produce a more protective environment for fixation. (Mittermeier *et al.*, 1999).

Muthukumar *et al.* (2010) isolated the nine bacterial endophytes from stem and root portions of chillies and tested for their efficacy against *Pythium aphanidermatum* (Edson) Fitzp. inciting chilli damping-off under glasshouse condition. Out of these nine bacterial endophytes, EBC 5, EBC 7 and EBC 6 recorded the minimum mycelial growth (28.00, 30.66 and 33.33 mm, respectively) with maximum inhibition zone of (12.33, 11.66 and 11.08 mm, respectively) of pathogen over control.

Rohani *et al.* (2013) successfully isolated the thirty endophytic fungi from leaf, rhizome, root, and stem of red ginger plant. Antagonistic activity was tested against *Fusarium oxysporum*, a pathogenic fungus on plants, using an antagonistic assay. The antagonistic activity of isolated endophytic fungi against *F. oxysporum* varied with the inhibition value range from 1.4 to 68.8%.

Ajay *et al.* (2016) isolated the fourteen endophytic bacterial isolates from the rhizome of *Curcuma longa* L. All the strains produced IAA and solubilized phosphate and only two strains produced siderophore (*Bacillus* sp. and *Pseudomonas putida*) during plant growth promoting trait analysis. The endophytic strains effectively inhibit the growth of *Escherichia coli*, *Klebsiella pneumoniae* and some of the fungal strain like *Fusarium solani* and *Alternaria alternata*.

2.6 COMPATIBILITY OF ENDOPHYTES WITH PLANT PROTECTION CHEMICALS

In any integrated plant disease management programme, its components must be compatible with each other. Otherwise, the desired output of the programme may not be achieved. This is more pertinent when biocontrol agents are used as one of the components in the integrated disease management strategy.

2.6.1 Fungicides

T. harzianum Rifai was tolerant to most of the fungicides and was used for the integrated control of many plant diseases (Henis *et al.*, 1979; Papavizas and Lewis, 1981; Papavizas, 1982). Papavizas (1985) observed the integrated approach could be successful only if the antagonists were compatible with the fungicides and biopesticides. According to Mukhopadhyay *et al.*, (1986) *T. harzianum* could tolerate fungicides like metalaxyl (0.1 per cent) and carbendazim (0.0065 per cent). Similar results were reported by Mukherjee *et al.* (1989). Kay and Stewart (1994) observed *T. harzianum* C 52 was insensitive to thiram and mancozeb.

Krishnamoorthy and Bhaskaran (1994) found that captan was compatible to *T. viride*, while it had little effect on *T. harzianum*. They also observed in copper oxy chloride poisoned medium *T. harzianum* showed normal growth and sporulation. Mondal *et al.* (1995) noticed inhibition of mycelial growth of *Trichoderma* sp. to a greater extent with addition of 200 to 500 ppm of mancozeb (Dithane M-45) after three days of incubation. Shanmugham (1996) reported that Bordeaux mixture completely inhibited the growth of *T. viride*.

Sarma and Anandaraj (1999) observed that copper fungicides were toxic to *Trichoderma* sp. and *Gliocladium virens*. Paciulyte *et al.* (2000) tested the sensitivity of fungicides, copper sulphate and copper oxychloride and found that both fungicides were active against fungi and it was also found that *Trichoderma* sp. were more sensitive to copper oxychloride than copper sulphate.

Akbari and Prakhia (2001) reported that thiram, mancozeb, tridemorph, metalaxyl mz and fosetyl Al were non inhibitory to *T. harzianum*, *T. viride* and *G. virens* at all concentrations tested. Mclean (2001) tested *in vitro* sensitivity of spores of *T. harzianum* to eight fungicides commonly applied to onions and indicated that *T. harzianum* was least sensitive to pyrocymidone and captan and most sensitive to mancozeb, tebuconazole and thiram.

Sharma *et al.* (2001) found that among two systemic and six non-systemic fungicides tested, tolerance of *T. harzianum* for metalaxyl was seven times higher than carbendazim. Jeyakumar *et al.* (2003) noticed that *Trichoderma* sp. were compatible with metalaxyl mz even at 1000 µg/ml. Vijayaraghan and Abraham (2003) reported that Bordeaux mixture at all concentrations completely inhibited the growth of *T. harzianum*, *T. viride* and *T. longibrachiatum* while, metalaxyl + mancozeb, potassium phosphonate (Akomin), mancozeb (Indofil M-45) and antracol at different concentrations showed varying degrees of inhibition.

Bhavani (2004) studied the biological management of *Phytophthora* pod rot and according to him potassium phosphonate (Akomin) and mancozeb (Indofil M-45) were compatible with fungal antagonists like *Trichoderma* sp. at recommended dose of concentrations.

Bhattiprolu (2007) and Madhusudhan *et al.* (2010) found that *T. viride* is compatible with mancozeb (Dithane M-45) and incompatible with carbendazim, hexaconazole, thiophanate methyl and tridemorph. Gaikwad *et al.* (2011) and Ahanger *et al.* (2014) reported that *T. viride* isolates were incompatible with seed dressing fungicides like captan, vitavax, carbendazim and found compatible with wettable sulphur, mancozeb and cymoxalin 8% + mancozeb 64% (Curzate) found compatible.

Pandian *et al.* (2013) conducted an experiment to study the compatibility of copper hydroxide (Kocide 3000) with bacterial and fungal biocontrol agents under *in vitro* conditions. Bacterial biocontrol agents *viz.*, *Pseudomonas fluorescens* and *Bacillus subtilis* were compatible with copper hydroxide (Kocide

3000) even at a high concentration of 300 ppm. Fungal biocontrol agent, *Trichoderma viride* was inhibited by copper hydroxide at a concentration above 2500 ppm. The fungal biocontrol agent was highly compatible with the fungicide than the bacterial biocontrol agents.

Vimi *et al.* (2016) carried out a study to evaluate the compatibility of *P. fluorescens* (KAU strain) with eleven fungicides commonly used in management of banana diseases by poisoned food technique. Each fungicide was tested at four concentrations *viz.*, 0.05%, 0.1%, 0.2% and 0.3%. The results of the study proved that, seven fungicides *viz.*, propiconazole, hexaconazole, tebuconazole, difenconazole, azoxystrobin, carbendazim and famoxadone + cymoxanil compatible at all tested concentrations. Kresoxim methyl was less compatible and mancozeb, copper oxychloride and copper hydroxide are not compatible with *P. fluorescens*.

Basamma and Shripad (2017) assessed the compatibility of bacterial biocontrol agent *Bacillus subtilis* with commonly used chemical fungicides *viz.*, carbendazim, mancozeb, metalaxyl mz, wettable sulphur, hexaconazole, difenconazole and tebuconazole. The compatibility was assessed at different concentrations and the concentration of 50, 100, 250, 500, 1000, 2000, 3000 and 5000 mg/lit for solid formulation fungicides and µl/lit for liquid formulation fungicides respectively. The compatibility tests revealed that among the solid formulation fungicides, the *B. subtilis* showed more tolerance to carbendazim and among the liquid formulation fungicides hexaconazole and kresoxim methyl showed maximum compatibility upto 3000 µl/lit concentration. The fungicides *viz.*, carbendazim, difenconazole and hexaconazole compatible with *B. subtilis* at concentrations which were recommended for plant disease management *i.e.*, 1000 mg carbendazim/lit, 500 µl difenconazole/lit, 1000 µl hexaconazole/lit seem to be safe tolerance limit for *B. subtilis*.

2.6.2 Insecticides

Sharma and Mishra (1995) studied the compatibility of the biocontrol agent *T.harzianum* with aldicarb, phorate and carbofuran which was applied for

management of nematodes and mealy bugs in black pepper. The study indicated that these insecticides were less toxic. Jebakumar *et al.* (2000) found that phorate and chlorpyrifos could be safely applied with *T. harzianum*.

Sushir and Pandey (2001) opined that among four insecticides tested *in vitro*, chlorpyrifos (Durret 20 EC) was found to be more toxic even at 50 µl ml⁻¹ which showed growth inhibition of 55.55 and 57.77 per cent respectively.

Sharma (2003) reported that *Trichoderma* sp. is compatible with potassium phosphonate and chlorpyrifos and therefore indicated their potential for IDM with dual mode of action in suppressing both pathogenic fungi and plant parasitic nematodes.

According to Mathew (2003) *P. fluorescens* was compatible with recommended dose of imidacloprid, etofenprox, chlorpyrifos and triazophos. Vijaraghavan (2003) found that three species of *Trichoderma* viz., *T. viride*, *T. harzianum* and *T. longibrachiatum* were incompatible with phorate and carbofuran at all concentrations tested. monocrotophos and quinalphos were incompatible with these antagonists and chlorpyrifos, endosulfan, dimethoate and cypermethrin were showed varying degree of inhibition at different concentrations.

Bhavani (2004) reported *T. viride* and *T. harzianum* were found incompatible with endosulfon, chlorpyrifos and phorate at recommended concentrations in management of insect pests. Gowdar *et al.* (2007) reported that species of *T. harzianum* rifai exhibited maximum inhibition at 0.75 per cent concentration of imidacloprid and they concluded that it was incompatible.

Bhai and Thomas, (2010) reported partial compatibility of *T. harzianum* with insecticides quinalphos with 55.84 per cent inhibition. Ranganathaswamy *et al.* (2011) evaluated the compatibility of *T. harzianum* and *T. virens* with different insecticides. They reported that *Trichoderma* sp. were incompatible with quinalphos and chloripyriphos, while dimethoate and endosulfan was found to be least compatible with showing 70 per cent inhibition on radial growth of the fungal isolate.

Ahila Devi (2013) conducted an experiment to know the compatibility of azoxystrobin 25 SC with biocontrol agents by poisoned food technique and turbidity method and with insecticides by emulsion stability test. The biological compatibility was done under glass house condition to find the percent injury. The compatibility showed that *Pseudomonas fluorescens* and *B. subtilis* were compatible with azoxystrobin 25 SC at 5, 10, 50, 100 and 250 ppm, concentration. The physical compatibility studies showed that azoxystrobin 25 SC was highly compatible commonly used with insecticides viz., profenphos, dichlorvos, monocrotophos, carbaryl, dimethoate, triazophos and quinalphos. The results from the biological compatibility revealed that insecticides, dichlorvos and profenphos were found to be less compatible with azoxystrobin 25 SC when compared to other insecticides.

2.7 SCREENING STUDIES

At present the rhizome rot disease was managed by using the fungicides which lead to development of resistant strains besides the environmental pollution and also the residue problem on final produce. Use of resistant varieties is the most economic and easily adoptable method in integrated disease management. Identification of high yielding turmeric accessions which are relatively resistant or tolerant to major diseases is of utmost importance for increasing the production and productivity of turmeric in Andhra Pradesh.

Senapati and Ghose (2005) screened 134 varieties of ginger against rhizome rot complex. The results indicated that, only one variety ZO-16 was found to be resistant (percentage disease incidence, PDI, - 0.0), eight moderately resistant (PDI -1.0 to 5.0), 19 tolerant (PDI - 6.0 to 15.0), 56 moderately tolerant (PDI -16.0 to 25.0), 39 susceptible (PDI - 26.0 to 40.0) and 11 highly susceptible (PDI - above 40.0).

The cardamom (*Elettaria cardamomum*) variety, IISR Avinash (RR-1) was developed at the Indian Institute of Spices Research, Cardamom Research Centre, Appangala (Karnataka), through clonal selection and recommended for release for its resistance to rhizome rot disease and high yield. The average yield

of IISR Avinash was 643 kg ha⁻¹ (dry) with a potential yield of 979 kg ha⁻¹ (dry) with good quality dark green capsules (Venugopal *et al.*, 2006).

Sharma *et al.* (2011) reported the rhizome rot and wilt disease complex is the important production constraints of ginger cultivation in hill agro-ecological region of West Bengal. In the present study, none of the locally available germplasm tested was found to be tolerant against rhizome rot and wilt disease complex of ginger, however, cultivar “Majauley” may be considered as moderately susceptible and “Bhaisey” and “Gorubathaney” are considered as highly susceptible germplasm in ginger.

Joshi *et al.* (2012) conducted a survey at Uttarakhand and Uttar Pradesh states in India, for collection of soil and plant samples. A total of eighty isolates of *Fusarium* were isolated from these samples. Pathogenicity test on chilli was conducted. One isolate of *F. oxysporum* was found most virulent pathogen, while eleven isolates were non- pathogenic isolates. Isolate no. 65 was found most antagonistic towards *F. oxysporum*, under *in-vitro* dual culture assay. Thirty chilli varieties were screened for evaluation of resistance. Among these, two varieties were found resistant against the *Fusarium* wilt. The present investigation focused on recovery of antagonistic *Fusarium* and resistant varieties of chilli, for controlling and resisting wilt and improving the soil health.

Pratap *et al.* (2013) reported twenty five varieties of ginger (*Zingiber officinale*) were screened in the field against *Pythium aphanidermatum* for two consecutive years during 2010–2011 at High Altitude Research Station, Orissa University of Agriculture and Technology, Pottangi, Koraput, Odisha. These varieties were screened in lab and net house condition. Out of 25 varieties only 3 varieties showed resistance to rhizome rot, of which Sargiguda was resistant and China and Varada were partially resistant, providing good material for developing rhizome rot resistant ginger varieties.

Field screening studies were conducted over a period of four years i.e. 2008-2012 in augmented block design with 295 available germplasm lines of turmeric (*Curcuma longa* L. and *Curcuma aromatic* L.) at Horticultural Research

Station, Jagtial and Turmeric Research Station, Kammarpally. These lines were screened against the leaf spot caused by *Collectotrichum capsici* under natural conditions. The severity of leaf spot is ranged from 0.00 to 75.28 per cent irrespective cultures screened. Among the germplasm cultures screened, cultures like Kasturi Joguripadu, PCT-3, Kasturi Ethakotta, Kasturi-3, Kasturi-2, Jupalli Kasturi, Shillong kasturi, Jorhat local in short duration, CLI-321, CLI-325, CLI-330, CLI-331, CLI-334 in medium duration and JTS-1, JTS-2, JTS-401, JTS-402, NDH-14,2-A, 15-B, Chennur local, Gadida pasupu, Kankipadu, Upparpalli in long duration group were free from leaf spot disease. More disease severity (susceptible reaction) of leaf spot was observed in long duration group (Narasimha Rao *et al.*, 2015).

Chapter III

MATERIAL AND METHODS

The present investigation entitled “Studies on Endophytic microbial diversity and antagonistic effect on rhizome rot pathogens in turmeric (*Curcuma longa* L.)” was carried out at the Department of Horticultural Plant Pathology, College of Horticulture, Anantharajupeta during 2016-2017. The details of materials used, methodologies adopted and techniques employed during the course of investigation are outlined in this chapter.

3.1 GENERAL

All the *in vitro* experiments were conducted in aseptic conditions. The equipments used for the experiments which included glassware, inoculation loops and needles, syringes, etc were sterilized by keeping them in an hot air oven at 180⁰C for at least six hours before starting each experiment.

3.1.1 Glassware

Borosil and Corning glasswares were used throughout the experiments. Tarson's disposable petriplates and microtips were used for serial dilution.

3.1.2 Cleaning/Sterilization of the glasswares

The glasswares used for all the experiments were thoroughly washed with potassium dichromate or cleaning solution and washed with tap water. Later they were rinsed with distilled water, allowed to dry and sterilized in the hot air oven at 180⁰C for 6 hours.

3.1.3 Replication and incubation

Three/four replications were maintained for each treatment, in the *in vitro* experiments and the petriplates were incubated at room temperatures (28 ± 2⁰C) unless otherwise specified.

3.1.4 Media used for the different experiments

The media required for the various experiments were prepared in steel containers and distributed equally into 250 ml. conical flasks @ 100 ml/flask and autoclaved at 15 psi pressure for 20 minutes. (Appendix III). The media like Potato Dextrose Agar, Nutrient Agar, King's B Agar, Ken Knight's Agar, Martin's Rose Bengal Streptomycin Agar are used for the different experiments (Plate 3.1).

3.2 DISEASE SURVEY

A roving survey was conducted during 2016-2017 in the turmeric growing tracts of A.P. in South India to study the intensity of rhizome rot disease in turmeric. The period of survey included both monsoon and post monsoon seasons. Different locations from five districts *viz.*, Kurnool, Kadapa, Guntur, West Godavari and Visakhapatnam were chosen for the survey. Diseased plant samples were collected randomly from the farmer's fields from different locations of the above stated districts of AP. In each district three mandals and in each mandal three villages were surveyed for the rhizome disease. In each field row (each 10 meters long) were selected randomly. A total of 45 different locations in 5 districts of A.P. were covered. In each row total number of plants and number of diseased plants were counted and expressed in terms of percentage. The plants showing symptoms of rhizome rot such as yellowing of the foliage, rotting of the pseudo stem, root rot etc. were identified and recorded. Per cent disease incidence was assessed by counting the number of affected plants out of the total plants. The representative samples of infected plants were collected for isolation and identification of pathogens for further studies.

A proforma was prepared to collect and record the information on seed treatments, planting time, previous crop, fertilizer and fungicide application, crop rotation and intercrops and other diseases in turmeric.

3.3 ISOLATION OF PATHOGENS FROM INFECTED RHIZOMES

The pathogens causing rhizome rot disease of turmeric were isolated from the infected rhizomes collected from different locations of the above stated

districts of A.P. The pathogens were isolated by tissue segment method (Rangaswami, 1958) on Potato Dextrose Agar medium. The diseased rhizome samples were brought to the laboratory, washed under tap water and the infected area along with healthy portion were cut into small bits (1.0-1.5 cm). The bits were surface sterilized with one per cent sodium hypochlorite solution for one minute followed by washing in three changes of sterile distilled water. The surface sterilized bits were placed on Potato Dextrose Agar (PDA) medium supplemented with streptomycin sulphate, under aseptic conditions in Petri dishes to prevent bacterial contamination and incubated at room temperature ($26 \pm 2^{\circ}\text{C}$). When the fungal growth was visible, small bits of mycelia were transferred to PDA mediated Petri dishes and the isolates were purified by single hyphal tip method. These purified cultures were maintained in PDA slants for further studies. The cultural and morphological characters of the isolates were also studied.

3.4 PATHOGENICITY

Inoculum of pathogens were prepared by placing 50 g sorghum seed in 250 ml conical flasks, soaking the seed overnight in water, pouring off the excess water, autoclaving twice on successive days and then inoculating the seed with mycelial disc of 10 mm diameter were taken from seven day old cultures of the respective organisms on PDA. The inoculum was used after the respective fungal colony had fully colonised the substrate (usually 7-10 days at 25°C) (Plate 3.2).

The steam-sterilized potting mixture containing soil, sand and farm yard manure (1:1:1) was filled in six numbers of clay pot. In treated set soil were inoculated separately with inoculums of each isolate at the rate of 50 g /kg of soil. The six pots were separated into two sets, one set was control and another one set was treated (inoculated). Three replications were kept for each set. The healthy rhizomes of turmeric were planted in all pots filled with sterilized potting mixture. The plants without inoculum served as control treatment (Johnston and Booth, 1983). The water was irrigated in pots once in a week and the trial pot was protected from insects and animals throughout the cultivation period under controlled condition. Plants were grown in treated pots showed the symptoms

three months after a seed piece had been planted in each pot. The rhizome rot symptoms like the development of water soaked lesions on pseudo stem and subsequent yellowing of the leaves showed by these plants were observed carefully and were recorded at regular intervals. The plants which showed symptoms of rhizome rot, those rhizomes were collected and used for the reisolation of the pathogens to prove the Pathogenicity and their cultural and morphological characters were studied and compared with that of original ones.

3.5 SYMPTOMATOLOGY OF RHIZOME ROT DISEASE OF TURMERIC AND IDENTIFICATION OF THE PATHOGENS

The symptomatology of rhizome rot disease of turmeric was studied both under natural and artificial conditions. The pathogen was identified based on cultural and morphological characters growing on the Potato Dextrose Agar medium and for further, confirmation the cultures were sent to NCFT, New Delhi. Morphological characters of mycelia spore bearing structures and spores were studied by slide culture method.

3.6 ISOLATION AND ENUMERATION OF ENDOPHYTIC MICROFLORA FROM TURMERIC

Endophytic microbes were isolated from healthy rhizome samples of turmeric collected from various locations of A.P. (Table 3.1). Fresh rhizome samples brought in separate polythene bags were washed under tap water, and used for isolation within 48 h. of collection.

Table 3.1 Locations of collections of endophytic microflora of turmeric

Sl. No	Crop	District	Place of collection
1	Turmeric	Kadapa	College of Horticulture, Anantharajupeta
2		Kurnool	Mahanandi
3		Visakhapatnam	HRS, Chintapalli
4		Gunur	Lam Farm
5		West Godavari	HRS, Kovvur

3.6.1 Surface sterilization

The surface of turmeric harbor a lot of epiphytes, thorough surface sterilization was needed to eliminate them. The concentration of sterilant and time of exposure were standardized so as to get the maximum number of endophytes with no growth on sterility check. Three different concentrations of sodium hypochlorite *viz.*, one, two and three per cent were tried for three different exposure times *viz.*, two, five and ten minutes. Further, three different weights of rhizome samples *viz.*, 0.5 gm, 1.0 gm and 2.0 gm were also tried. Since the isolation from the 2 gm of sample after surface sterilization with two per cent sodium hypochlorite for 10 min yielded good number of colonies with no growth in sterility check, it was selected for further studies.

3.6.2 Isolation and enumeration of endophytes

For the isolation of endophytes from healthy rhizomes of turmeric, samples were weighed out and exposed to sterilent (sodium hypochlorite) followed by washing in three changes of sterile water and blot dried. The rhizome bits were then transferred to sterilized mortar containing 8 ml sterile Potassium phosphate buffer (PB 0.1M, p^H 7.0) washed thoroughly in the buffer (Appendix IV). From the final buffer wash, one ml was pipetted out and poured into sterile Petri plate. To this molten and cooled medium was added and this served as a sterility check. If microbial growth was observed in sterility check with in four days, the isolates obtained from particular samples were discarded. The surface sterilized rhizomes of turmeric were triturated (McInroy and Kloepper, 1995) using sterile mortar and pestle with 8 ml of sterile buffer. The triturate was serially diluted in sterile PB up to 10⁻⁷. One ml of diluted triturate was pipetted in to sterile Petri plate and suitable medium was poured. The plates were incubated at room temperature for various intervals. The dilution and medium used for enumeration of each group microorganism are furnished in (Table 3.2).

Table 3.2 Dilution and media used for isolation and enumeration of endophytic microflora

Sl. No	Organism	Dilution	Medium	Period of incubation (Days)
1.	Fungi	10^{-4}	Potato Dextrose Agar	2
2.	Bacteria	10^{-6}	Nutrient Agar	2
3.	Fluorescent Pseudomonads	10^{-6}	King's B Agar	2
4.	Actinomycetes	10^{-1}	Ken Knight's Agar	7

Representative colonies of endophytes based on colony morphology were selected from the dilution plates and transferred to slants to establish pure cultures by the following standard protocols. Further, representative colonies of fungi, bacteria were selected and sub cultured and maintained in the respective media for further studies.

3.6.2.1 Fungi

The single colonies of fungi showing similar colony characters were selected from the dilution plates and transferred to Petri dishes containing PDA. Pure cultures of the isolates were maintained on PDA slants.

3.6.2.2 Bacteria

Representative bacterial colonies developed in dilution plates were picked and streaked on nutrient agar to get single colonies. The pure cultures were maintained on NA slants.

3.6.2.3 Fluorescent Pseudomonads

The bacterial colonies developed in the dilution plates on King's B agar were observed under U.V transilluminator for the presence of greenish yellow fluorescent pigment and only such representative fluorescent colonies were selected, they were purified and such cultures were maintained on King's B slants.

3.6.2.4 Actinomycetes

No actinomycetes colonies were observed during study.

Microbial population in each dilution was recorded and the number of colonies in each sample was calculated using the following equation given by Tan *et al.* (2006).

$$\text{Number of cfu /g sample} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume plated (ml)}}$$

3.7 IN VITRO ANTAGONISTIC EFFECT OF ENDOPHYTES AGAINST THE PATHOGENS OF TURMERIC

The *in vitro* antagonistic effect of endophytes towards the pathogen of turmeric was tested by dual culture method. As an initial step 58 endophytic isolates obtained from experiments 3.6.2.1, 3.6.2.2, 3.6.2.3 and 3.6.2.4 were subjected to preliminary screening to know whether they possess antagonistic properties or not.

3.7.1 Preliminary screening of endophytic microflora against the pathogens of turmeric

Mycelial disc of the pathogen isolated from the turmeric spice were taken from seven day old culture on PDA plate and incubated for 2 days. For screening of endophytic bacteria, four isolates were inoculated one each on the four sides of the pathogen as a line of streak one cm away from the periphery of the Petri dish. Similarly for screening of endophytic fungi, the mycelial discs from four isolates were placed one each on four sides of the pathogen at equidistance from the periphery of the Petri dish one cm away from the edge of Petri dish. Plates with pathogen alone served as control. The inoculated plates were incubated at room temperature and observed for inhibition of the pathogen for five days or when there was full growth in the control.

3.8 IN VITRO EVALUATION OF ANTAGONISTIC ENDOPHYTES AGAINST PATHOGENS OF TURMERIC

The endophytes which showed antagonistic activity in preliminary screening were further, tested individually to select the most efficient ones.

3.8.1 In vitro evaluation of antagonistic endophytic fungi against pathogens of turmeric

Four fungal isolates were evaluated for their antagonistic potential against the pathogens of turmeric by dual culture method (Skidmore and Dickson, 1976) in comparison with standard culture of *Trichoderma viride*. The organisms were inoculated on dual cultures after giving due consideration of their growth rate. Mycelial disc of the pathogen from seven day old culture grown on PDA was placed on one side of the plate and incubated at room temperature ($26 \pm 2^{\circ}\text{C}$) for two days. The mycelial disc (10 mm) of antagonistic fungi were placed on other side of the plate, four cm away from the pathogen and incubated. Three replications were maintained for each isolate. The pathogen grown as monoculture served as control. The plates were observed daily after 24 h. of inoculation of antagonists till the pathogen grew and covered the plate kept as control. The per cent inhibition of the pathogen was calculated using the formula suggested by Vincent (1927).

$$\text{PI} = \frac{\text{C} - \text{T}}{\text{C}} \times 100$$

PI = Per cent inhibition, C = Growth of the pathogen in control (mm), T = Growth of the pathogen in dual culture (mm)

Based on the per cent inhibition of mycelial growth of the pathogen, the efficient antagonists were selected for further studies. The nature of antagonistic action of endophytic fungi against the rhizome rot pathogens of this rhizome spice was assessed by the method of Purkayastha and Bhattacharya (1982) and assigned to four categories.

- A- Overgrowth : Pathogen overgrown by test organism
- B- Homogeneous : Free intermingling of hyphae
- C- Cessation of growth : Cessation of the growth at line of contact
- D- Aversion : Development of clear inhibition zone

3.8.2 *In vitro* evaluation of antagonistic endophytic bacteria

All the 6 bacterial isolates of endophytes along with the standard culture of *Pseudomonas fluorescens* were evaluated for their antagonistic effect by dual culture method (Utkhede and Rahe, 1983). Mycelial disc (10 mm) taken from seven day old culture of the pathogen grown on PDA was placed at the centre PDA mediated Petri dish and incubated for two days. The endophytic bacteria were inoculated as a line of streak on both sides, one cm away from the edge of Petri dish. For each isolate three replications were maintained. Plates with pathogen alone served as control. The plates were incubated at room temperature and observed daily, until the control exhibited full growth of the pathogen. The per cent inhibition was calculated as given in 3.8.1. Based on the per cent inhibition the efficient ones were selected.

3.9 MECHANISM OF ANTAGONISM OF SELECTED ANTAGONISTS ON PATHOGENS OF TURMERIC

3.9.1 Fungi

The standard dual culture technique was employed to study the mechanism of antagonism of selected fungal antagonists on pathogens of turmeric. For this PDA medium was plated on sterile Petri dishes and allowed to solidify. Mycelial disc of 8 mm diameter of respective pathogens of turmeric taken from an actively growing culture were inoculated at one end of the Petri dish 48 h. prior to inoculation of the antagonists, which was placed two centimeters away from the pathogen. The plates were incubated at room temperature and observations taken at regular intervals until there was hyphal intermingling. Microscopic observation for hyphal interaction was done by cutting out one sq. cm portion of the intermingling hyphal growth of antagonists and pathogens which was mounted in cotton blue lactophenol on slides.

3.9.1.1 Antibiosis test for the production of diffusible, nonvolatile inhibitory metabolites

Antibiosis test for production of diffusible, nonvolatile inhibitory metabolite was carried out using cellophane paper method described by Dennis and Webster (1971). Cellophane paper of 9 cm diameter was taken and sterilized in autoclave at 121°C for 15 min and then each sterilized disc was aseptically placed over the PDA inoculated plates. Ten mm discs were taken from the growth of each isolate of endophytes and was placed at the centre of the cellophane paper and incubated for 72 h. After this, the cellophane paper along with adhering antagonists was removed carefully and 8 mm disc of pathogens of turmeric was immediately placed on the medium at the central position previously occupied by antagonist. The growth of the pathogen was calculated from 48 h. upto seven days and the growth was compared with that in control. Three replications were maintained and the per cent inhibition of the pathogen was calculated.

3.9.2 Bacteria

The ability of selected endophytic bacterial antagonists and standard culture of *P. fluorescens* were tested for the production of siderophore, hydrogen cyanide (HCN), ammonia production and Indole acetic acid (IAA).

3.9.2.1 Siderophore production

Siderophore production by the bacterial isolates were detected by the method of Kloepper *et al.* (1980). The King's B agar was amended with two concentrations of $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ at the rate of 1 and 10 mg lit^{-1} . The sterilized medium was poured into each Petri dish. The test cultures were streaked on the surface of the medium. Three replications were maintained. The inoculated plates were incubated at room temperature for 48 h. and observed for the production of greenish yellow fluorescent pigment.

3.9.2.2 HCN production

HCN production by bacterial isolates were detected by the method of Baker and Schipper (1987). The King's B agar was amended with 4.4 gm⁻¹ of glycine and sterilized. The sterile medium was poured into dishes and allowed to solidify and the isolates were inoculated. Whatman No.1 filter paper disc (90 mm diameter) was soaked in picric acid solution (2.5 g picric acid + 12.5 g Na₂CO₃ in 1000 ml of water) and placed on the lid of each plate. Three replications were maintained for each isolate. Petri dishes were sealed with parafilm and incubated at room temperature for four days. The uninoculated plate served as control. An observation on colour change of filter paper from deep yellow to orange brown and to red indicates the production of HCN.

3.9.2.3 Ammonia production

Selected endophytic bacterial isolates were tested for their potential for production of ammonia following the method of Dye (1962). The bacterial isolates were grown in 10 ml of peptone water (Appendix III) and incubated at 30°C for four days. Three replications were maintained for each bacterial isolate. After incubation, 50 µl of Nessler's reagent was added to the broth. The change in the colour of the broth from faint yellow to deep yellow or brown colour indicated the production of ammonia. The reaction was scored as nil, low, medium and high in 1-4 scale based on intensity of colour.

3.9.2.4 Antibiosis test for production of volatile inhibitory metabolites

Antibiosis test for volatile metabolite production was carried out by sealed Petri plate technique with slight modification as described by Dennis and Webster (1971). For this, two Petri dish bases of 90 mm diameter were taken and molten cooled PDA poured on both dishes. One dish containing the PDA was inoculated with fungal antagonist and allowed to grow for three days. However, for bacterial antagonists, lawn of the candidate endophytic isolate was prepared by pour plate method. In the second Petri dish base plated with PDA medium, eight mm disc of seven days old pathogens of turmeric was placed at centre of the dish and two dishes were sealed together with parafilm and incubated at room

temperature in such a way that the antagonist fungus or bacteria lie under the lower dish. This allowed the volatile compounds produced by the antagonists to reach the pathogen growing in the upper dish. Similarly sealed dishes with pathogen inoculated in one plate and no antagonist on the other plate served as control. Three replications were maintained for each isolate. The growth of the pathogen was measured at 48 h interval upto seven days. The per cent inhibition of the pathogen by endophytes was calculated, using the formula given in 3.8.1.

3.9.2.5 IAA Production

Bacterial isolates inoculated in five ml of King's B broth supplemented with L-tryptophan @ 100 $\mu\text{g ml}^{-1}$ (100 $\mu\text{g ml}^{-1}$ L-tryptophan in 50 per cent ethanol), were incubated for 42 h. Growth of the isolates were removed by centrifugation at 5000 rpm for 10 min. One ml aliquot of supernatant was mixed thoroughly with four ml Salkowski's reagent and allowed to stand for 20 min at room temperature. The absorbance was read at 535 nm. IAA concentration was calculated from the standard curve. The isolates were also scored based on IAA production by following the scale *viz.*, IAA concentration $>5 < 10 \mu\text{g ml}^{-1} = 1$; $> 10 < 25 \mu\text{g ml}^{-1} = 2$; $> 25 < 30 \text{ mg } 50 \text{ ml}^{-1} = 3$ and $> 30 \mu\text{g ml}^{-1} = 4$.

3.9.2.6 Biochemical characterization and identification of isolates

Endophytic bacterial Isolates were identified by morphological and biochemical characterization according to Bergey's manual of Determinative Bacteriology (Kumar *et al.* 2015).

3.10 COMPATIBILITY OF SELECTED ANTAGONISTS TO PLANT PROTECTION CHEMICALS USED IN TURMERIC

The *in vitro* compatibility of the selected fungal and bacterial antagonists to plant protection chemicals commonly used in turmeric was tested.

3.10.1 Fungicides

The fungicides listed below were used for the *in vitro* evaluation by using poison food technique for fungi, inhibition zone technique for bacteria.

Sl. No.	Chemical name	Trade name	Concentrations (per cent)
1	Copper oxychloride 50 % WDP	Blitox	0.1, 0.2, 0.3
2	Mancozeb 4 % + Metalaxyl-M 64 % w/w	Ridomil Gold MZ 68 WG	0.05, 0.1, 0.2
3	Carbendazim 12 % + Mancozeb 64% WP	Saaf	0.05, 0.1, 0.2
4	CuSO ₄ + Lime + Water	Bordeaux mixture	0.5, 1.0, 1.5
5	Mancozeb 75 % WP	Indofil-M45 WP	0.2, 0.25, 0.3
6	Fenamidone 10% + Mancozeb 50% WG	Sectin	0.05, 0.1, 0.2
7	Cyamoxanil 8% + Mancozeb 64% WP	Curzate M-8	0.1, 0.2, 0.3

3.10.2 Insecticides

The insecticides listed below were selected for *in vitro* evaluation by poisoned food technique for fungi and inhibition zone technique in case of bacteria (Vincent, 1947).

Sl. No.	Chemical name	Trade name	Concentrations (per cent)
1.	Dimethoate 30 % EC	Rogor	0.05, 0.1, 0.2
2.	Chloropyrifos 20 % EC	Dursban	0.05, 0.1, 0.2
3.	Thiomethoxam 25% WG	Cruiser	0.1, 0.2, 0.3
4.	Malathion 50% EC	Malathion	0.05, 0.1, 0.2
5.	Phosphamidon 40% SL	Demecron	0.05, 0.1, 0.2

3.10.2.1 Fungal antagonists

The quantity of fungicides, insecticides needed to get the desired concentration was added to 100 ml sterilized, molten PDA medium, mixed well and poured in sterilized Petri dishes at the rate of 15-20 ml per plate. To avoid contamination, all ten fungicides were exposed to UV light for a period of 30 min before adding it into the medium. After solidification of the medium, mycelial discs of 8 mm diameter from actively growing fungal antagonists and *Trichoderma viride* were cut and placed at the centre of the each Petri dish. Control consisted of PDA medium alone inoculated with the antagonist. Three replications were maintained for each concentration. The inoculated plates were incubated at room temperature and observations on the mycelial growth of the fungal antagonists were taken when control plates showed full growth. The per cent inhibition of growth of the antagonists were calculated by using the formulae as described in the section 3.8.1.

3.10.2.2 Bacterial antagonists

Sterile filter paper discs of 8 mm diameter were soaked in different concentrations of each fungicide and insecticide. The discs were placed at the center of Petri dishes containing the NA medium seeded with 48 h. old culture of the three different isolates of the bacterium. Control consisted of filter paper disc soaked in sterile distilled water. Three replications were maintained. The inoculated plates were incubated at room temperature and the observations on inhibition zone were recorded after 48 h. The per cent inhibition of growth of bacterial antagonists was calculated using the formula given in 3.8.1.

3.11 IDENTIFICATION OF EFFICIENT ENDOPHYTIC ANTAGONISTS

The cultural and morphological characters of fungal isolates were studied for identification. Further, confirmation of the identified fungus was done at National Centre for Fungal Taxonomy (N.C.F.T) New Delhi.

3.11.1 Fungi

A total of two fungi were selected as promising antagonists and they showed more than 60 per cent inhibition of the pathogen. The cultural characters of the antagonists like growth, colony colour, and pigmentation were studied on PDA medium and morphological characters of the mycelium, spores and spore bearing structures were studied.

3.11.2 Bacteria

The four promising bacterial antagonists selected from turmeric were characterized based on the cultural morphological and biochemical characters. Cultural characters like Gram reaction, production of fluorescent pigments, growth at 4°C and 41°C, catalase activity, oxidase test, levan production, starch hydrolysis, nitrate reduction, urease activity and arginine hydrolase activity were tested following the procedures suggested by Manual of American Phytopathologists along with the standard cultures of *Pseudomonas fluorescens*. The Hi Assorted TM biochemical test kit for Gram negative rods were also employed for characterization of the endophytic isolates and compared with the interpretation chart given in the manual. Three replications were maintained for each isolate.

3.12. VARIETAL RESISTANCE (FIELD TRIALS)

A screening trial was conducted during *kharif*, 2016-17 under field conditions and pot cultures at College of Horticulture, Anantharajupeta determined the reaction of turmeric germplasm lines against rhizome rot.

3.12.1 Geographical location of experimental site

The experimental site was located at College of Horticulture, Anantharajupeta that falls under tropical zone of Rayalaseema region of Andhra Pradesh with an average rainfall of 700 mm and is situated at an altitude of 162 meters (531 feet) above mean sea level. The geographical situation is 13.980 N latitude & 79.400 E longitudes.

3.12.2 Characteristics of soil

The soil of the experimental area was red sandy. The p^H of the soil was 7.5. The characteristics of the soil under the experimental plot was analyzed in the soil testing Laboratory, COH, Anantharajupeta and details of the soil characteristics are presented in Appendix-I.

3.12.3 Weather conditions

The meteorological data for the experimental period (June 2016 to March 2017) pertaining to weekly mean rainfall, minimum and maximum temperature and relative humidity were recorded at meteorological observatory of College of Horticulture, Anantharajupeta are presented in Appendix-II.

3.12.4 Planting materials

In this research work, the rhizomes of turmeric germplasm lines were used as planting materials. The seeds (rhizomes) of the turmeric were collected from Horticultural Research Stations in Kovvur, Anantharajupeta and Chintapalli. The rhizomes of turmeric were broken into small pieces bearing 1-2 buds. The average weight of individual pieces was 25-50 g.

3. 12. 5 Treatment Details and Design of Experiment

3.12.5.1 Under natural field conditions

Field experiment for screening of turmeric germplasm lines were conducted at College of Horticulture, Anantharajupeta during 2016-17. During the present study 120 turmeric lines collected from Horticultural Research Stations at Kovvur, Chintapalli and A.R.Peta (Table 3.3) were included for screening against the rhizome rot under natural conditions. These lines were sown at first fortnight of June in shallow, red loamy soils. No plant protection measures were taken during the crop growth period. The trial was laid out in augmented block design using 120 lines including the 3 susceptible checks namely Duggirala, Mydukur, Tekuripeta. These highly susceptible Check plants were interplanted as “spreader rows” along with rows of the test material to create the natural disease infection. Sown three highly susceptible checks at

intervals, after every 10 rows of the test lines, at the same time as the test lines. Uniform sized forty fingers (fingers with 5-6 cm length and weighing about 20-25 g each) were sown in 2x2 m size plots for two varieties at spacing of 30 x15 cm in four rows accommodating 40 rhizomes/plot (Fig. 3.1). Application of 20 tonnes of farm yard manure and inorganic fertilizers like nitrogen, phosphorous and potassium were applied @ 190 kg, 75 kg and 120 kg per hectare in the form of urea, single super phosphate and murate of potash, respectively, as per the recommendations of the Dr YSR Horticultural University. Germination percentage counts at 45 days after planting (DAP) and percentage of rhizome rot incidence at 120 DAP, at harvest were performed (Plate 3.3).

Table 3.3 Source of germplasm collections

S. No.	Place of collection or name of the HRS	No. of Collections
1.	Kovvur, Coastal Andhra Pradesh	71
2.	Anantharajupeta, Rayalaseema Zone	17
3.	Chintapalli, Coastal Andhra Pradesh	32
	Total	120

3.12.5.2 Under pot culture conditions

In pot culture, the sterilised potting mix to which 40 g of moist sorghum seed colonised by *Fusarium solani* (equivalent to 20 g dry seed) had been added and turmeric rhizomes were planted in the 4-L pots. Thirty different germplasms of turmeric viz., Pratibha, Rajendra Sonia, Morthapuzta, Salem, Sports, GL-Puram, Kasturi, Roma, KTS-6, BSR-2, KTS-7, TCP-70, TCP-64, TCP-129, PTS-8, CL-1, CL-10, VK-9, RH-9/90, NB-60, NDH-8, CLI-335, ACC-79, CL-12, IC-416941, IC-330113, 1C-033007, IC- 212606, IC-211647, IC-211641 were planted in the second fortnight of June, 2016. Apparently healthy rhizomes of each variety were planted in pots and replicated three times. After emergence of sprout from the turmeric, close observation was kept for 45 days at regular intervals (Plate 3.4).

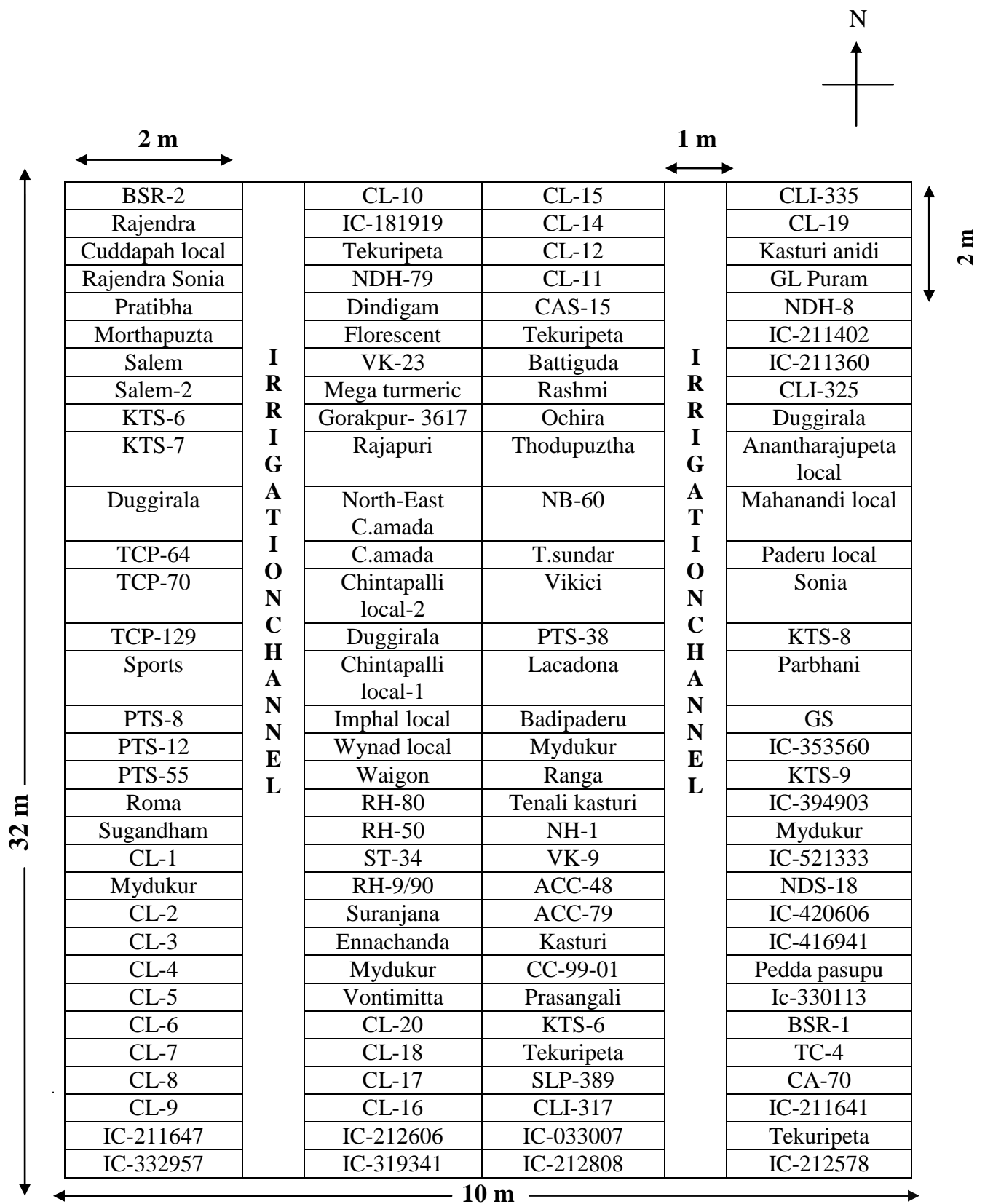


Fig.3.1. Field layout of the Experimental plot

3.12.6 Observations

3.12.6.1 Estimation of disease incidence (%)

According to Pratap *et al.* (2013), finally on the basis of per cent disease incidence (PDI) the germplasms were screened and categorically grouped into different types of reactions as described in table 3.4.

Table 3.4 Disease scale used for screening

Scoring Scale	Disease Reaction	Disease incidence (%)
0	Resistant (R)	0%
1	Tolerant/ Moderately resistant (MR)	1 - 10%
2	Moderately susceptible (MS)	11 - 25%
3	Susceptible (S)	26 - 50%
4	Highly susceptible (HS)	>50%

$$\% \text{ Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

3.12.6.2 Yield of Rhizome

After nine months of planting, the weight of rhizome per variety was recorded in field conditions. The yield was taken as the fresh weight of the rhizomes. Weight of the good and marketable rhizomes harvested from each plant variety was recorded and the data were expressed in kilogram (Singh, 2002).

3.13 STATISTICAL ANALYSIS

The data was analyzed as per the standard statistical procedures. The data obtained for different characters were statistically analyzed to find out the significance of difference among the treatments. The mean values of all characters were evaluated and analysis of variance was performed by 'F' value

test. The significance of the difference among the treatment combinations means was estimated by the Duncan's Multiple Range Test (DMRT) at 0.01% level of probability for lab experiment and 0.05% level of probability for field experiment (Singh and Gomez, 2001). The results have been depicted graphically wherever necessary.

Chapter IV

RESULTS AND DISCUSSION

An attempt to reveal the results of the present investigation "**Studies on Endophytic microbial diversity and antagonistic effect on rhizome rot pathogens in turmeric (*Curcuma longa* L.)**" was carried out at the Department of Horticultural Plant Pathology, College of Horticulture, Anantharajupeta during 2016-17. The results obtained during the course of present investigation are being presented statistically in the form of the tables and depicted in the form of graphs. The results along with discussion are presented under the following sub-headings with detailed discussion where ever necessary.

4.1 DISEASE SURVEY

A survey was conducted during monsoon and post monsoon seasons of 2016-17 to estimate rhizome rot incidence under field conditions in major turmeric growing areas of Guntur, Kadapa, Kurnool, West Godavari and Visakhapatnam districts. It is evident from the data (Table 4.1) that the disease incidence varied considerably among different locations, with a range of 1.87% to 45.56%. The mean maximum disease incidence was observed in Guntur district (16.12%) followed by West Godavari district (12.68%). Whereas, 4.54% and 3.27% mean disease incidences was observed in Kadapa and Kurnool districts and in Visakhapatnam district least mean disease incidence (2.18%) was noticed (Plate 4.1).

4.1.1 Incidence of rhizome rot disease in Andhra Pradesh during 2016-17

Among the 48 locations surveyed in Andhra Pradesh, the highest disease incidence was recorded from Atmakur (45.56%) of Mangalagiri mandal of Guntur district followed by Gundlapalem (32.22%) and Visadala (25.67%) of Medikondur mandal, while Morampudi of Tenali rural mandal of the same district was found to be free from disease (Plate 4.2a). The initial symptoms of rhizome rot disease was noticed after one month of planting and the highest

incidence of the disease was recorded during August-October, when secondary finger rhizome formation starts. In Kadapa, the disease incidence varied from 1.87%-12.60% (Table 4.1). Nagiripadu of Chitvel mandal (12.60%) showed maximum incidence while Immanur of Pullampeta mandal showed minimum disease incidence (1.87%). The disease incidence in other locations was comparatively low. The rhizome rot disease was observed to be high during September-November. In Kurnool, the disease incidence ranged from 2.90%-5.56%. No disease incidence was recorded from Alamur and Kondamayapalle of Rudravaram mandal. The highest disease incidence was recorded from Kalugotlapalle (5.56%) of Chagalamarri mandal. In West Godavari, disease incidence varied from 4.56%-21.0%. The lowest incidence was reported in Chodavaram (4.56%) of Nallajerla mandal and the highest in Ananthapalle (21.00%) of same mandal. In all the surveyed mandals of West Godavari, the time of appearance of the rhizome rot disease was noticed one month after planting. The highest incidence of the rhizome rot disease was observed during September-October when the secondary sprout and finger formation starts. In Visakhapatnam, the disease incidence ranged from 2.00%-3.36%. No disease incidence was recorded from Mallingavalasa and Nandigummi of Ananthagiri mandal. The highest disease incidence was reported from Cherapalli (3.66%) of Chintapalli mandal and the incidence of rhizome rot disease was noticed during July-September.

The progression of the disease in all the districts was correlated with rainfall. The disease was noticed when the newly formed sprouts were infected. This period varied with the different districts. The appearance of the disease coincided with the South-West Monsoon in districts like West Godavari, Vishakhapatnam and Guntur and North-East Monsoon in Kadapa and Kurnool districts of A.P. Shankariah *et al.* (1991) reported that continuous rain for a week in September induced rhizome rot disease in turmeric. They also reported a positive correlation between continuous rain and rhizome rot occurrence in Guntur district of A.P. The survey conducted in these areas shows that the rainfall during finger formation increases the chance of infection. The infected younger tip of the newly formed finger rhizomes and infected young sprouts

reinforce this view (Plate 4.2). Atmakur (45.56%) of Mangalagiri mandal of Guntur district that showed the highest disease incidence was severely affected by the rainfall received one week before the survey. In Chitvel and Pullampeta mandals of Kadapa, the severity of the disease incidence was reported to be higher during North- East monsoon. In most cases the symptoms were reported to appear in the initial stages of the crop when seed borne inoculum plays a major role (Shankariah *et al.*, 1991).

For the better understanding of the disease it is very essential to know about the cultivation practices of the crop which in turn will help to manage the disease to certain extent. The details of cultural practices followed during the cultivation of turmeric were collected with the objective of understanding the disease incidence under each cultivation practices. One of the major differences observed in cultivation practices is the use of the raised bed system (Kadapa, Vishakhapatnam) and ridge and furrow system (Guntur, West Godavari and Kurnool). In Vishakhapatnam, where raised bed system is practiced, comparatively less disease incidence was observed. Moreover, since it is a rainfed crop in Vishakhapatnam, the disease occurrence was found only during the rainy season when soil moisture was high. The bed system is supposed to help the water to drain off easily. In ridge and furrow system, the irrigation sometimes causes flooding increasing the chance of infection irrespective of the season. The information regarding intercrops and mixed crops also help to understand the biology of the pathogen. The intercrops and crop rotation may be helpful in reducing the primary inoculum for the next season. All the farmers included in the survey followed crop rotation and used suitable and traditionally cultivated crops.

Similar line of works carried out by Anoop *et al.* (2014) in major turmeric growing tracts *viz.*, A.P, Karnataka, Kerala and Tamil Nadu of South India and Rekha *et al.* (2015) in major ginger growing areas of Malnad districts *viz.*, Shimoga, Coorg, Chikkamagalore, Hassan and Uttara Kannada of Karnataka for the prevalence of rhizome rot disease.

4.1.2 Frequency distribution of fungi associated with rhizome rot of turmeric

A total of 144 samples collected from different locations used for the isolation of organisms associated to rhizome rot disease of turmeric. *Pythium* sp. and *Fusarium* sp. were the major organisms isolated from the infected samples (Table 4.2). Among the organisms isolated, *Fusarium* sp. dominated in majority of the diseased samples followed by *Pythium* sp. Out of total 144 samples, 56 samples (38.88%) showed *Pythium* sp. and 88 samples (61.11%) showed *Fusarium* sp.

All the samples collected from Kadapa yielded *Fusarium*. It showed more frequency during isolation of the organisms from infected samples. Most of the samples collected from Visakhapatnam district yielded *Pythium*. Out of 25 samples collected from Chintapalli, Paderu and Ananthagiri mandals of Visakhapatnam, *Pythium* was isolated from 24 samples (96.00%) showing its predominance. Only one sample from Munchingiputtu of Paderu yielded only *Fusarium*. In West Godavari also, the isolates from diseased samples were dominated by *Pythium* sp. Out of 28 samples collected from three mandals, 18 samples were found to be colonized by *Pythium* sp. (64.28%) whereas *Fusarium* was isolated from 10 samples (35.71%). Samples collected from Guntur showed the dominance of *Fusarium* sp. Out of the 32 samples collected from four mandals, 24 samples (75%) yielded *Fusarium* whereas *Pythium* was isolated from 8 samples (25%). Samples collected from Kurnool showed the dominance of *Fusarium* sp. Out of the 26 samples collected from three mandals, 20 samples (76.92%) yielded *Fusarium* whereas *Pythium* was isolated from 6 samples (23.07%). The overall data showed the dominance of *Fusarium* sp. (Table 4.2).

In majority of the samples, which were collected at the initial stage of the crop colonization mainly by *Pythium* sp. showing its significance as the primary causal organism of the disease (Plate 4.2). The sample which yielded *Fusarium* was at the advanced stage having completely decayed rhizomes with fibrous vascular tissues and was also colonized by maggots.

The data revealed that the isolation from samples at the initial stage of the disease increases the frequency of getting *Pythium* sp. This is supported by the reports of Park (1934) and Ramakrishnan and Sowmini (1954). Almost similar isolation frequency of the two organisms from Nuthakki of Mangalagiri samples can be substantiated by the advanced stage of the infected samples which were colonized by maggots producing foul smell (Plate 4.2). The complete colonization of *Fusarium* in the Atmakur sample might have suppressed the colonization of other fungi and also those which caused earlier infection. In West Godavari, the predominance of *Pythium* may be due to the collection of samples at the initial stage of the disease. The frequency of *Fusarium* was found to be more in Devarapalli mandal due to the collection at the end of December and at the later stage of the disease. The variation in the frequency of these isolates can be well substantiated by the samples from Kadapa district where the sampling was done during first week of February *i.e.*, almost at end of the season. No *Pythium* was isolated from samples which were of completely decayed and in a dry condition. The samples yielded only *Fusarium*. In Kovvur samples, the frequencies of these organisms were almost same since the sampling and isolation were made during the second fortnight of December. There are several reports of simultaneous occurrence of *Pythium* and *Fusarium* together associated with disease complexes (Bharadwaj *et al.*, 1988; Chauhan and Patel, 1990; Harvey *et al.*, 2008; Duarte *et al.*, 2007).

Anandam *et al.* (1996) reported the occurrence of *Fusarium* sp. along with *Pythium* sp. wherein the latter was repeatedly and consistently associated with rotted samples collected from West Godavari district of Andhra Pradesh which supports the present results. They could prove pathogenicity only with *P. aphanidermatum*. The primary involvement of the maggots in the disease can be excluded since the samples with the initial stages of infection were devoid of maggots. Hence, they are considered as secondary invaders causing the aggravation of the disease.

Sharma and Jain (1977) who had observed the frequency of *Fusarium* sp. was however maximum in ginger rhizomes as compared to other fungi *viz.*,

Fusarium oxysporum, *Pythium deliense*, *P. myriotylum* and *Pseudopapulaspora kendrickii* in Kerala.

4.2 ISOLATION OF PATHOGENS FROM TURMERIC

Surveys were conducted at different locations of Guntur, Kadapa, Kurnool, West Godavari and Vishakhapatnam districts for the collection of diseased specimens of turmeric. The organisms causing rhizome rot disease of turmeric were isolated from infected rhizomes collected from different locations of Andhra Pradesh (A.P). The isolates were purified by hyphal tip method and maintained on Potato Dextrose Agar (PDA) slants by periodical sub culturing. Pathogenicity of the organisms isolated from the turmeric plant was proved by artificial inoculation on healthy plants showed typical symptoms as observed in natural condition and re-isolation from artificially inoculated rhizomes of plants yielded organisms having the same characters as the original one. A total of two organisms from turmeric were isolated and their pathogenicity was proved (Plate 4.4a, b). The pathogenicity results of *P. ultimum* are in close agreement with those described for *P. aphanidermatum* and *P. myriotylum* where symptoms on the host appeared 3 to 5 days after inoculation (Lacava *et al.*, 2008).

In this study, the recovered isolates from diseased turmeric rhizomes were identified on their morphological characteristics as well as colony growth as *Pythium ultimum* and *Fusarium solani*. Pathogenicity test showed that the *Pythium ultimum* and *Fusarium solani* from diseased turmeric were pathogenic fungi and caused the same symptoms in turmeric plants. This is in accordance with the findings of Premkumar *et al.* (1982) and Koya (1990). They could prove pathogenicity with *P. aphanidermatum* and *Fusarium solani* in turmeric and ginger crops.

4.3 SYMPTOMATOLOGY OF RHIZOME ROT DISEASE OF TURMERIC AND IDENTIFICATION OF PATHOGENS

Observations on the symptoms developed by rhizome rot pathogens on turmeric were recorded from the naturally infected plants and also from the

artificially inoculated plants. The pathogens were tentatively identified based on the cultural and morphological characters and also by comparing the characters given in CMI Descriptions of Pathogenic Fungi and Bacteria.

4.3.1 Rhizome rot disease

In the present study, the leaves of affected plants exhibit gradual drying along the margins. This ultimately results in complete drying of all the leaves. The collar region of the pseudostem becomes soft and water soaked, resulting in collapse of the plant. The root system is very much reduced and its tissues are also affected. In severe condition, the infection spreads to rhizomes which decompose and turn into a decaying mass of tissues. The development of rhizomes is poor. The disease may appear in isolated plants or may involve several adjacent clumps resulting in appearance of diseased patches in the field (Plate 4.3 a, b).

The findings of the present study corroborates with the findings of Anandaraj and Sarma (1993). They reported the in well differentiated turmeric clumps, the infection starts at the collar region of the pseudostem as water soaked area which generally spreads both upwards and downwards.

4.3.2 Morphological Studies

4.3.2.1 *Pythium ultimum* Trow

The fungus produced cottony and fluffy colonies on PDA. The hyphae were long, slender, hyaline, profusely branched and aseptate measuring 2.1-4.2 μm in diameter. The sporangia were rare in culture but were obtained abundantly in 0.01 potassium nitrate solution. The sporangia were hyaline, globose to oval and apapillate. The sporangia measured $9.9 \times 19.8 \mu\text{m}$ in diameter. The sporangiophores were long and produce sporangia terminally. The sporangia germinated by a germ tube. The oospores were spherical and measured 14.0-18.0 μm in diameter. On the basis of these characters the pathogen was tentatively identified as *Pythium ultimum* (Plate 4.5).

The studies on the cultural and morphological characters of the isolated by pathogens showed its close identity with *P. ultimum* which was described by earlier workers (Lucas, 1975; Mehrotra and Aggrawal, 2004; Rangaswami and Mahadevan, 2005 and Gaur and Chauhan, 2007). These morphological characters are in conformity with those recorded by Sparrow, 1960.

4.3.2.2 *Fusarium solani* (Mar.) Sacc.

F. solani appears light yellow, moist appearance, red with cottony and orange brown mycelium, with light brown exudate on PDA medium. Macroconidia have three to five septa (35-55x4.5-6 µm), abundant, sickle shaped with blunt end. Microconidia have 0-1septas (8-16x2-4 µm), abundant, oval to kidney shaped, and formed in false heads on very long and thin, approximately 100 µm or more monophialides. Colonies are fast growing (4-5 days on PDA). Chlamydospores were smooth to rough walled and both intercalary and terminal, single or in pairs on short lateral branches in mycelium (Plate 4.6). Based on these characters and further confirmation from N.C.F.T, New Delhi with ID.NO (9009.17) the organism was tentatively identified as *Fusarium solani*. All the morphological characters of *Fusarium solani* are in close conformity to Booth (1971).

Table 4.3. Morphological characteristics of fungi associated with rhizome rot of turmeric

Fungi	Morphological characters						
<i>Pythium ultimum</i>	Hyphae (µm) 2.1-4.2	Sporangia (µm) 9.9-19.8		Oospores (µm) 14.0-18.0			
<i>Fusarium solani</i>	Hyphae (µm) 2.1-4.2	Micro Conidia (µm) 8-16x2-4	No. of Septa 0-1	Macro conidia (µm) 35-55x4.5-6	No. of Septa 3-5	linear growth Pinnotal type	Pigmentation light yellow to orange brown

4.4 ISOLATION AND ENUMERATION OF ENDOPHYTIC MICROFLORA FROM TURMERIC

Rhizome samples collected from different locations of turmeric growing areas of the A.P. State were used in isolation of endophytes. Isolation of endophytic microorganisms needs elimination of surface contaminants. Hence, the first step was surface sterilization followed by isolation (Araujo *et al.*, 2002). Different kinds of surface sterilization methods have been used by various workers (Bell *et al.*, 1995; Fischer *et al.*, 1992; Shishido *et al.*, 1999; Balan, 2009 and Kurian, 2011). In this study, the sterilization by using two per cent sodium hypochlorite solution for 10 min gave good results. The total endophytic microflora *viz.*, fungi, bacteria and fluorescent Pseudomonads were quantitatively estimated by dilution plate method. However, actinomycetes could not be isolated from any of the samples collected.

4.4.1 Fungi

Population of endophytic microbes isolated from healthy rhizome samples of turmeric are given in (Table 4.4). The data revealed that the population of endophytic fungi varied among the samples collected from different locations which ranged from 1 to 11×10^4 cfu g⁻² of rhizome tissue. The population was highest in samples collected from Mahanandi of Kurnool district (11.0×10^4 cfu g⁻²). The samples from HRS, Kovvur of West Godavari and COH, Anantharajupeta of Kadapa district and HRS, Chintapalli of Visakhapatnam also harboured comparatively higher fungal population. But the least population was in samples from Lam farm, Guntur district (1×10^4 cfu g⁻²).

4.4.2 Bacteria

The highest number of endophytic bacteria was recorded in samples collected from Mahanandi of Kurnool district (Table 4.4) (20.54×10^6 cfu g⁻² of plant tissue) followed by those from HRS, Chintapalli of Visakhapatnam (12.5×10^6 cfu g⁻²). The lowest population was observed in samples taken from Immanur of Kadapa district (1.50×10^6 cfu g⁻²).

4.4.3 Fluorescent Pseudomonads

Compared to endophytic bacteria, the population of fluorescent Pseudomonads was found more in turmeric rhizome samples and it ranged from 10 to 18.10×10^6 cfu g⁻² of rhizome tissue (Table 4.4). The highest number was in samples collected from Mahanandi of Kurnool district and lowest (10×10^6 cfu g⁻²) in Medikondur of Guntur district. Samples from HRS, Chintapalli of Visakhapatnam and COH, Anantharajupeta of Kadapa district harbored a population of 15×10^6 cfu g⁻² of plant tissue.

In the present study, endophytic organisms were isolated from healthy rhizomes of turmeric. The population of fungal endophytes was less compared to bacteria and fluorescent Pseudomonads but no actinomycetes were obtained. This is supported by an earlier report of Fisher *et al.* (1992) that though several fungi and bacteria were present as endophytes, no antagonistic actinomycetes were isolated from maize. Kurian (2011) reported that population of antagonistic bacteria is more as compared with fungi in the cocoa. Recently Hatem *et al.* (2013) reported *Acremonium* sp. as an endophytic bioagent against *Fusarium* wilt of date palm caused by *Fusarium oxysporum* f. sp. *albedenis*.

4.5 IN VITRO ANTAGONISTIC EFFECT OF ENDOPHYTES AGAINST THE PATHOGENS OF TURMERIC

Evaluation on antagonistic effect of endophytes against the pathogens of turmeric was carried out in *in vitro* condition.

4.5.1 Preliminary screening of endophytic microflora against the pathogens of turmeric

Isolation of endophytic microorganisms from turmeric yielded 58 endophytes. These were subjected to preliminary screening as described in 3.7.1 in order to evaluate their antagonistic property towards the pathogens of turmeric. Out of 58 isolates screened 34 isolates did not exhibit any antagonism against any pathogens and 24 isolates showed varying degrees of antagonism.

They were further evaluated for their antagonistic property against each pathogen under *in vitro* condition.

Similar line of work was carried out by Sturz *et al.* (2000) who tested the endophytic flora from potato and clover against *R. solani* and found that out of several bacteria tested, 74 per cent showed *in vitro* antibiosis. Kurian (2011) who reported antagonistic nature of endophytes against *Phytophthora palmivora* of cocoa.

4.5.2 *In vitro* evaluation of antagonistic endophytes against pathogens of turmeric

Fifteen fungi, five bacteria and four fluorescent Pseudomonads of endophytic origin were isolated from turmeric.

4.5.2.1 Endophytic fungi

Out of the 15 endophytic fungal isolates evaluated in the preliminary screening only 4 were showed antagonistic property against the pathogens of turmeric. These were further evaluated for the antagonistic property in terms of per cent inhibition against the fungal pathogens of turmeric namely *Pythium ultimum* and *Fusarium solani* and their efficiency were compared with the reference culture of *Trichoderma viride*.

The per cent inhibition on the growth of the pathogen by the antagonist over control was calculated when the growth of pathogen attained 9 cm growth on Petri dish. A perusal of the data given in Table 4.5 and Fig. 4.1 revealed that the 4 endophytic fungi exhibited varying levels of inhibition on the growth of the pathogens which was in the range of 16.33 to 72.33 per cent. Among the isolates Tc ed f 4 significantly exerted the highest mycelial (more than 70 per cent) inhibition of two pathogens tested and it recorded over growth type of antagonistic reaction against *Pythium ultimum* where as in case of *F. solani*, it recorded cessation type of antagonistic reaction.

The isolate Tc ed f 1 showed the lowest inhibition of 22.11 per cent against *F. solani* followed by the same isolate against the *P. ultimum* (16.33 per cent). The reference culture *T. viride* recorded more than 65 per cent inhibition against two pathogens (Plate 4.7a, b).

4.5.2.2 Endophytic bacteria

Of the five bacterial isolates only three were antagonistic towards the pathogens and they were further evaluated for their efficacy against fungal pathogens of turmeric in comparison with standard culture *P. fluorescens* (Table 4.6 and Fig. 4.2).

The per cent inhibition on the growth of the pathogens of turmeric by the three endophytic bacteria was in the range of 22.55 to 86.66 per cent. The endophytic bacteria Tc ed b 2 and the standard culture of *P. fluorescens* recorded more than 70 per cent inhibition against two pathogens. The lowest inhibition of 22.55 per cent was recorded by Tc ed b1 against *P. Ultimum* (Plate 4.8a, b).

4.5.2.3 Endophytic fluorescent Pseudomonads

Out of 4 fluorescent Pseudomonads isolates evaluated in the preliminary screening only three were antagonistic towards the pathogens of turmeric (Table 4.7 and Fig. 4.3). Among them Tc ed b 3 recorded more than 80 per cent inhibition against two pathogens of turmeric in the range of 15.00 to 82.33 per cent. The standard culture *P. fluorescens* recorded more than 70 per cent inhibition against two pathogens. The lowest inhibition of 15 per cent was recorded by Tc ed b 2 against *Pythium ultimum* (Plate 4.9a, b).

After the preliminary screening, four isolates of endophytic fungi were selected from turmeric. They were further evaluated along with the standard culture of *T. viride* against the test pathogens. Among them only one isolate *i.e.*, Tc ed f 4 recorded more than 70 per cent inhibition against two test pathogens. Among, endophytic bacteria and fluorescent Pseudomonads three isolates each were selected after the preliminary screening. On further evaluation along with standard culture of *P. fluorescens* one isolate of fluorescent Pseudomonads *i.e.*,

Tc ed b 3 and one isolate of endophytic bacteria *i.e.*, Tc ed b2 were selected as it recorded more than 80 per cent inhibition against two test pathogens. Similar line of works like isolation of endophytes and evaluation against pathogens were carried out in nutmeg and cinnamon by earlier workers like Sopalaun *et al.* (2003) and Gary *et al.* (2001) respectively.

4.6 MECHANISM OF ANTAGONISM OF SELECTED ANTAGONISTS ON PATHOGENS OF TURMERIC

4.6.1 Fungal antagonists

The mechanism of action of the selected fungal antagonists *viz.*, Tc ed f 1, Tc ed f 2, Tc ed f 3 and Tc ed f 4 along with the standard culture *T. viride* against the pathogens of turmeric was studied.

The hyphae of the antagonists along with the pathogens at the meeting point were observed under the microscope. Free intermingling and coiling of hyphae of antagonists and pathogen was noticed which resulted in malformation of hyphae of pathogens. Microscopic observations also revealed the penetration of hyphae of pathogens by their antagonists which resulted in the lysis of hyphae of pathogens (Plate 4.10).

4.6.1.1 Inhibition of pathogen by diffusible, non-volatile metabolites

Production of non-volatile metabolites by promising endophytic antagonists in comparison with reference cultures (*T. viride* and *P. fluorescens*) was tested by following the cellophane paper method as described in section 3.9.

After 7 days of inoculation in case of *P. ultimum* and *F. solani* of turmeric, the fungal antagonist Tc ed f 4, recorded per cent inhibition of 67.44% and 66.66%. The highest per cent inhibition (77.77%) over *P. ultimum* of turmeric recorded by fungal antagonist Tc ed f 3 (Table 4.8 and Fig. 4.4).

4.6.1.2 Inhibition of the pathogen by production of volatile inhibitory metabolites

None of the selected endophytes showed the production of volatile inhibitory metabolites.

Observations on the inhibitory response in dual culture suggested that all four selected fungal antagonists did not produce the same inhibitory effect. Hence, this test was done to compare the promising isolates based on diffusible inhibitory metabolites. All the isolates tested produced inhibitory substances at varying levels which was diffused into the medium through the cellophane and inhibited the growth of pathogens of turmeric. Chet (1990) observed involvement of compounds of microbial origin in the suppression. However, Kurian (2011) noted the production of both volatile and non volatile compound by endophytes of cocoa.

In present study, selected fungal antagonist overgrew the pathogen and showed interactions like coiling, penetration and disintegration of the host hyphae, which ultimately lead to death of the pathogen. Similar type of antagonistic reactions reported by Vijayaraghavan (2003) with *Trichoderma* sp. against *Phytophthora* sp. Bhavani (2004) reported similar mechanism of action of antagonistic endophytes of cocoa against *P. palmivora*. The results are in line with the results of Manka and Fruzynska (1989) who reported the effect of *T. viride* and *T. harzianum* against growth of wilt pathogen in carnation.

4.6.2 Bacterial antagonists

The selected potential endophytic bacterial isolates viz., Tc ed b 1, Tc ed b 2 and Tc ed b 3 were subjected to various tests to study the mechanism of antagonism along with standard culture of *Pseudomonas fluorescens* (Plate 4.11).

4.6.2.1 Siderophore production

The endophytic bacterial isolates and standard culture of *P. fluorescens* were grown on King's B medium supplemented with $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ at different

concentrations. All the four bacterial isolates are siderophore negative (Table 4.9).

In present study, none of the endophytic isolates showed siderophore production, which is in contradictory to the earlier findings of Bhavani (2004), Balan (2009) and Kurian (2011), who observed siderophore production by bacterial endophytes. The results are in accordance with the findings of Sgroy *et al.* (2009) whose had reported that the endophytic *B. subtilis* isolate from halophyte *Prosopis srtombulifera* did not produce siderophores. Forchetti *et al.* (2010) and James (2015) also not observed siderophore production by bacterial endophytes isolated from sunflower and tomato respectively.

4.6.2.2 Production of HCN

The potential endophytic bacterial isolates were tested for their ability to produce hydrogen cyanide (HCN). It was observed that none of the isolates produced hydrogen cyanide (Table 4.9).

HCN is considered as a possible and perhaps frequent mechanism by which bacteria suppress plant pathogens (Ross and Ryder, 1994; Maurhofer *et al.*, 1994). However, it was noticed that all the isolates tested were unable to produce HCN. This result confirmed earlier report by Nejad and Johnson (2000), who found that most of the endophyte isolates from oil seed rape were HCN negative. But they have suggested volatiles other than HCN may be involved in antagonism expressed by endophytic isolates. Kurian (2011) and James (2015) also did not observed the cyanogenic nature of the endophytic bacteria used in their study.

4.6.2.3 Ammonia Production

Production of ammonia by potential isolates was detected by change in colour of peptone broth media on addition of Nessler's reagent. The isolates produced varying amount of ammonia as evidenced by colour change of the medium (Table 4.9). The isolate Tc ed b 1 produced low ammonia as evidenced by the colour change of the medium to yellow and scored as 1, the isolates Tc ed

b 2 and Tc ed b 3 produced moderate amount of ammonia since the colour changed to orange these were scored as 3. Standard culture *P. fluorescens* (COH) produced more ammonia as colour of the medium changed to brownish orange and therefore scored as 4.

The capacity to produce ammonia is an attribute which is directly related to antagonistic ability of a biocontrol organism. In the present investigation, all the isolates tested produced ammonia. The result also closely matched with the report of the Samanta and Dutta (2004) whose had already proved the role of ammonia production by PGPR in suppressing *Sclerotinia sclerotium*. The ammonia production by certain endophytic bacteria has been also reported by Balan (2009), Kurian (2011), and James (2015). But according to Ryu *et al.* (2003), it is not sure whether endophyte can produce volatiles like ammonia in plants.

4.6.2.4 IAA production

All the isolates, three each of endophytic bacteria and the reference culture *P. fluorescens* produced varying levels of IAA, ranging from 7.5 to 19.1 $\mu\text{g ml}^{-1}$ (Table 4.9). Among the bacterial antagonists the maximum quantity of IAA was produced by Tc ed b 3 (17.1 $\mu\text{g ml}^{-1}$) followed by Tc ed b 2 (16.5 $\mu\text{g ml}^{-1}$) and Tc ed b 1 isolate produced 7.5 $\mu\text{g ml}^{-1}$. The reference culture *P. fluorescens* recorded 19.1 $\mu\text{g ml}^{-1}$ IAA production.

Production of phytohormones as one of the mechanism involved in the growth promotion by endophytes as suggested by Sturz *et al.*, (2000) and Sessitsch *et al.*, (2002). The findings of the present study are supported by Mendes *et al.* (2007) who found that IAA production is more prevalent among the bacterial endophytes isolated from rhizosphere than those from aerial parts. Several other workers also reported IAA production by endophytic bacteria in different crops. Kuklinksky-Sobral *et al.* (2004) and Hung *et al.* (2007) reported IAA production by endophytic bacteria from soyabean. Similarly Balan (2009) reported the IAA production of most isolates from anthurium and Kurian (2011) from cocoa.

After two different levels of screening four isolates of antagonistic fungi and three isolates of antagonistic bacteria were selected as the efficient ones against the two pathogens of turmeric. Their mechanisms of antagonism were studied in detail. Samanta and Dutta (2004) reported the production ammonia, IAA and HCN plays a significant role in the antagonistic potential of an organism. Several researchers have studied the production of these substances by endophytes (Nejad and Johnson, 2000; Sturz *et al.*, 2000; Sessitsch *et al.*, 2002; Kuklinsky-Sorbal *et al.*, 2004; Bhavani, 2004; Balan, 2009 and Kurian, 2011).

4.7 COMPATIBILITY OF SELECTED ANTAGONISTS TO PLANT PROTECTION CHEMICALS USED IN TURMERIC

Different fungicides and insecticides commonly used in turmeric fields were evaluated at three different concentrations to assess their compatibility with the selected one fungal and bacterial antagonist.

4.7.1.1 Compatibility of turmeric endophytic fungal antagonist (Tc ed f 4) with fungicides

Seven fungicides *viz.*, copper oxychloride 50% WDP (Blitox), mancozeb 4% + metalaxyl 64% W/W (Ridomil Gold MZ), carbendazim 12% + mancozeb 64% WP (Saaf), Bordeaux mixture, mancozeb 75% WP (Indofil M-45), fenamidone 10% + mancozeb 50% WG (Sectin) and cyamoxanil 8% + mancozeb 64% WP (Curzate M-8) each at three different concentrations were evaluated for this study (Table. 4.10 and Fig. 4.5). The different fungicides showed varied level of per cent inhibition, on the growth of Tc ed f 4. From the data it was noticed that concentration of fungicides increased, there was a corresponding increase in inhibition. Tc ed f 4 was incompatible with Bordeaux mixture and carbendazim (12%) + mancozeb (64%) (Saaf) at all concentrations tested and showed cent per cent inhibition on the growth of the antagonist. Among these fungicides tested cyamoxanil (8%) + mancozeb (64%) WP (Curzate M-8) at 0.1 per cent concentration recorded lowest inhibition of 13.58 per cent on the growth of the bioagent (Plate 4.12).

These findings are in tune with the reports of many workers who studied the compatibility of fungal antagonists with different fungicides. (Mondal *et al.*, 1995; Shanmugam, 1996; Rajan and sarma, 1997; Sarma and Anadaraj, 1999; Mclean *et al.*, 2001; Vijayaraghavan and Abraham, 2003; Bhavani, 2004; Bhattiprolu, 2007; Madhusudhan *et al.*, 2010; Gaikwad *et al.*, 2011; Ahanger *et al.*, 2014).

4.7.1.2 Compatibility of turmeric endophytic fungal antagonist (Tc ed f 4) with insecticides

The sensitivity of insecticides *viz.*, thiomethoxam 25% WG (Cruiser), chloropyrifos 20% EC (Dursban), dimethoate 30% EC (Rogor), malathion 50% EC (Malathion), phosphamidon 40% SL (Demecron) each at three different concentrations was tested under *in vitro* conditions (Plate 4.13).

The response of the fungal isolate Tc ed f 4 to different insecticides differed significantly. Cent per cent inhibition was noticed at three concentrations of phosphamidon (40%) (Demecron). The lowest inhibition on growth of antagonist was noticed with dimethoate (30% EC) (Rogar) at 0.05 per cent concentration (19.75%) followed by thiomethoxam (25%) (Cruiser) at 0.1% recorded lowest inhibition of 21.36 per cent which were statistically on par with each other on the growth of the bioagent where as in others the reduction in growth was in range of 24.70 to 84.44 per cent over control (Table 4.11).

The results of the compatibility study of fungal antagonist with insecticides revealed that in general all insecticides showed varying levels of compatibility. Bhai and Thomas (2010) conducted similar studies with Quinalphos (Fig. 4.6).

4.7.2.1 Compatibility of turmeric endophytic bacterial antagonist (Tc ed b 3) with fungicides

The bacterial antagonist selected from the *in vitro* evaluation is Tc ed b 3 and was tested for their compatibility to seven different fungicides each at three different concentrations.

The response of isolate *Pseudomonas* sp. (Tc ed b 3) to different fungicides at three different concentrations varied significantly (Table 4.12). The bacterial isolate compatible with mancozeb (4%) + metalaxyl-M (64%) (Ridomil Gold MZ), carbendazim (12%) + mancozeb (64%) (Saaf), cyamoxanil (8%) + mancozeb (64%) (Curzate M8) and mancozeb (75%) (Indofil M-45) at all the three concentrations followed by lower concentration of fenamidone (10%) + mancozeb (50%) (Sectin) at 0.05 per cent concentration with zero per cent inhibition. The other fungicides recorded the inhibition in the growth of the antagonist in the range of 9.5 to 21.35 per cent.

From data given in (Table 4.12 and Fig. 4.7) it revealed that Bordeaux mixture at all the concentrations showed higher inhibitory effect on the bacterial antagonist and among them 1.5 per cent concentration of Bordeaux mixture recorded the highest inhibition of 21.35 per cent on the growth of the bioagent.

Similar reports were made by Mohiddin and Khan (2013) with respect to tolerance of bacterial agents to six fungicides commonly used in the control of soil borne plant pathogens. They evaluated at different concentrations and reported that maximum tolerant concentration for *P. fluorescens* was maximum in Mancozeb (5000 mg/l) followed by Carbendazim (3200 mg/l). Whereas, Bordeaux mixture and Blitox were more inhibitory and supporting the findings of present study.

4.7.2.2 Compatibility of turmeric endophytic bacterial antagonist (Tc ed b 3) with insecticides

The *in vitro* sensitivity of five insecticides viz., thiomethoxam 25% WG (Cruiser), chlorpyrifos 20% EC (Dursban), dimethoate 30% EC (Rogor), malathion 50% EC (Malathion), phosphamidon 40% SL (Demecron) each at three concentrations were tested against one bacterial endophytic antagonist. The response of isolate *Pseudomonas* sp. (Tc ed b 3) to different insecticides at three concentrations varied significantly. From data given in (Table 4.13 and Fig. 4.8) it revealed that the antagonist was found compatible with thiomethoxam (25%) (Cruiser) and phosphamidon (40%) (Demecron) at all the three concentrations

with zero per cent inhibition. The other insecticides like chlorpyrifos (20%) (Dursban), dimethoate (30%) (Rogar) and malathion (50%) at all three concentrations showed the inhibition on the growth of antagonist in the range of 7.77 and 24.44 per cent.

On comparing the effect of different insecticides tested it was found that, except highest concentration of dimethoate (30%) and malathion (50%) (0.2 per cent) all other fungicides recorded less than 20 per cent inhibition on the growth of all bacterial antagonists. Hence they may be considered as incompatible with their antagonist.

A similar type of study was conducted by Elkins and Lindow (1999). They found the bacterial antagonists were compatible with thiomethoxam and phosphamidon at various concentrations. Therefore, these insecticides could be recommended for the insect control without much adverse effect against the bacterial antagonists. Mathew (2003) reported *P. fluorescens* was incompatible with recommended doses of dimethoate, chlorpyrifos and malathion.

The result of the present screening would help in the selection of biological control agents, which can be used, with reduced dose of selected fungicides for the control of plant pathogenic fungi. Therefore care should be taken while selecting components in the integrated disease management programme.

So it is evident that there lies in the potential of two biocontrol agents to be used along with plant protection chemicals as a control of integrated control packages. However, the performance of these selected bioagents in different turmeric growing areas is to be ascertained before recommending to the farming community as an eco-friendly management practice against the disease. Fungicides those are inhibitory against a narrow spectrum of plant pathogen but not against biocontrol agent offer a chance for integration of chemical and biocontrol agents.

4.8 IN VIVO SCREENING OF TURMERIC (*Curcuma longa* L.) GERMPLASM FOR RESISTANCE TO RHIZOME ROT

4.8.1 Under natural field conditions

Field screening studies were conducted within a period of one year for screening the available germplasm/cultures/lines against rhizome rot under natural conditions. These germplasm/cultures/lines were grouped into three categories *i.e.*, short duration (matured before 210 days or 7 months), medium duration (matured between 210 to 240 days or 7-8 months) and long duration (matured after 240 days or above 8 months) based on the time taken from planting to attain maturity period or harvesting time.

First observation was recorded during middle of August after four months of sowing at tiller initiation stage. After a fortnight, second observation was taken at active tillering stage. Third observation was recorded during middle of September at the beginning of rhizome bulking stage. Mean of three observations were recorded as annual PDI of each varieties. Crop duration is an important factor to determine the cropping sequence of the specific region.

In the present investigation, out of 120 turmeric lines screened 42 lines were short duration, 30 lines were medium duration and 48 lines were long duration. The germplasm lines like Dindigam, VK-23, Kasturi, IC-211641, Tenali Kasturi, Sonia, GS, IC-420606, IC-319341, IC-033007, Vikici, PTS-8, NB-60, ACC-48, Enna Chanda, IC-212606, IC-394903, IC-330113, IC-332957, IC-211647, Florescent, T. Sundar, IC-211402, IC-521333, CA-1711, ST-34, IC-353560, IC-416941, CAS-15, VK-9, Parbhani, CA-70, Rajendra, SLP-389-1, Kasturi Anidi, IC-181919, GL Puram, IC-211360, IC-212808, Sugandham, Battiguda and Paderu local were found to be short duration types, Pratibha, Thodupuzha, ACC-79, Prasangali, KTS-9, KTS-8, BSR-2, TCP-64, TCP-129, Gorakpur-3617, Rajapuri, NDH-8, CLI-3611, TCP-70, CLI-317, CL-325, Sports, Morthapuzta, Rashmi, Ochira, CLI-335, RH-50, KTS-6, Rajendra Sonia, RH-80, Suranjana, Pedda pasupu, PTS-55, KTS-7 and RH-9/90 were found to be medium in duration, Salem, PTS-12, CL-3, CL-9, Ranga, NH-1, Salem-2, CL-4,

CL-8, CL-10, Wagon, Chintapalli Local-2, Imphal local, Vontimitta, Mydukur, Duggirala, Roma, CL-1, CL-7, CL-18, CL-16, CL-14, CL-11, CC-91-01, CL-19, NDS-18, CL-2, CL-6, CL-17, CL-15, PTS-38, BSR-1, CL-12, Cuddapah Local, TC-4, Tekuripeta, NDH-79, Laccadona, Badipaderu, CL-20, CL-5, North-East *C. amada*, Mahanandi Local, Mega Turmeric, *C. amada*, Chintapalli Local-1, Wynad Local and Anantharajupeta Local were fallen in late duration group. These findings are in confirmations with Shanmugasundaram *et al.* (2001) who had recorded considerable variation in the duration of different genotypes of turmeric.

In the present investigation, thirty lines (14 short duration, 5 medium duration, 11 long duration) were found resistant to rhizome rot disease. IC-319341, Tenali Kasturi, VK-23, GS, IC-420606, IC-033007, IC-211641, PTS-8, Vikici, Dhindigam, ACC-48, Sonia, NB-60, Kasturi in short duration group, Prathibha, Thodupuzha, KTS-9, Prasangali, ACC-79 in medium duration group and NH-1, Ranga, Salem, Salem-2, Wagon, PTS-12, CL-8, CL-9, CL-10, CL-3, CL-4 in long duration group were resistant to rhizome rot showed 0.0% diseases incidence (Plate 4.14). It can be seen from the table 4.14, that all the germplasm lines in different duration groups under study were recorded the incidence of rhizome rot disease under natural conditions. The disease incidence of rhizome rot is ranged from 0.00 to 92.86 per cent irrespective cultures and different duration groups under study.

4.8.1.1 Long duration varieties

The results recorded on forty eight long duration turmeric lines revealed that, the incidence of rhizome rot ranged from 0.00 to 92.86 per cent. Eleven out of forty eight (22.91%) cultures were resistant to rhizome rot. Four out of forty eight (8.33%) were showed the moderately resistant reaction, Seventeen out of forty eight (35.41%) were showed the moderately susceptible reaction and eight out of forty eight (16.66%) were showed the susceptible reaction and eight out of forty eight (16.66%) were showed the highly susceptible reaction to rhizome rot disease. In long duration group germplasm lines like NH-1, Ranga, Salem,

Salem-2, Wagon, PTS-12, CL-8, CL-9, CL-10, CL-3, CL-4 were resistant to rhizome rot, showed 0.0% disease incidence whereas, Chintapalli local-2, Vontimitta, Mydukur, Imphal local were moderately resistant to rhizome rot disease. The germplasm lines like Tekuripeta, TC-4, NDH-79, CL-12, CL-20, Laccadona, Badipaderu, Cuddapah local were susceptible to rhizome rot whereas, Anantharajupeta local, Wynad local, Mahanandi local, *C.amada*, North-East *C.amada*, Chintapalli local-1, Mega turmeric, CL-5 were highly susceptible to rhizome rot and NDS-18, PTS-38, Duggirala, BSR-1, CL-19, CL-16, CL-6, CC-91-01, Roma, CL-2, CL-1, CL-18, CL-17, CL-15, CL-14, CL-11, CL-7 were moderately susceptible to rhizome rot (Table 4.14).

4.8.1.2 Medium duration varieties

The results of thirty medium duration lines showed that the rhizome rot disease incidence is ranged from 0.00 to 72.72 per cent. Five out of thirty (16.66%) cultures were resistant to rhizome rot. Nine out of thirty (30.00%) were showed the moderately resistant reaction, seven out of thirty (23.33%) were showed the moderately susceptible reaction and eight out of thirty (26.66%) were showed the susceptible reaction and one out of thirty (3.33) were showed the highly susceptible reaction to rhizome rot disease. In this group the germplasm lines *viz.*, Prathibha, Thodupuztha, KTS-9, Prasangali, ACC-79 were found resistant to rhizome rot, showed 0.0% disease incidence whereas, Rajapuri, KTS-8, TCP-64, TCP-70, TCP-129, CLI-3611, BSR-2, NDH-8, Gorakpur-3617 were moderately resistant to the rhizome rot disease. The germplasm lines like Pedda pasupu, RH-80, RH-50, Rajendra Sonia, Suranjana, PTS-55, KTS-7, KTS-6 were susceptible to rhizome rot whereas RH-9/90 was highly susceptible and CLI-317, CLI-335, CLI-325, Rashmi, Ochira, Morthapuzta, Sports were moderately susceptible to rhizome rot (Table 4.14).

4.8.1.3 Short duration varieties

In case of short duration groups, rhizome rot disease incidence is ranged from 0.00 to 64.15 per cent. Fourteen out of forty two (33.33%) were resistant to rhizome rot. Eleven out of forty two (26.19%) germplasm showed moderately

resistant reaction to rhizome rot. It was found that majority of short duration group were in between resistant and moderately resistant to rhizome rot. In short duration group, the germplasm lines like IC-319341, Tenali Kasturi, VK-23, GS, IC-420606, IC-033007, IC-211641, PTS-8, Vikici, Dhindigam, ACC-48, Sonia, NB-60, Kasturi were resistant to rhizome rot, showed 0.0% disease incidence. Whereas, Ennachanda, IC-211647, T. Sundar, IC-332957, IC-330113, CA-1711, IC-394903, IC-211402, IC-212606, IC-521333, Florescent were moderately resistant to rhizome rot disease. The germplasm lines like GL- Puram, IC-211360, IC-181919, IC-212808, Sugandham, Battiguda were susceptible to rhizome rot whereas, Paderu local was highly susceptible and SLP-389-1, Kasturi Anidi, Rajenrda, Parbhani, CA-70, VK-9, IC-416941, CAS-15, IC-353560, ST-34 were moderately susceptible to rhizome rot (Table 4.14).

In the present study, large number of germplasm lines (120) were screened at Anantharajupeta, Kadapa district, and it brought out location specific varietal reaction against rhizome rot of turmeric in the eastern ghat high land zone of Andhra Pradesh. The resistance of the resistant lines and other moderately resistant lines may be attributed to their genetic background with higher metabolic/gene activity unsuitable to the rhizome rot pathogens. The reason for this might be the antifungal compounds such as phenolics produced by resistant lines/germplasm was more potent than other compounds, especially those produced by susceptible lines/germplasm (Iftikhar *et al.*, 2005; Jamil *et al.*, 1996; Sahi *et al.*, 2000). In addition to environmental conditions, amount of inocula, types of phytoalexins and the genetic structure of a plant also affect the resistance of plants against the pathogen. The longer incubation period of the resistant and moderately resistant accessions/lines compared to the susceptible ones might be responsible for either delaying the initial infection of the disease or slow down of the rate of wilting. (Sahi *et al.*, 2000). The results were in conformity with the results of Parey *et al.* (2013) under West Bengal conditions in turmeric.

4.8.1.4 Yield

In case of rhizome yield, under field conditions medium duration variety RH-50 (1.69 kg/plant) was significantly superior over all other genotypes evaluated. It was followed by long duration varieties CL-17 (1.41 kg/plant) and CL-11 (1.31 kg/plant). Low yield was recorded in long duration variety Mahanandi Local (0.14 kg/plant), which was statistically on par with medium duration varieties TCP-70 (0.17 kg/plant) and Gorakpur- 3617, short duration variety IC-332957 (0.19 kg/plant) (Table 4.15).

In case of long duration group, the rhizome yield was highest in CL-17 (1.41kg/plant) followed by CL-11 (1.31kg/plant) and low yield of rhizome was noticed in Mahanandi local (0.14 kg/plant). In medium duration group the rhizome yield was highest in RH-50 (1.69 kg/plant) followed by TCP-64 (1.17kg/plant) and low rhizome yield was noticed in TCP-70 (0.17 kg/plant). In short duration group the rhizome yield was highest in IC-353560 (1.13 kg/plant) followed by Katuri Anidi (0.98 kg/plant) and low yield was noticed in IC-332957 (0.19 kg/plant).

The variation in yield among the turmeric lines grown under same agro-climatic conditions can be attributed to the genetic factors which may be due to its genetic potential. This variation is probably attributable to influence of climate, soil and nutrition (Anandaraj *et al.*, 2014). However, RH-50 and CL-17 though susceptible and moderately susceptible to rhizome rot disease recorded higher yield *i.e.*, 166.91 and 139.25 t ha⁻¹ respectively, as compared to other germplasm lines which may be due to its genetic potential. These findings also supported by (Venugopal *et al.*, 2006) who had screened the varieties of cardamom against rhizome rot disease.

4.8.2 Under pot culture conditions

Thirty varieties/lines of turmeric *viz.*, Pratibha, Rajendra Sonia, Morthapuzta, Salem, KTS-6, BSR-2, KTS-7, TCP-70, TCP-64, TCP-129, PTS-8, Roma, CL-1, CL-10, VK-9, RH-9/90, NB-60, NDH-8, Sports, CLI-335, GL-Puram, Kasturi, ACC-79, CL-12, IC-416941, IC-330113, 1C-033007, IC-

212606, IC-211647, IC-211641 were evaluated for their relative resistance to rhizome rot during March-April, 2017. The data were recorded on per cent incidence of rhizome rot in pots and summarized in Table 4.16.

Under pot culture conditions, out of all the turmeric cultivars screened, Salem and Prathibha were found resistant to rhizome rot with 0% infection (Plate 4.15). KTS-7 (20%), TCP-129 (20%), Sports (6%), IC-330113 (10%), IC-212606 (8%) and VK-9 (20%) were moderately resistant to the disease. Rajendra Sonia (80%), Morthapuzta (80%), KTS-6 (100%), BSR-2 (80%), TCP-64 (80%), Roma (100%), CL-1 (100%), CL-10 (100%), NDH-8 (100%), NB-60 (80%), RH-9/90 (64%), CLI-335 (64%), GI-Puram (55%), IC-416941 (62%), IC-211647 (55%) and CL-12 (64%) were highly susceptible whereas TCP-70 (50%), PTS-8 (50%) and IC-033007 (50%) were moderately susceptible and Kasturi (37%), ACC-79 (33%), IC-21164 (28%) were susceptible (Table 4.16).

The screening study indicated that two turmeric varieties were found resistant and six varieties were moderately resistant to the rhizome rot disease. Since the variation in the percent of disease incidence between various varieties was wide, it was not possible to fix the upper and lower limits of each group as constant values. Hence, the mean and standard deviation of the percent of disease incidence was used for fixing various categories of resistance/susceptibility. The categorization based on the extent of variation from the mean (positive or negative) reduced the probabilities of resistant/susceptible varieties.

Under field and pot culture conditions, medium duration variety Prathibha and long duration variety Salem were found to be superior and resistant to rhizome rot infection in turmeric while medium duration variety RH-9/90 was highly susceptible both under field and pot culture conditions. Screening by different methods has confirmed the performance of lines, which are now being used extensively in crossing programs at ICRISAT and elsewhere. The present study provides the breeders with a wide choice of varieties resistant to rhizome rot.

Singh *et al.* (2002) reported that the differences in resistance among turmeric cultivars to rhizome rot might be attributed to differences in genetic makeup of the cultivars and environmental factors that might have affected the host pathogen interactions. Similar line of work carried out by Pratap *et al.* (2013) in ginger germplasm against rhizome rot.

Identification of diverse and stable field source resistance to rhizome rot is imperative and pre-requisite to a resistance breeding programme. The use of resistant variety is beneficial not only in reducing the losses due to diseases but these sources are also useful to minimize the fungicidal toxicity (Parey *et al.*, 2013; Manu *et al.*, 2014).

4.9 IDENTIFICATION OF EFFICIENT ENDOPHYTIC ANTAGONISTS

4.9.1 Fungal antagonists

The fungi *viz.*, Tc ed f 1, Tc ed f 2, Tc ed f 3 and Tc ed f 4 was selected as efficient antagonists against the pathogens of turmeric. The cultural and morphological characters of these antagonists were studied on PDA medium for their mycelial characters comparing that of given in CMI descriptions of Pathogenic Fungi and Bacteria.

4.9.1.1 Tc ed f 2 (Turmeric endophytic fungus)

Colonies radially sulcate, velutinous to floccose, conidiogenesis moderate to heavy in greyish blue green shades exudates abundant, pale to bright yellow, similar soluble pigment, reverse yellow to brown. Conidiophores 200-300 x 3-4 µm, walls smooth, terminating in terverticillate penicilli with 1-2 rami, less commonly bi- or quarter verticillate; rami 15-20 x 3-4 µm; metulae 3-5 per verticil, 8-12 x 2.5-4 µm; phialides 4-7 per metula, ampulliform, 7-8x2-2.5 µm; conidia ellipsoidal to subspheroidal, 2.5-4 x 2-3.5 µm; smooth walls, borne in long irregular columns. Based on these characters and coupled with confirmation of identification from National Centre for Fungal Taxonomy (N.C.F.T) New Delhi with the reference (Id no. 9010.17) the antagonist was identified as *Penicillium chrysogenum* (Plate 4.16).

4.9.1.2 Tc ed f 3 (Turmeric endophytic fungus)

Colonies were dark green, fast growing, formed smooth sparse mycelial mat, which later became hairy and formed loose scanty aerial hyphae. Mycelium hyaline, smooth walled, septate and much branched. Conidiophores arise in compact or loose tuft, main branches produced several side branches in groups of two to three. Based on these characters and coupled with confirmation of identification from National Centre for Fungal Taxonomy (N.C.F.T) New Delhi with reference (Id no. 6765.15) identified as *Trichoderma viride* (Plate 4.16).

4.9.1.3 Tc ed f 4 (Turmeric endophytic fungus)

Colonies fast growing white green to bright green with the sporulation in concentric rings as time proceeds. Hyphae hyaline, septate, smooth with 3.84-4.24 μm width. Conidiphores are loose tuft, main branches produced numerous side branches at right angles. Phialides sharp pointed. Phialospores accumulated at the tip of phialides, subglobose, short obovoid, with truncate base, smooth, pale green with 1.35 μm in size. Based on these characters and coupled with confirmation of identification from National Centre for Fungal Taxonomy (N.C.F.T) New Delhi with reference Id no. (6764.15) identified as *Trichoderma harzianum* (Plate 4.16).

4.9.1.4 Tc ed f 1 (Turmeric endophytic fungus)

Colonies fast growing, mycelium loose textured, bearing long stalked conidial structures, aerial mycelium white to pale green to buff, conidial heads radiate. Conidiophores: long up to 4-5 mm but generally less than 2.5 mm. Vesicles: subglobose, fertile all over or upper 3/4th, (40-50) μm diam. Sterigmata commonly in one series or both uni- and biseriate. Primaries 8-12 x 4-5 μm and secondary's 8-10x3.0-3.5 μm or in uniseriate heads 12-15 x 3-5 μm , often inflated in older structures. Conidia: elliptical to globose, varying, 4.5-7.0 μm size. Based on these characters and coupled with confirmation of identification from National Centre for Fungal Taxonomy (N.C.F.T) New Delhi with reference Id no. (9011.17) identified as *Aspergillus oryzae* (Plate 4.16).

The selected endophytic antagonists which were proved as the efficient organisms against the pathogens of turmeric were identified based on cultural, morphological and biochemical characters. The fungal antagonists viz., Tc ed f 1, Tc ed f 2, Tc ed f 3 and Tc ed f 4 were cultured on PDA medium and cultural and morphological characters were studied in detail. Based on this the isolates were tentatively identified and was confirmed from N.C.F.T, New Delhi. Accordingly the fungal antagonists viz., Tc ed f 1, Tc ed f 2, Tc ed f 3 and Tc ed f 4 were identified as *Aspergillus oryzae* (N.C.F.T. ID.NO. 9011.17), *Pencilium chrysogenum* (N.C.F.T. ID.NO. 9010.17), *Trichoderma viride* Pers. Ex. S. F. Gray (N.C.F.T. ID.NO. 6765.15) and *Trichoderma harzianum* Rifai. (N.C.F.T. ID.NO. 6764.15) respectively. There are several reports by many workers like (Galindo, 1992; Vijayaraghavan, 2003; Bhavani, 2004 and James, 2015) as *Trichoderma* sp. as endophytes and established their role in antagonistic nature against *Phytophthora* pod rot diseases of cocoa and *Ralstonia* wilt of Tomato respectively. Hatem *et al.* (2013) reported the antagonistic nature of *Acremonium kilense* and *Pencilium chrysogenum* against the *Fusarium* wilt of date palm caused by *Fusarium oxysporum* f. sp. *albedenis*.

4.9.2 Bacterial antagonists

4.9.2.1 Characterization of bacterial antagonists

The cultural and biochemical characters of the bacterial isolates Tc ed b 1 = Turmeric endophytic bacteria 1, Tc ed b 2 = Turmeric endophytic bacteria 2, Tc ed b 3 = Turmeric endophytic bacteria 3 and *P. fluorescens* (COH) are presented in Table 4.17.

4.9.2.2 *Bacillus* sp. (Tc ed b 2)

Colonies were large, irregular, dry, flat and cream coloured with undulated margin, Gram positive, rod shaped, forms endospore. Positive for biochemical tests including for catalase, gelatin liquefaction, starch hydrolysis, utilization of citrate, lysine and ornithine, nitrate reduction and utilization of glucose, sucrose, fructose, lactose, and arabinose negative for indole production,

denitrification, urease and H₂S production. Based on these tests, the bacterium was tentatively identified as *Bacillus* sp (Plate 4.16).

4.9.2.3 *Pseudomonas* sp. (Tc ed b 3, Tc ed b 1)

The isolates were Gram negative short rods, showed growth at 4°C, failed to grow at 41°C. The isolate were positive in catalase activity, oxidase reaction, levan production, starch hydrolysis, urease activity, arginine hydrolase and nitrate reduction reaction, Gram negative reaction. Based on the cultural and biochemical characters the isolates were tentatively identified as *Pseudomonas* sp (Plate 4.16).

The cultural and biochemical characters of bacterial antagonists were studied and based on that the antagonists Tc ed b 2 was identified as *Bacillus* sp. The other three different bacterial antagonists viz., Tc ed b 1 and Tc ed b 3 were identified as *Pseudomonas* sp.

There are several reports by many workers like (Attafuah, 1965 and Galindo, 1992) as *P. fluorescens* and *Bacillus* sp. as endophytes and established their role in antagonistic nature against *Phytophthora* pod rot diseases of cocoa.

Table 4.1. Details of locations and per cent disease incidence (PDI) of turmeric in Andhra Pradesh during 2016-17

District in A.P.	Time of Collection	Location		PDI*	Longitude & Latitude		
		Mandal	Village				
Guntur	Oct, 2016	Medikondur	Gundlapalem	32.22 (34.54)	80°31'E, 16°34'N		
			Medikondur	24.33 (29.45)	80°30'E, 16°34' N		
			Visadala	25.67 (30.38)	80°32'E, 16°35' N		
		Tenali rural	Kolakaluru	10.56 (18.89)	80°61'E, 16°30' N		
			Morampudi	0.00 (00.00)	80°62'E, 16°35' N		
			Penumuli	10.33 (18.66)	80°44'E, 16°31' N		
		Mangalagiri	Atmakur	45.56 (42.44)	80°58'E, 16°42' N		
			Pedavadlapudi	11.00 (19.33)	80°60'E, 16°41' N		
			Nuthakki	5.00 (12.85)	80°65'E, 16°41' N		
		Duggirala	Gudivada	3.33 (10.41)	80°65'E, 16°28' N		
			Nandivelugu	6.00 (14.05)	80°64'E, 16°29' N		
			Duggirala	19.50 (25.90)	80°62'E, 16°32' N		
		Mean				16.12 (23.66)	

Contd...

District in A.P.	Time of Collection	Location		PDI*	Longitude & Latitude
		Mandal	Village		
Kadapa	Feb, 2016	Pullampeta	Reddipalle	3.66 (10.84)	79°22'E, 14°09' N
			Rangampalle	3.33 (10.41)	79°20'E, 14°06' N
			Immanur	1.87 (06.90)	78°82'E, 14°46' N
		Chitvel	Nagavaram	4.56 (12.26)	79°36'E, 14°02' N
			Nagiripadu	12.60 (20.53)	79°35'E, 14°13' N
			Thimmayapalm	4.11 (11.59)	79°36'E, 14°14' N
		Mydukur	Vonipenta	2.90 (9.421)	78°77'E, 14°79' N
			Sivapuram	5.00 (12.85)	78°53'E, 13°84' N
			Ganjikunta	2.88 (09.63)	77°38'E, 15°00' N
		Mean			
Kurnool	Oct, 2016	Rudravaram	Alamur	0.00 (00.00)	77°53'E, 14°64' N
			Kondamayapalle	0.00 (00.00)	78°04'E, 15°82' N
			Pedda Kambaloor	4.00 (11.30)	78°61'E, 15°30' N
		Chagalamarri	Kalugotlapalle	5.56 (13.62)	78°57'E, 14°97' N
			Madduru	2.90 (9.421)	78°38'E, 15°42' N
			Pedda Bodhanam	4.00 (11.30)	78°54'E, 15°03' N
		Allagadda	Padakandla	4.60 (11.91)	78°52'E, 15°14' N
			Palasagaram	4.33 (11.86)	78°53'E, 15°12' N
			Bachepalle	4.11 (11.59)	78°04'E, 15°82' N
		Mean			

Contd...

District in A.P.	Time of Collection	Location		PDI*	Longitude & Latitude
		Mandal	Village		
West Godavari	Dec, 2016	Nallajerla	Ananthapalle	21.00 (27.20)	78°48'E, 17°40' N
			Chodavaram	4.56 (12.26)	82°02'E, 16°85' N
			Gundepalle	6.00 (14.05)	81°47'E, 16°95' N
		Kovvur	Thogummi	15.56 (23.21)	81°73'E, 16°97' N
			Pasivedala	11.00 (19.31)	81°70'E, 16°99' N
			Kovvur	10.33 (18.66)	81°72'E, 17°01' N
		Devarapalli	Bandapuam	19.50 (25.90)	82°15'E, 16°35' N
			Duddukuru	5.33 (13.34)	81°59'E, 17°04' N
			Devarapalle	20.67 (26.99)	81°56'E, 17°03' N
		Mean			
Visakhapa-tnam	Nov, 2016	Chintapalli	Vamigadda	2.88 (09.63)	82°15'E, 16°35' N
			Antharla	3.00 (09.91)	81°69'E, 16°74' N
			Cherapalli	3.66 (10.84)	82°58'E, 17°30' N
		Paderu	Gabbangi	2.90 (9.421)	82°15'E, 16°35' N
			Iskali	1.87 (06.90)	81°60'E, 16°42' N
			Munchingiputtu	3.33 (10.41)	83°25'E, 18°70' N
		Ananthagiri	Nandigummi	0.00 (00.00)	81°60'E, 16°42' N
			Patakota	2.00 (07.63)	82°15'E, 16°35' N
			Mallingavalasa	0.00 (00.00)	81°12'E, 16°86' N
		Mean			
Overall mean				8.28 (16.72)	

*Mean of three replications

Figures in parenthesis are arc sin transformed values

Table 4.2. Sample wise isolation of organisms from infected rhizomes

District in A.P.	Location		No. of Samples Collected	Organisms isolated	
	Mandal	Village		<i>Py</i>	<i>Fu</i>
Guntur	Medikondur	Gundlapalem	3	1	2
		Medikondur	3	1	2
		Visadala	3	1	2
	Tenali rural	Kolakaluru	2	-	2
		Morampudi	2	-	2
		Penumuli	3	-	3
	Mangalagiri	Atmakur	2	-	2
		Pedavadlapudi	3	1	2
		Nuthakki	2	1	1
	Duggirala	Gudivada	3	1	2
		Nandivelugu	2	-	2
		Duggirala	4	2	2
Kadapa	Pullampeta	Reddipalle	6	-	6
		Rangampalle	5	-	5
		Immanur	4	-	4
	Chitvel	Nagavaram	7	-	7
		Nagiripadu	3	-	3
		Thimmayapalem	2	-	2
	Mydukur	Vonipenta	1	-	1
		Sivapuram	3	-	3
		Ganjikunta	2	-	2
Kurnool	Rudravaram	Alamur	3	1	2
		Kondamayapalle	3	-	3
		Pedda Kambaloor	3	-	3
	Chagalamarri	Kalugotlapalle	3	-	3
		Madduru	4	-	4
		Pedda Bodhanam	5	-	5
	Allagadda	Padakandla	1	1	-
		Palasagaram	2	2	-
		Bachepalle	2	2	-

Contd...

District in A.P.	Location		No. of Samples Collected	Organisms isolated	
	Mandal	Village		<i>Py</i>	<i>Fu</i>
West Godavari	Nallajerla	Ananthapalle	2	2	-
		Chodavaram	3	3	-
		Gundepalle	3	3	-
	Kovvur	Thogummi	3	3	-
		Pasivedala	2	2	-
		Kovvur	4	2	2
	Devarapalli	Bandapuam	5	1	4
		Duddukuru	4	1	3
		Devarapalle	2	1	1
Visakhapatnam	Chintapalli	Vamigadda	1	1	-
		Antharla	2	2	-
		Cherapalli	2	2	-
	Paderu	Gabbangi	2	2	-
		Iskali	3	3	-
		Munchingiputtu	3	2	1
	Ananthagiri	Nandigummi	3	3	-
		Patakota	4	4	-
		Mallingavalasa	5	5	-
Total			144	56	88
Percentage Frequency (%)				38.88	45.83

Py- *Pythium* sp., *Fu*- *Fusarium* sp.

Table 4.4. Population of endophytes of turmeric at different locations

Sl. No	District	Location	Fungi ($\times 10^4$ cfu g^{-2})*	Bacteria ($\times 10^6$ cfu g^{-2})*	Fluorescent Pseudomonads ($\times 10^6$ cfu g^{-2})*
1	Kadapa	COH, Anantharajupeta	6.00	12.00	15.00
		Immanur	2.00	1.50	11.00
2	Guntur	Lam Farm	1.00	9.00	12.00
		Medikondur	2.00	8.80	10.00
3	West Godavari	HRS, Kovvur	8.00	8.50	12.70
4	Kurnool	Mahanandi	11.00	20.54	18.10
5	Visakha -patnam	HRS, Chintapalli	8.00	12.50	15.00
Overall Mean			5.42	10.40	13.40

*Average of three replications

Table 4.5. *In vitro* evaluation of turmeric endophytic fungal isolates against pathogens of turmeric

Sl. No	Fungal isolate	Radial growth of mycelium (mm)*		Per cent inhibition over control*			
		<i>Py</i>	<i>Fs</i>	<i>Pythium ultimum</i>	AR	<i>Fusarium solani</i>	AR
1	Tc ed f 1	75.30	70.10	16.33 (23.83)**	AV	22.11 (28.04)	AV
2	Tc ed f 2	51.40	53.60	42.88 (40.89)	CS	40.44 (39.47)	LY
3	Tc ed f 3	33.00	34.70	63.33 (52.76)	AV	61.44 (51.59)	OG
4	Tc ed f 4	27.00	24.90	70.00 (56.77)	OG	72.33 (58.24)	CS
5	<i>T. viride</i> (COH)	31.20	27.80	65.33 (53.99)	OG	69.11 (56.21)	LY
6	Control	90.00	90.00	-		-	
	S.Em ±	0.781	0.676	0.535		0.463	
	CD (P = 0.05)	2.337	2.025	1.602		1.387	

*Mean of four replications

** Figures in parentheses are angular transformed values

TC ed f = Turmeric endophytic fungi

AV = Aversion; OG = Over growth; LY = Lysis; AR = Antagonistic reaction;

CS = Cessation of growth

Table 4.6. *In vitro* evaluation of turmeric endophytic bacterial isolates against pathogens of turmeric

Sl. No	Bacterial isolate	Radial growth of mycelium (mm)*		Per cent inhibition over control*	
		<i>Py</i>	<i>F_s</i>	<i>P. ultimum</i>	<i>F. solani</i>
1	Tc ed b 1	69.70	68.20	22.55 (28.34)**	24.22 (29.47)
2	Tc ed b 2	15.00	12.00	83.33 (65.88)	86.66 (68.55)
3	Tc ed b 3	31.10	29.00	65.44 (53.97)	67.77 (55.39)
4	<i>P. f</i> (COH)	21.10	20.50	76.55 (61.01)	77.22 (61.47)
5	Control	90.00	90.00	-	-
	S.Em ±	0.349	1.139	0.265	0.709
	CD (P = 0.05)	1.062	3.466	0.807	2.208

*Mean of four replications

** Figures in parentheses are angular transformed values

Tc ed b = Turmeric endophytic bacteria

P. f = *Pseudomonas fluorescens*

Table 4.7. *In vitro* evaluation of turmeric endophytic fluorescent Pseudomonads isolates against pathogens of turmeric

Sl. No	Bacterial isolate	Radial growth of Mycelium (mm)*		Per cent inhibition over control*	
		<i>Py</i>	<i>Fs</i>	<i>P. ultimum</i>	<i>F. solani</i>
1	Tc ed b 1	44.70	61.00	50.33 (45.17)**	32.22 (34.57)
2	Tc ed b 2	76.50	75.60	15.00 (22.78)	16.00 (23.57)
3	Tc ed b 3	15.90	18.00	82.33 (65.12)	80.00 (63.41)
4	<i>P. f</i> (COH)	21.0	20.50	76.66 (61.09)	77.22 (61.47)
5	Control	90.00	90.00	-	-
	S.Em ±	0.889	0.505	0.741	0.424
	CD (P=0.05)	2.704	1.537	2.254	1.290

*Mean of four replications

** Figures in parentheses are angular transformed values

Tc ed b = Turmeric endophytic bacteria on King's B medium

P. f = *Pseudomonas fluorescens*

Table 4.8. *In vitro* inhibition of pathogens of turmeric by non-volatile metabolites of endophytic fungi

Sl. No	Fungal antagonist	Linear mycelia growth in (mm)*		Per cent inhibition over control*	
		<i>Py</i>	<i>Fs</i>	<i>P. ultimum</i>	<i>F. solani</i>
1	Tc ed f 1	41.00	35.00	54.44 (47.53)**	61.11 (51.40)
2	Tc ed f 2	35.50	40.00	60.55 (51.07)	55.55 (48.17)
3	Tc ed f 3	20.00	25.00	77.77 (61.85)	72.22 (58.17)
4	Tc ed f 4	29.30	30.00	67.44 (55.19)	66.66 (54.71)
5	<i>T. viride</i> (COH)	22.90	26.50	74.55 (59.68)	70.55 (57.11)
6	Control	90.00	90.00	-	-
	S.Em \pm	0.887	1.087	0.632	0.732
	C D (P = 0.05)	2.655	3.253	1.893	2.192

*Mean of four replications

** Figures in parentheses are angular transformed values

Table 4.9. Mechanism of action of selected bacterial antagonists

Sl. No.	Bacterial antagonist	Siderophore production	HCN production	Ammonia production		IAA produced ($\mu\text{g ml}^{-1}$) *
				Colour change	Score	
1	Tc ed b 1	–	–	Yellow	1	7.5
2	Tc ed b 2	–	–	Orange	3	16.5
3	Tc ed b 3	–	–	Orange	3	17.1
4	<i>P. fluorescens</i> (COH)	+	+	Brownish orange	4	19.1

* Mean of three replications

Table 4.10. Compatibility of selected fungal antagonist with fungicides

Sl. No	Fungicides	Concentration (Per cent)	Tc ed f 4	
			Mean diameter of colony (mm)*	PIOC*
1	Copper oxychloride 50% WDP	0.1	54.44	39.51 (38.93)**
		0.2	48.00	46.66 (43.07)
		0.3	40.22	53.31 (46.88)
2	Mancozeb 4% + Metalaxyl 64% W/W	0.05	72.22	19.75 (26.38)
		0.1	67.77	24.70 (29.79)
		0.2	50.9	43.44 (41.21)
3	Carbendazim 12% + Mancozeb 64% WP	0.05	0	100 (89.97)
		0.1	0	100 (89.97)
		0.2	0	100 (89.97)
4	Bordeaux mixture	0.5	0	100 (89.97)
		1	0	100 (89.97)
		1.5	0	100 (89.97)
5	Mancozeb 75% WP	0.2	72.22	19.75 (26.38)
		0.25	40.00	55.55 (48.17)
		0.3	15.00	83.33 (65.88)
6	Fenamidone 10% + Mancozeb 50% WG	0.05	60.00	33.33 (35.25)
		0.1	50.9	43.44 (41.21)
		0.2	34.44	61.73 (51.76)
7	Cyamoxanil 8% + Mancozeb 64% WP	0.1	77.77	13.58 (21.62)
		0.2	44.44	50.62 (45.34)
		0.3	37.00	58.88 (50.10)
8	Control	-	90	-
	S.Em ±		0.905	0.839
	C D (P = 0.05)		2.588	2.399

* Mean of three replications

**Figures in parenthesis are angular transformed values

PIOC = Per cent Inhibition Over Control

Table 4.11. Compatibility of selected fungal antagonist with insecticides

Sl. No	Insecticides	Concentration (per cent)	Tc ed f 4	
			Mean diameter of the colony (mm)*	PIOC*
1	Dimethoate 30% EC	0.05	72.22	19.75 (26.38) **
		0.1	67.77	24.70 (29.79)
		0.2	67.00	25.55 (30.35)
2	Chloropyrifos 20% EC	0.05	44.44	50.62 (45.34)
		0.1	15.55	82.72 (65.41)
		0.2	14.00	84.44 (66.74)
3	Thiomethoxam 25% WG	0.1	70.77	21.36 (27.52)
		0.2	32.00	64.44 (53.37)
		0.3	25.00	72.22 (58.17)
4	Malathion 50% EC	0.05	50.9	43.44 (41.21)
		0.1	25.00	72.22 (58.17)
		0.2	15.55	82.72 (65.41)
5	Phosphamidon 40% SL	0.05	0	100 (89.97)
		0.1	0	100 (89.97)
		0.2	0	100 (89.97)
6	Control	-	90	-
	S.Em ±		1.019	0.732
	C D (P = 0.05)		2.949	2.117

* Mean of three replications

**Figures in parenthesis are angular transformed values

PIOC = Per cent Inhibition Over Control

4.12. Compatibility of selected bacterial antagonist with fungicides

Sl. No	Fungicides	Concentration (Per cent)	Tc ed b 3	
			Mean diameter of Inhibition zone (mm)*	PIOC*
1	Copper oxychloride 50% WDP	0.1	10.00	11.11 (3.40)**
		0.2	12.55	13.94 (3.80)
		0.3	13.22	14.68 (3.89)
2	Mancozeb 4% + Metalaxyl 64% W/W	0.05	0	0 (0.71)
		0.1	0	0 (0.71)
		0.2	0	0 (0.71)
3	Carbendazim 12% + Mancozeb 64% WP	0.05	0	0 (0.71)
		0.1	0	0 (0.71)
		0.2	0	0 (0.71)
4	Bordeaux mixture	0.5	8.55	9.5 (3.16)
		1	12.55	13.74 (3.77)
		1.5	19.22	21.35 (4.67)
5	Mancozeb 75% WP	0.2	0	0 (0.71)
		0.25	0	0 (0.71)
		0.3	0	0 (0.71)
6	Fenamidone 10% + Mancozeb 50% WG	0.05	0	0 (0.71)
		0.1	9	10 (3.24)
		0.2	12.55	13.94 (3.8)
7	Cyamoxanil 8% + Mancozeb 64% WP	0.1	0	0 (0.71)
		0.2	0	0 (0.71)
		0.3	0	0 (0.71)
8	Control	-	0	0 (0.71)
	S.Em ±		0.236	0.037
	C D (P = 0.05)		0.673	0.105

* Mean of three replications

**Figures in parenthesis are square root $\sqrt{+0.5}$ transformed values

PIOC = Per cent Inhibition Over Control

Table 4.13. Compatibility of selected bacterial antagonist with insecticides

Sl. No	Insecticides	Concentration (per cent)	Tc ed b 3	
			Mean diameter of Inhibition zone (mm)*	PIOC*
1	Dimethoate 30% EC	0.05	10	11.11 (3.40)**
		0.1	15.55	17.27 (4.21)
		0.2	21.22	23.57 (4.90)
2	Chloropyrifos 20% EC	0.05	6.00	6.66 (2.67)
		0.1	10.00	11.11 (3.40)
		0.2	14.22	15.8 (4.03)
3	Thiomethoxam 25% WG	0.1	0	0 (0.71)
		0.2	0	0 (0.71)
		0.3	0	0 (0.71)
4	Malathion 50% EC	0.05	7.00	7.77 (2.87)
		0.1	15.00	16.66 (4.14)
		0.2	22.00	24.44 (4.99)
5	Phosphamidon 40% SL	0.05	0	0 (0.71)
		0.1	0	0 (0.71)
		0.2	0	0 (0.71)
6	Control	-	0	0 (0.71)
	S.Em ±		0.388	0.044
	C D (P = 0.05)		1.123	0.128

* Mean of three replications

**Figures in parenthesis are square root $\sqrt{+0.5}$ transformed values

PIOC = Per cent Inhibition Over Control

Table 4.15. Rhizome rot reaction and yield parameter in natural field condition of turmeric germplasm in Andhra Pradesh

Sl. no.	Name of the Germplasm	Percent Disease Incidence (%)*	Fresh Rhizome Yield* (kg/plant)	Projected yield (t ha ⁻¹)*	Reaction Category	Duration
1	Duggirala	15.38 (23.08)	0.40	39.50	MS	Long
2	Cuddapah Local	28.57 (32.30)	0.61	60.24	S	Long
3	Pratibha	0.00 (0.00)	0.52	51.35	R	Medium
4	Salem	0.00 (0.00)	0.91	89.87	R	Long
5	KTS-8	7.14 (15.49)	0.52	51.35	MR	Medium
6	BSR-2	8.33 (16.77)	0.58	57.28	MR	Medium
7	TCP-64	6.25 (14.47)	1.17	115.55	MR	Medium
8	TCP-129	5.00 (12.92)	0.55	54.32	MR	Medium
9	PTS-12	0.00 (0.00)	0.59	58.27	R	Long
10	Roma	21.42 (27.56)	0.34	33.58	MS	Long
11	CL-1	20.00 (26.55)	0.39	38.51	MS	Long
12	CL-3	0.00 (0.00)	0.51	50.37	R	Long
13	CL-5	66.67 (54.72)	1.00	98.76	HS	Long
14	CL-7	11.11 (19.46)	0.41	40.49	MS	Long
15	CL-9	0.00 (0.00)	0.90	88.88	R	Long
16	IC-181919	28.57 (32.30)	0.76	75.06	S	Short
17	Dindigam	0.00 (0.00)	0.75	74.07	R	Short
18	VK-23	0.00 (0.00)	0.38	37.53	R	Short

Contd...

Sl. no.	Name of the Germplasm	Percent Disease Incidence (%)*	Fresh Rhizome Yield* (kg/plant)	Projected yield (t ha ⁻¹)*	Reaction Category	Duration
19	Gorakpur-3617	9.09 (17.54)	0.19	18.76	MR	Medium
20	North-East <i>C.amada</i>	66.66 (54.71)	0.29	28.64	HS	Long
21	Chintapalli Local-2	8.01 (16.43)	0.30	29.62	MR	Long
22	Imphal Local	9.82 (18.26)	0.69	68.14	MR	Long
23	Wagon	0.00 (0.00)	0.54	53.33	R	Long
24	RH-50	30.77 (33.68)	1.69	166.91	S	Medium
25	RH-9/90	72.72 (58.49)	0.43	42.46	HS	Medium
26	Enna Chanda	5.50 (13.56)	0.45	44.44	MR	Short
27	CL-18	23.08 (28.70)	0.55	54.32	MS	Long
28	CL-16	12.5 (20.70)	0.84	82.96	MS	Long
29	CL-14	22.22 (28.11)	0.69	68.14	MS	Long
30	CL-11	21.42 (27.56)	1.31	129.38	MS	Long
31	Kasturi	0.00 (0.00)	0.33	32.59	R	Short
32	Ranga	0.00 (0.00)	0.49	48.39	R	Long
33	NH-1	0.00 (0.00)	0.62	61.23	R	Long
34	ACC-48	0.00 (0.00)	0.27	26.66	R	Short
35	CC-91-01	22.22 (28.11)	0.79	78.02	MS	Long

Contd...

Sl. no.	Name of the Germplasm	Percent Disease Incidence (%)*	Fresh Rhizome Yield* (kg/plant)	Projected yield (t ha⁻¹)*	Reaction Category	Duration
36	KTS-6	46.47 (42.96)	0.57	56.29	S	Medium
37	CLI-317	14.28 (22.19)	0.75	74.07	MS	Medium
38	CL-19	15.38 (23.08)	0.62	61.23	MS	Long
39	GL Puram	30.76 (33.67)	0.93	91.85	S	Short
40	IC-212606	8.33 (16.77)	0.34	33.58	MR	Short
41	IC-211360	57.14 (49.09)	0.57	56.29	S	Short
42	Mahanandi Local	92.86 (74.47)	0.14	13.82	HS	Long
43	Paderu Local	64.15 (53.20)	0.57	56.29	HS	Short
44	ST-34	15.38 (23.08)	0.96	94.81	MS	Short
45	IC-353560	11.76 (20.05)	1.13	111.60	MS	Short
46	CL-325	12.50 (20.70)	0.61	60.24	MS	Medium
47	IC-394903	2.88 (9.77)	1.13	111.60	MR	Short
48	NDS-18	11.00 (19.36)	0.78	77.03	MS	Long
49	IC-416941	18.18 (25.23)	0.53	52.34	MS	Short
50	IC-330113	8.01 (16.43)	0.77	76.04	MR	Short
51	IC-332957	9.09 (17.54)	0.19	18.76	MR	Short
52	TC-4	33.33 (35.25)	1.17	115.55	S	Long
53	IC-211647	9.82 (18.26)	0.48	47.40	MR	Short
54	IC-211641	0.00 (0.00)	0.63	62.22	R	Short

Contd...

Sl. no.	Name of the Germplasm	Percent Disease Incidence (%)*	Fresh Rhizome Yield* (kg/plant)	Projected yield (t ha ⁻¹)*	Reaction Category	Duration
55	Sports	13.64 (21.67)	0.56	55.30	MS	Medium
56	IC-212808	26.08 (30.70)	0.47	46.41	S	Short
57	Tekuripeta	28.57 (32.30)	0.60	59.25	S	Long
58	Rajendra Sonia	30.77 (33.68)	0.68	67.16	S	Medium
59	Morthapuzta	21.42 (27.56)	0.89	87.90	MS	Medium
60	Salem-2	0.00 (0.00)	0.68	67.16	R	Long
61	Sugandham	42.85 (40.87)	0.22	21.72	S	Short
62	CL-2	13.64 (21.67)	0.25	24.69	MS	Long
63	CL-4	0.00 (0.00)	0.34	33.58	R	Long
64	CL-6	23.08 (28.70)	0.45	44.44	MS	Long
65	CL-8	0.00 (0.00)	0.29	28.64	R	Long
66	CL-10	0.00 (0.00)	0.52	51.35	R	Long
67	NDH-79	28.76 (32.42)	0.72	71.11	S	Long
68	Florescent	5.26 (13.75)	0.23	22.71	MR	Short
69	Mega Turmeric	64.42 (53.36)	0.36	35.55	HS	Long
70	Rajapuri	8.00 (16.42)	1.11	109.62	MR	Medium
71	<i>C.amada</i>	55.00 (47.85)	0.52	51.35	HS	Long
72	Chintapalli Local-1	62.50 (52.22)	0.62	61.23	HS	Long
73	RH-80	22.58 (28.36)	0.60	59.25	S	Medium

Contd...

Sl. no.	Name of the Germplasm	Percent Disease Incidence (%)*	Fresh Rhizome Yield* (kg/plant)	Projected yield (t ha⁻¹)*	Reaction Category	Duration
74	Wynad Local	90.00 (71.54)	1.06	104.69	HS	Long
75	Laccadona	37.00 (37.45)	0.91	89.87	S	Long
76	Suranjana	33.00 (35.05)	0.55	54.32	S	Medium
77	Vontimitta	8.12 (16.55)	0.48	47.40	MR	Long
78	CL-17	16.66 (24.08)	1.41	139.25	MS	Long
79	CL-15	11.42 (19.74)	1.00	98.76	MS	Long
80	CL-12	28.76 (32.42)	0.65	64.19	S	Long
81	CAS-15	18.75 (25.65)	0.88	86.91	MS	Short
82	Rashmi	15.62 (23.27)	1.12	110.61	MS	Medium
83	Thodupuzta	0.00 (0.00)	0.67	66.17	R	Medium
84	T. Sundar	5.00 (12.920)	0.38	37.53	MR	Short
85	PTS-38	40.00 (39.22)	0.62	61.23	MS	Long
86	Mydukur	7.22 (15.58)	0.50	49.38	MR	Long
87	Tenali Kasturi	0.00 (0.00)	1.03	101.72	R	Short
88	VK-9	13.79 (21.79)	0.38	37.53	MS	Short
89	ACC-79	0.00 (0.00)	0.94	92.83	R	Medium
90	Prasangali	0.00 (0.00)	0.06	5.92	R	Medium
91	NDH-8	10.00 (18.43)	0.56	55.30	MR	Medium
92	IC-211402	8.12 (16.55)	0.56	55.30	MR	Short

Contd...

Sl. no.	Name of the Germplasm	Percent Disease Incidence (%)*	Fresh Rhizome Yield* (kg/plant)	Projected yield (t ha⁻¹)*	Reaction Category	Duration
93	Anantharaju peta Local	80.00 (63.41)	0.54	53.33	HS	Long
94	Sonia	0.00 (0.00)	0.18	17.77	R	Short
95	Badipaderu	28.76 (32.42)	1.08	106.66	S	Long
96	Parbhani	11.42 (19.74)	0.34	33.58	MS	Short
97	GS	0.00 (0.00)	0.50	49.38	R	Short
98	KTS-9	0.00 (0.00)	0.57	56.29	R	Medium
99	IC-521333	9.40 (17.85)	0.94	92.83	MR	Short
100	IC-420606	0.00 (0.00)	0.61	60.24	R	Short
101	Pedda pasupu	37.00 (37.45)	0.48	47.40	S	Medium
102	BSR-1	15.62 (23.27)	0.34	33.58	MS	Long
103	IC-319341	0.00 (0.00)	0.74	73.08	R	Short
104	IC-033007	0.00 (0.00)	0.05	4.93	R	Short
105	CA-70	30.00 (33.20)	0.27	26.66	MS	Short
106	CLI-3611	9.75 (18.19)	0.58	57.28	MR	Medium
107	Rajendra	21.11 (27.34)	0.52	51.35	MS	Short
108	CA-1711	7.44 (15.82)	0.54	53.33	MR	Short
109	Vickici	0.00 (0.00)	0.88	86.91	R	Short
110	PTS-8	0.00 (0.00)	0.27	26.66	R	Short
111	PTS-55	37.57 (37.79)	0.58	57.28	S	Medium

Contd...

Sl. no.	Name of the Germplasm	Percent Disease Incidence (%)*	Fresh Rhizome Yield* (kg/plant)	Projected yield (t ha ⁻¹)*	Reaction Category	Duration
112	Battiguda	60.43 (51.00)	0.78	77.03	S	Short
113	Ochira	11.59 (19.90)	0.44	43.45	MS	Medium
114	NB-60	0.00 (0.00)	0.77	76.04	R	Short
115	KTS-7	60.37 (50.97)	0.96	94.81	S	Medium
116	CL-20	22.58 (28.36)	0.56	55.30	S	Long
117	TCP-70	6.27 (14.50)	0.17	16.79	MR	Medium
118	SLP-389-1	18.79 (25.68)	0.27	26.66	MS	Short
119	CLI-335	11.52 (19.83)	0.61	60.24	MS	Medium
120	Kasturi Anidi	16.70 (24.11)	0.98	96.78	MS	Short
	S.Em ±	0.245	0.026	0.464		
	C D (P = 0.05)	0.683	0.073	1.294		
	CV (%)	1.963	3.569	10.613		

* Mean of three replications

Figures in parentheses are arc sine transformed values

R - Resistant, MR - Moderately resistant, MS - Moderately susceptible, S - Susceptible, HS - Highly susceptible

Table 4.16. Screening of turmeric germplasm against rhizome rot under pot culture conditions

Variety	Per cent disease incidence (%) (M ± SE)	Disease Reaction Category
Rajendra Sonia	80 ± 19.24	HS
Prathibha	0 ± 0	R
Morthapuzta	80 ± 11.11	HS
Salem	0 ± 0	R
KTS-6	100 ± 0	HS
KTS-7	20 ± 11.11	MR
BSR-2	80 ± 19.24	HS
TCP-64	80 ± 22.22	HS
TCP-70	50 ± 11.11	MS
TCP-129	20 ± 11.11	MR
PTS-8	50 ± 0	MS
Roma	100 ± 0	HS
CL-1	100 ± 0	HS
CL-10	100 ± 0	HS
VK-9	20 ± 11.11	MR
RH-9/90	64.42 ± 0.28	HS
NB-60	80 ± 11.11	HS
NDH-8	100 ± 0	HS
Sports	06.25 ± 0.02	MR
CLI-335	64.42 ± 0.28	HS
Gl-Puram	55.00 ± 0.57	HS
Kasturi	3+7.00 ± 0.57	S
ACC-79	33.00 ± 0.57	S
IC-416941	62.50 ± 0.12	HS
IC-330113	10.00 ± 0.57	MR
IC-033007	50 ± 0	MS
IC-212606	08.12 ± 0.11	MR
IC-211647	55.00 ± 0.57	HS
IC-211641	28.76 ± 0.1	S
CL-12	64.42 ± 0.28	HS

Mean of three replications

Resistant, MR - Moderately resistant, MS - Moderately susceptible, S - Susceptible, HS - Highly susceptible

Table 4.17. Cultural and biochemical characters of the bacterial isolates

Sl. No	Cultural and biochemical characters	Tc ed b 1	Tc ed b 2	Tc ed b 3	<i>P. f</i> (K)
1	Gram's reaction	-	+	-	-
2	Lysine utilization	-	-	+	-
3	Ornithine utilization	-	+	+	-
4	Urease production	+	+	+	+
5	Phenylalanine deamination	-	-	-	-
6	Nitrate reduction	+	+	-	-
7	H ₂ S Production	-	-	-	-
8	Glucose utilization	-	-	-	+
9	Adonitol utilization	-	-	-	+
10	Lactose utilization	-	-	-	+
11	Arabinose utilization	-	-	-	+
12	Sorbitol utilization	-	-	-	-
13	Citrate utilization	+	+	+	+

+ Positive reaction - Negative reaction

Table 4.14. Screening of turmeric germplasm against the rhizome rot disease under natural field conditions

Scale	Per cent disease incidence	Disease Reaction	Germplasm/lines		
			Short duration (200 to 230 days)	Medium duration (230 to 250 days)	Long duration (250 to 270 days)
0	0%	R	Dindigam, VK-23, Kasturi, IC-211641, Tenali Kasturi, Sonia, GS, IC-420606, IC-319341, IC-033007, Vickici, PTS-8, NB-60, ACC-48	Pratibha, Thodupuztha, ACC-79, Prasangali, KTS-9	Salem, PTS-12, CL-3, CL-9, Ranga, NH-1, Salem-2, CL-4, CL-8, CL-10, Wagon
1	1-10%	MR	Enna Chanda, IC-212606, IC-394903, IC-330113, IC-332957, IC-211647, Florescent, T. Sundar, IC-211402, IC-521333, CA-1711	KTS-8, BSR-2, TCP-64, TCP-129, Gorakpur-3617, Rajapuri, NDH-8, CLI-3611, TCP-70	Chintapalli Local-2, Imphal Local, Vontimitta, Mydukur
2	11-25%	MS	ST-34, IC-353560, IC-416941, CAS-15, VK-9, Parbhani, CA-70, Rajendra, SLP-389-1, Kasturi Anidi	CLI-317, CL-325, Sports, Morthapuzta, Rashmi, Ochira, CLI-335	Duggirala, Roma, CL-1, CL-7, CL-18, CL-16, CL-14, CL-11, CC-91-01, CL-19, NDS-18, CL-2, CL-6, CL-17, CL-15, PTS-38, BSR-1
3	26-50%	S	IC-181919, GL Puram, IC-211360, IC-212808, Sugandham, Battiguda	RH-50, KTS-6, Rajendra Sonia, RH-80, Suranjana, Pedda pasupu, PTS-55, KTS-7	CL-12, Cuddapah Local, TC-4, Tekuripeta, NDH-79, Laccadona, Badipaderu, CL-20
4	>50%	HS	Paderu Local	RH-9/90	CL-5, North-East <i>C. amada</i> , Mahanandi Local, Mega Turmeric, <i>C. amada</i> , Chintapalli Local-1, Wynad Local, Anantharajupeta Local

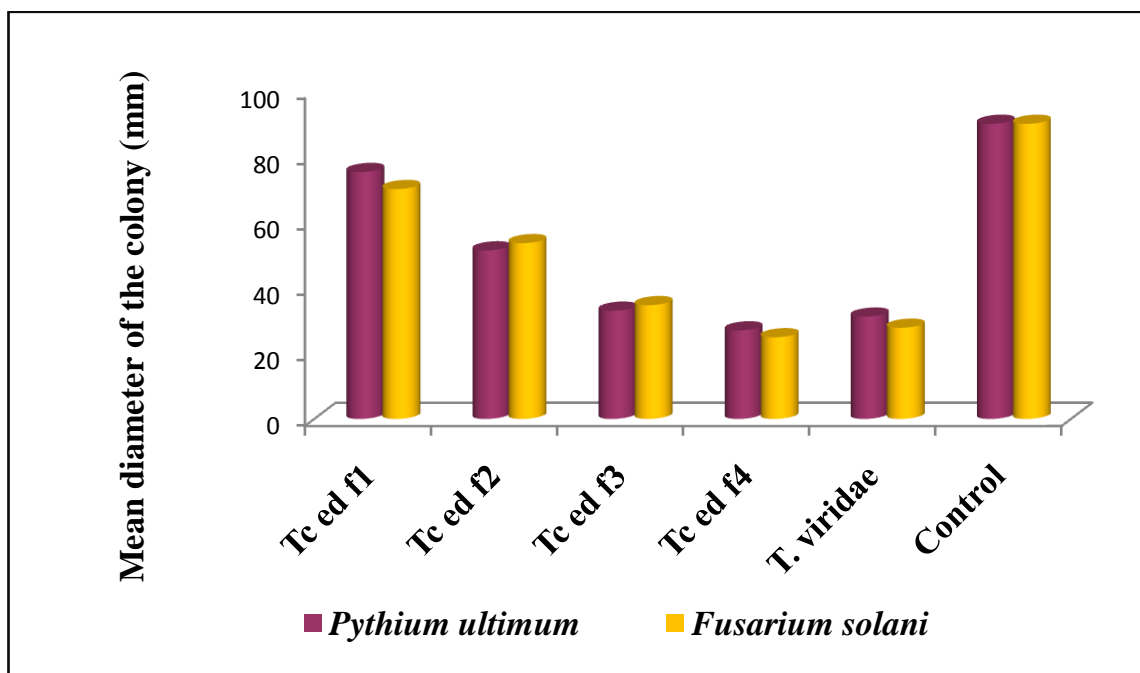


Fig. 4.1 Screening of endophytic fungal isolates against pathogens of turmeric

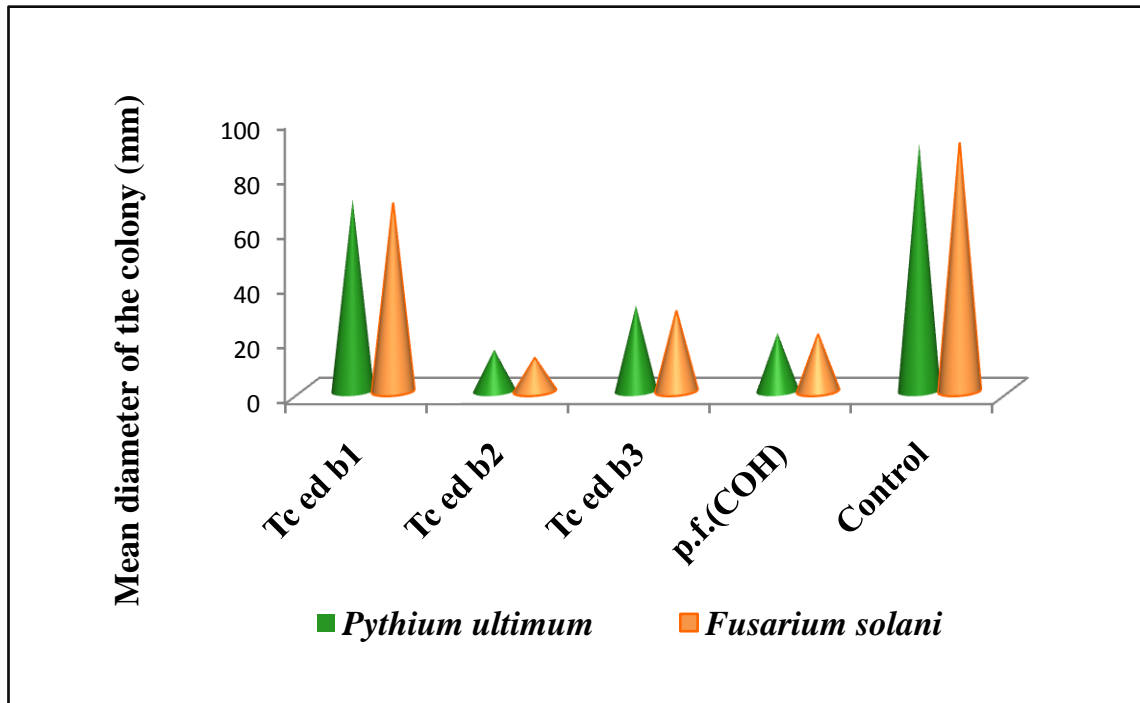


Fig. 4.2 Screening of endophytic bacterial isolates against pathogens of turmeric

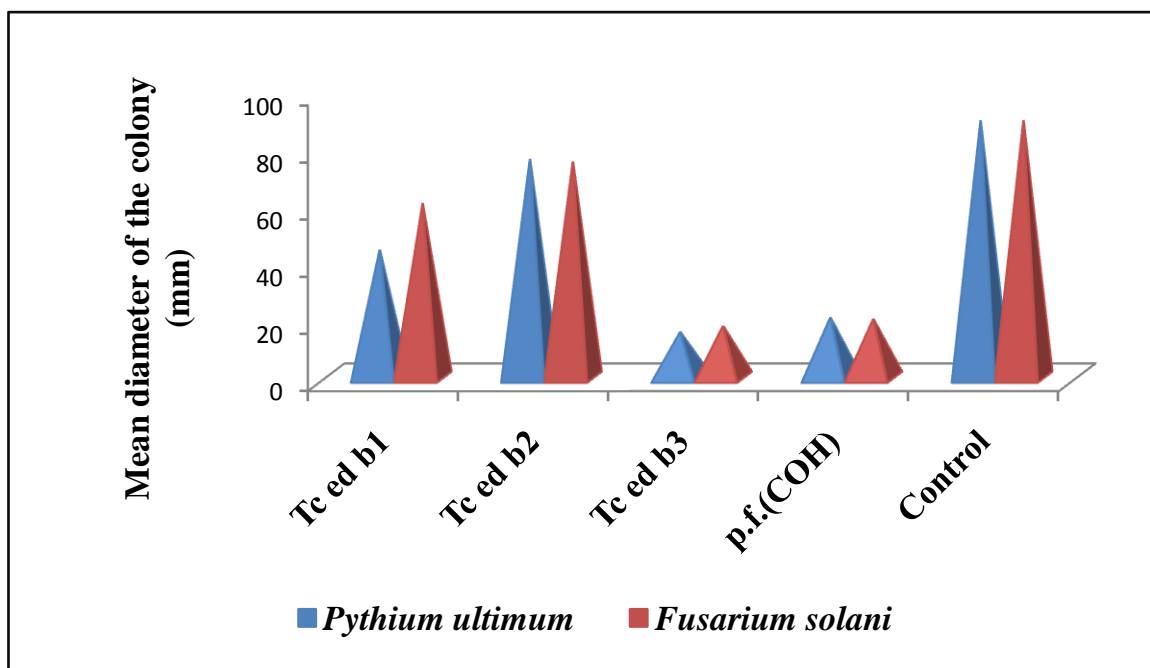


Fig. 4.3 Screening of endophytic fluorescent Pseudomonads isolates against pathogens of turmeric

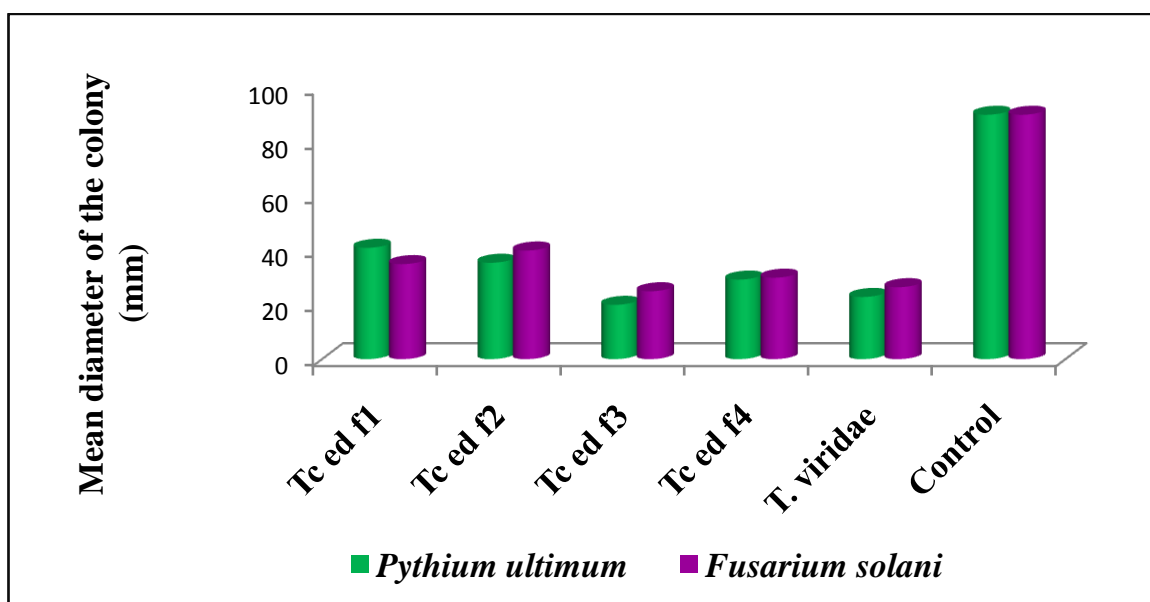


Fig. 4.4 Effect of non-volatile metabolites of endophytic fungi on growth of turmeric pathogens

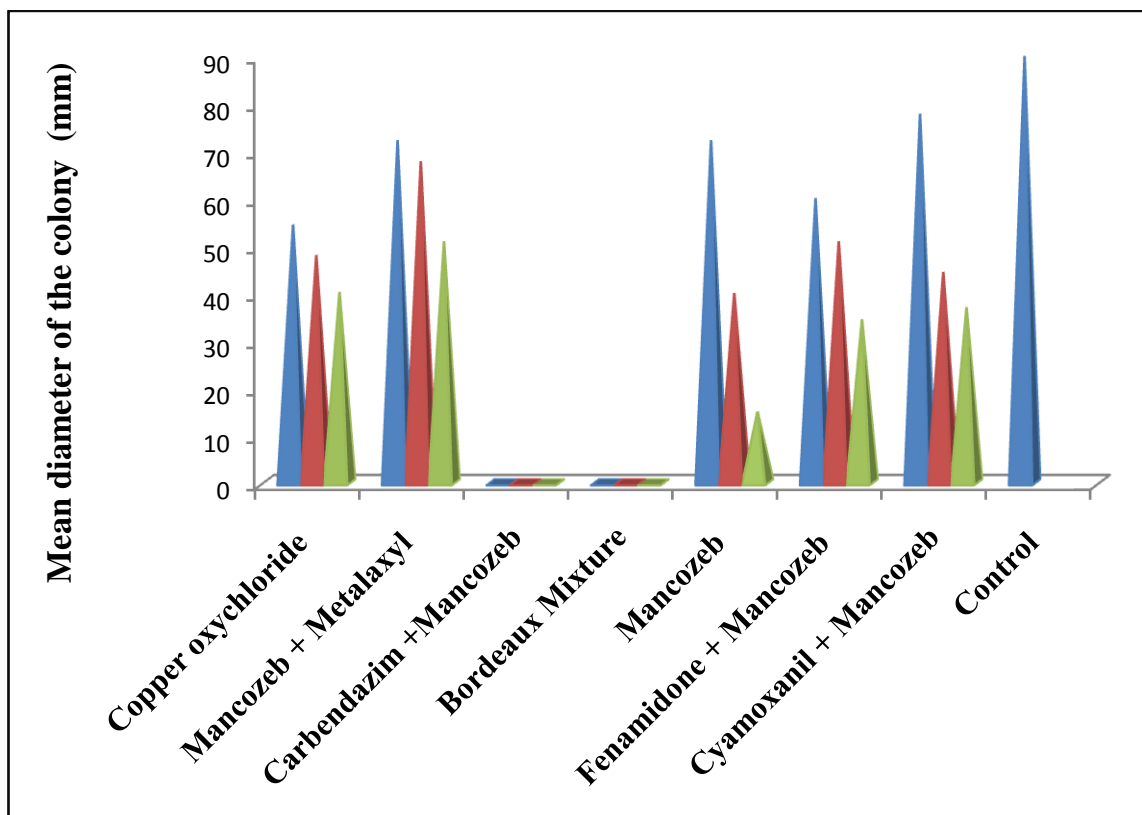
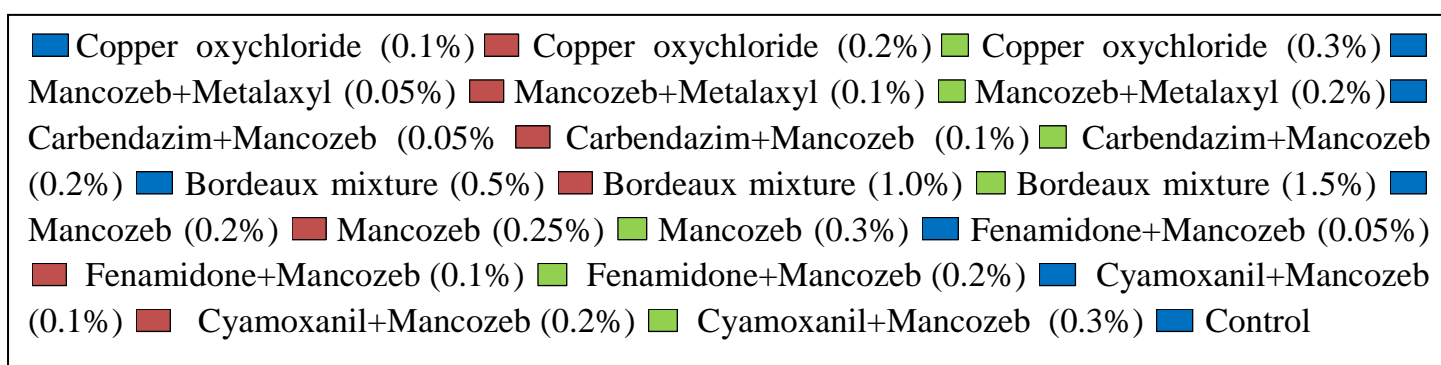


Fig. 4.5 Compatibility of *Trichoderma harzianum* (Tc ed f 4) with fungicides



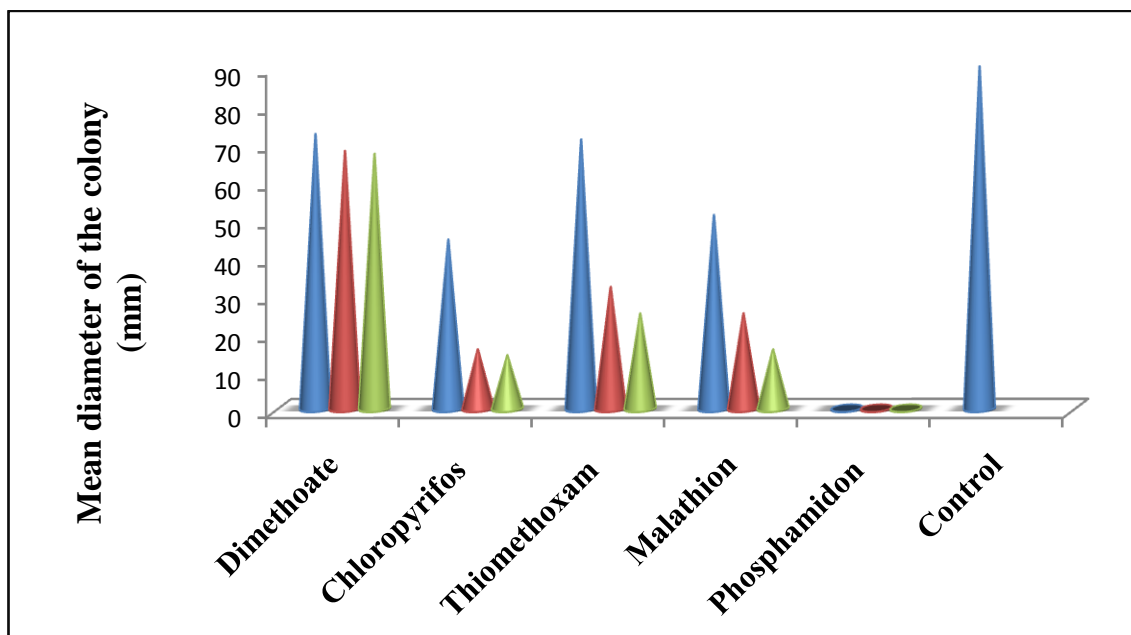
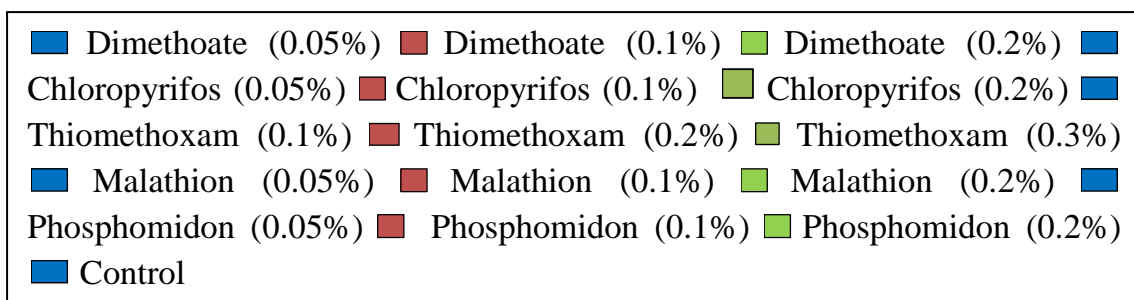


Fig. 4.6 Compatibility of *Trichoderma harzianum* (Tc ed f 4) with insecticides



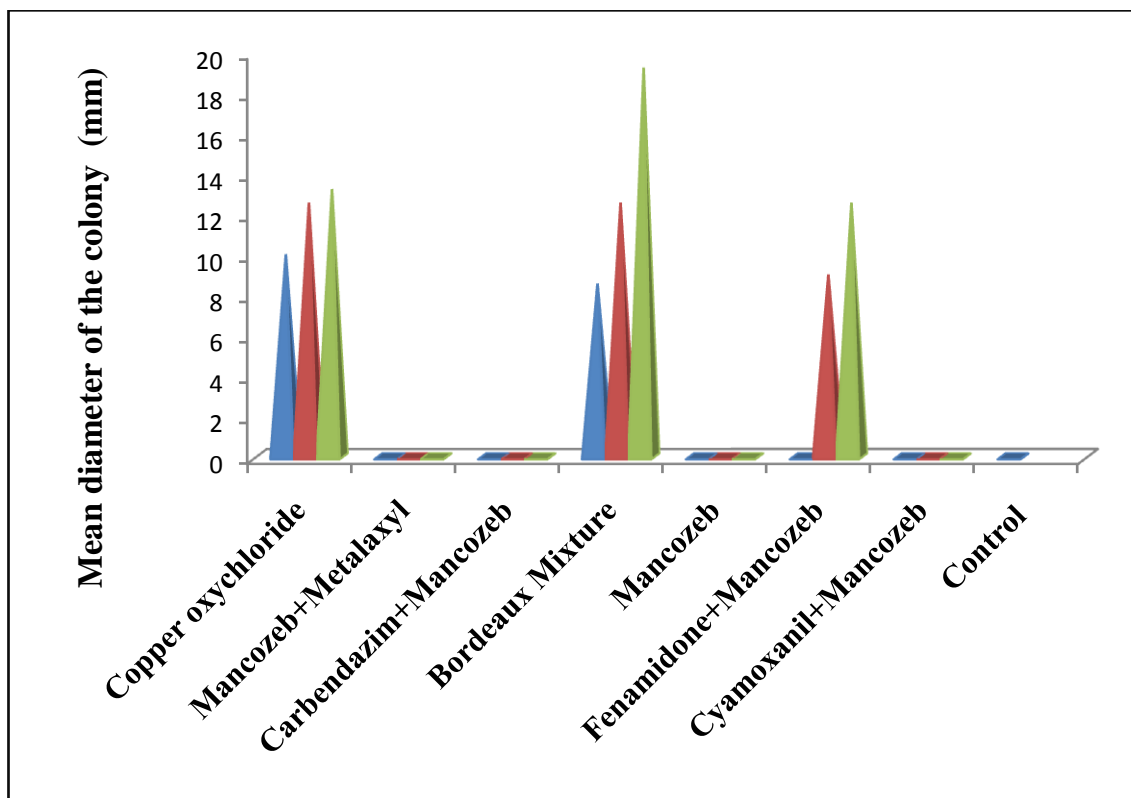


Fig. 4.7 Compatibilty of *Pseudomonas* sp. (Tc ed b 3) with fungicides

■ Copper oxychloride (0.1%) ■ Copper oxychloride (0.2%) ■ Copper oxychloride (0.3%) ■
 Mancozeb+Metalaxyl (0.05%) ■ Mancozeb+Metalaxyl (0.1%) ■ Mancozeb+Metalaxyl (0.2%) ■
 Carbendazim+Mancozeb (0.05%) ■ Carbendazim+Mancozeb (0.1%) ■ Carbendazim+Mancozeb
 (0.2%) ■ Bordeaux mixture (0.5%) ■ Bordeaux mixture (1.0%) ■ Bordeaux mixture (1.5%) ■
 Mancozeb (0.2%) ■ Mancozeb (0.25%) ■ Mancozeb (0.3%) ■ Fenamidone+Mancozeb (0.05%)
 ■ Fenamidone+Mancozeb (0.1%) ■ Fenamidone+Mancozeb (0.2%) ■ Cyamoxanil+Mancozeb
 (0.1%) ■ Cyamoxanil+Mancozeb (0.2%) ■ Cyamoxanil+Mancozeb (0.3%) ■ Control

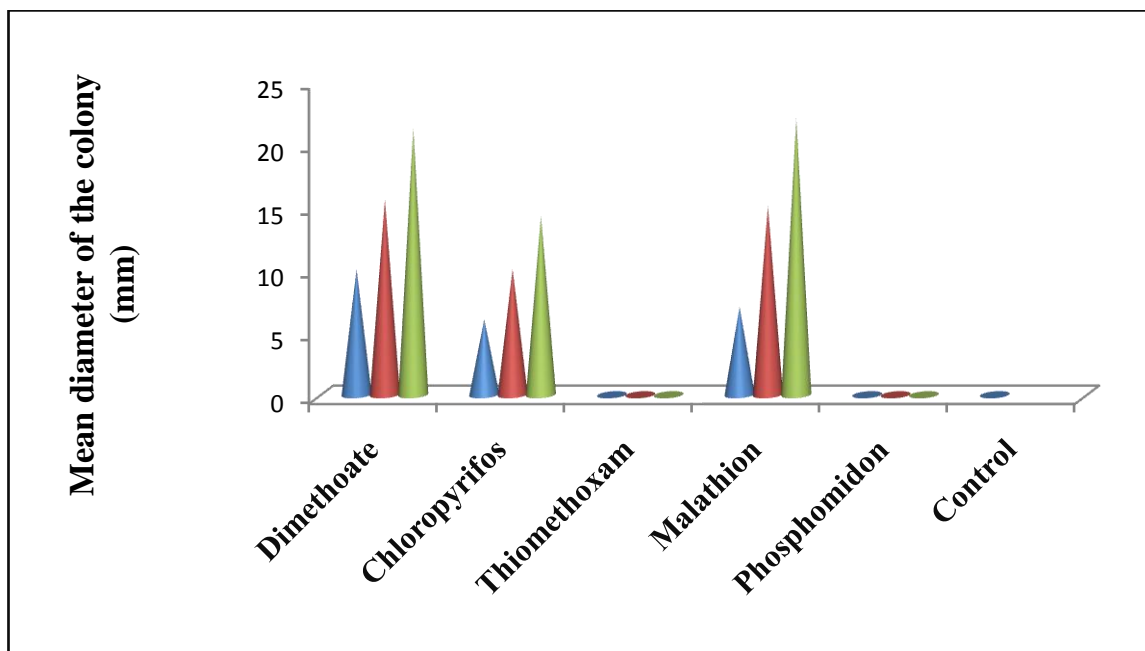
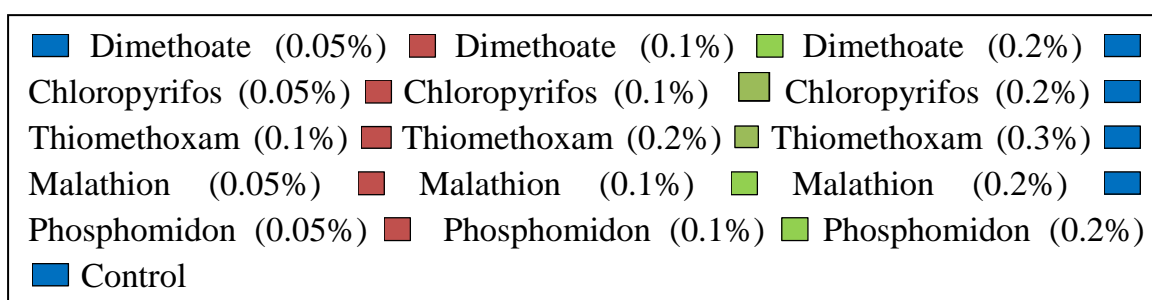


Fig. 4.8 Compatibilty of *Pseudomonas* sp. (Tc ed b 3) with insecticides



Chapter V

SUMMARY AND CONCLUSIONS

The experimental findings of the present investigation entitled "**Studies on Endophytic microbial diversity and antagonistic effect on rhizome rot pathogens in turmeric (*Curcuma longa* L.)**" was carried out at the Department of Horticultural Plant Pathology, College of Horticulture, Anantharajupeta during the year 2016-17. The major constraint faced by turmeric farmers of Andhra Pradesh is the prevalence of rhizome rot disease. Antagonistic endophytes are known to possess the beneficial attributes of other biological agents like the capacity to induce systemic resistance and growth promoting ability with additional advantage of systemic existence in plants. Hence, the present investigations are carried out to harness the potential of native endophytic microbes of turmeric for the management of rhizome rot disease. The salient findings of the study are summarized below.

1. A roving survey was conducted to estimate rhizome rot incidence under field conditions in five districts of A.P. The mean maximum disease incidence was observed in Guntur district (16.12%) followed by West Godavari district (12.68%) and in Visakhapatnam district least mean disease incidence (2.18%) was noticed.

2. The organisms causing rhizome rot disease of turmeric were isolated from infected rhizomes and their pathogenicity proved. Based on cultural and morphological characters, pathogens were identified as *Pythium ultimum* and *Fusarium solani*.

3. Enumeration of endophytic microorganisms from healthy turmeric rhizomes from various areas of A.P. state revealed the predominance of bacteria (10.40×10^6 cfu g^{-2}) and fluorescent Pseudomonads (13.40×10^6 cfu g^{-2}).

4. Altogether, 58 endophytes from turmeric were isolated. Among them 24 isolates (Fifteen fungi, five bacteria and four fluorescent *Pseudomonads*) were found to possess antagonistic activity against turmeric rhizome rot pathogens.

5. Antagonistic action of these endophytes against pathogens was further studied in comparison with that of reference cultures of *Pseudomonas fluorescens* (COH) and *Trichoderma viride* (COH). The study revealed that, only 3 bacterial and 4 fungal cultures showed efficient antagonist property against turmeric rhizome rot pathogens and they were selected further *in vitro* evaluation.

6. The selected endophytes were subjected to various tests to understand parameters that contributed to their antagonistic potential. All antagonists inhibited growth of the pathogen, produced IAA, ammonia and were negative to HCN and siderophore production. The fungal antagonists were positive to non volatile metabolites production and negative to volatile metabolites production.

7. The compatibility of the selected fungal antagonist Tc ed f 4 to seven fungicides was assessed. Bordeaux mixture and carbendazim (12%) + mancozeb (64%) were incompatible and showed cent percent inhibition on the growth of the antagonist.

8. With regard to insecticides, phosphamidon (40%) was incompatible with the selected fungal antagonist while dimethoate (30%) was highly compatible.

9. The compatibility of fungicides towards the selected bacterial antagonist Tc ed b 3 was studied. Mancozeb (4%) + metalaxyl-M (64%), carbendazim (12%) + mancozeb (64%), cyamoxanil (8%) + mancozeb (64%), mancozeb, lower concentration of fenamidone (10%) + mancozeb (50%) were compatible with bacterial antagonist. Among copper fungicides, Bordeaux mixture was more inhibitory than copper oxychloride (50%).

10. With regard to insecticides, all concentrations of thiomethoxam (25%) and phosphamidon (40%) were compatible with bacterial antagonist. Chlorpyrifos (20%), dimethoate (30%) and malathion (50%) were incompatible.

11. The fungal antagonists were identified as *Aspergillus oryzae* (Tc ed f 1), *Penicillium chrysogenum* (Tc ed f 2), *Trichoderma viride* (Tc ed f 3) and *Trichoderma harzianum* (Tc ed f 4). Three of the bacterial isolates (Tc ed b 1 and Tc ed b 3) were tentatively identified as *Pseudomonas* sp. while the other one (Tc ed b 2) as *Bacillus* sp.

12. Under field conditions, out of 120 turmeric lines screened, thirty lines (14 short duration, 5 medium duration, 11 long duration) were found resistant to rhizome rot disease. IC-319341, Tenali Kasturi, VK-23, GS, IC-420606, IC-033007, IC-211641, PTS-8, Vikici, Dhindigam, ACC-48, Sonia, NB-60, Kasturi in short duration group, Prathibha, Thodupuzha, KTS-9, Prasangali, ACC-79 in medium duration group and NH-1, Ranga, Salem, Salem-2, Wagon, PTS-12, CL-8, CL-9, CL-10, CL-3, CL-4 in long duration group were resistant to rhizome rot showed 0.0% infection. In case of rhizome yield, medium duration turmeric line RH-50 (1.69 kg/plant) was significantly superior over all other genotypes evaluated.

13. Under pot culture conditions, out of 30 turmeric lines screened, Salem and Prathibha were found resistant to rhizome rot with 0 % infection.

Future line of work:

- Survey and surveillance of rhizome rot disease of turmeric in major turmeric growing areas in India.
- Further studies can be taken up using antagonistic endophytic micro flora an alternative to fungicides under field conditions against the foliar and soil borne diseases.
- Germplasm cultures which showed less disease incidence needs to be studied further under *in vitro* by artificial inoculation for better evaluation and refinement.
- Needs to study the presence of races of the pathogen or resistant genes, as one variety showing the resistant reaction in one year and the same variety showing the susceptible reaction in another year in the same region.

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- The pattern of literature cited presented above is in accordance with the guidelines for thesis presentation for Dr. Y.S.R. Horticultural University, Venkataramannagudem.

Appendix-III

MEDIA COMPOSITION

(Ingredients per litre)

1. POTATO DEXTROSE AGAR

Composition:

Potato	:	200.0 g
Dextrose	:	20.0 g
Agar	:	20.0 g
Distilled Water	:	1000 ml

2. MARTIN'S ROSE BENGAL STREPTOMYCIN AGAR

Composition:

Dextrose	:	10.0 g
Peptone	:	5.0 g
KH ₂ PO ₄	:	1.0 g
MgSO ₄	:	0.5 g
Agar	:	20.0 g
Rose Bengal	:	0.03 g
Streptomycin	:	30 mg
Distilled water	:	1000 ml

3. NUTRIENT AGAR MEDIUM

Composition:

Glucose	:	5.0 g
Peptone	:	5.0 g
Beef extract	:	3.0 g
NaCl	:	5.0 g
Agar	:	20.0g
p ^H	:	6.5 to 7.5
Distilled water	:	1000 ml

4. KING'S B AGAR MEDIUM

Composition:

Peptone	:	20.0 g
Glycerol	:	10.0 ml
K ₂ HPO ₄	:	10.0 g
MgSO ₄ · 7H ₂ O	:	1.5 g
Agar	:	20.0 g
p ^H	:	7.2
Distilled water	:	1000 ml

5. KEN KNIGHT'S AGAR MEDIUM

Composition:

Dextrose	:	1.0 g
KH ₂ PO ₄	:	0.1 g
NaNO ₃	:	0.1 g
KCl	:	0.1 g
MgSO ₄	:	0.1 g
Agar	:	20.0 g
p ^H	:	7
Distilled water	:	1000 ml

6. Peptone water (p^H 7.0)

Composition:

Peptone	:	10.0 g
NaCl	:	15.0 g
Distilled water	:	1000 ml

Appendix – IV

Phosphate buffer composition

0.02 M Potassium Phosphate buffer: p^H 7.0

* Stock solutions:

A- 3.5 g of dibasic K_2HPO_4 in 1000 ml

B- 2.7 g of monobasic KH_2PO_4 in 1000 ml

For getting 0.02 M Potassium Phosphate buffer of p^H 7, mix 61 ml of solution A with 39 ml of solution B in 100 ml of water

10 Mm Solution Phosphate buffer: p^H 6.0

A- 0.2 M solution of monobasic sodium phosphate, it can be obtained by dissolving 27.8 g in 1000 ml distilled water.

B- 0.2 M solution of dibasic sodium phosphate, it can be obtained by dissolving 71.7 g of $Na_2HPO_4 \cdot 12H_2O$ in 1000 ml distilled water.

Appendix-II

Weekly meteorological data recorded during the period of study (June, 2016-March, 2017)

Standard week number	Period	Temperature (⁰ C)		Relative humidity (%)		Rainfall (mm)
		Maximum	Minimum	at 8 am	at 2 pm	
25	14-20 JUNE	36.80	26.60	82.57	35.57	8.4
26	21-27	34.85	24.71	83.83	35.16	34.00
27	28-4 JULY	33.5	24.25	80.5	39.75	4.0
28	5-11	32.28	21.85	86.14	47.14	--
29	12-18	33	25.71	89.14	35.14	29.4
30	19-25	29.85	24.57	86.85	36.71	7.0
31	26-1 AUG	31.5	25.5	86.14	37.14	--
32	2-8	30.16	24.83	87.42	35.57	--
33	9-15	34.57	26.42	86.04	35.25	--
34	16-22	33	26.42	87.71	35.85	--
35	23-29	32.57	24.62	88.28	35.57	1.0
36	30-6 SEP	30.16	23.66	88.57	36.42	4.0
37	7-13	29.28	24.57	89.28	36.57	--
38	14-21	28.14	23.85	83.85	35.42	29.00
39	22-30	29.57	24.28	83.00	33.42	37.00
40	01-07 OCT	25.42	20.85	83.57	33.28	--
41	08-14	28.71	22.14	83.71	33.71	--
42	15-21	28.57	25.71	82.42	32.25	22.60
43	22-28	29	25	84.42	34.71	53.00
44	29-04 NOV	27.57	21.57	87	37.57	12.00
45	05-11	26.85	19.71	83.85	40.85	--
46	12-18	27.57	22.14	73.85	35.85	55.2
47	19-25	27.42	21.14	85.85	35.85	--
48	26-02 DEC	25.71	18.42	89.42	44.14	--
49	03-09	29.71	17.71	89.42	41.57	
50	10-16	25.71	20	89.57	37.42	50.9
51	17-23	25.57	18.28	86.14	35.57	--
52	24-31	28.87	17.87	88.87	37.62	--
1	01-07 JAN	26.28	17.42	86	36.57	--
2	08-14	25.42	15.14	85.71	35.42	--
3	15-21	25.57	14.71	85.57	35.28	--
4	22-28	24.71	16.42	85.71	39	--
5	29-04 FEB	25.71	19	87.14	41.28	--
6	05-11	26.42	18.14	86.57	36.42	--
7	12-18	29.85	20.57	86.85	36.14	--
8	19-25	29.57	20	85.71	37.85	--
9	26-04 MAR	30	18.85	88.28	41.42	--
10	05-11	29.71	22.71	87.28	43.71	5.2
11	12-18	31	23.14	87.57	41.42	--

Appendix I

Results of mechanical and chemical analysis of soil of the experimental Field

A. Mechanical analysis:

Constituents	Per cent (%)
Sand	33.45
Silt	60.25
Clay	6.25
Textural class	Silt clay

B. Chemical analysis:

Soil properties	Amount
Soil p ^H	6.12
Organic Carbon (%)	1.32
Total nitrogen (%)	0.08
Available P (ppm)	20
Exchangeable K (%)	0.2



Plate 3.3 Field view of experimental plot

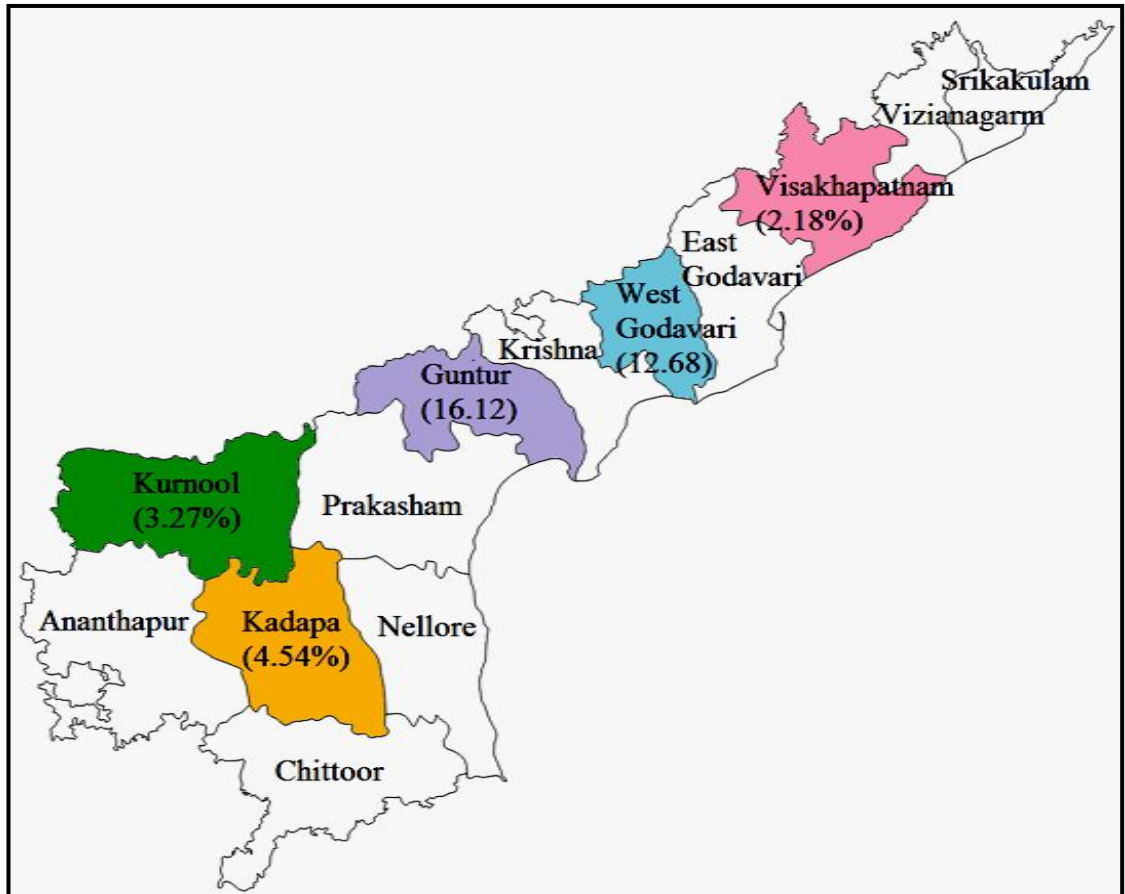


Plate 4.1 District mean per cent disease incidence of turmeric rhizome rot disease during 2016-17 in Andhra Pradesh



Plate 4.2a View of rhizome rot infected farmer's field during the survey in Guntur district



Plate 4.2b Rhizome rot infected field



c. Infected rhizome



d. Advanced stage of infected rhizome colonized by maggots



Plate 4.3a Healthy and diseased rhizome



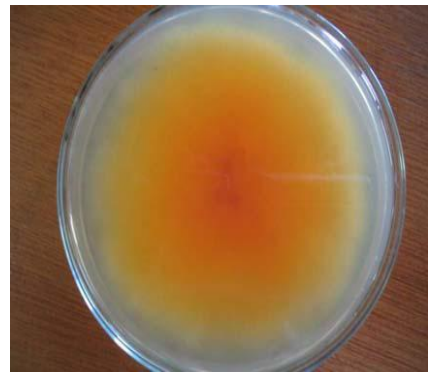
Plate 4.3b Healthy and rhizome rot infected turmeric plant



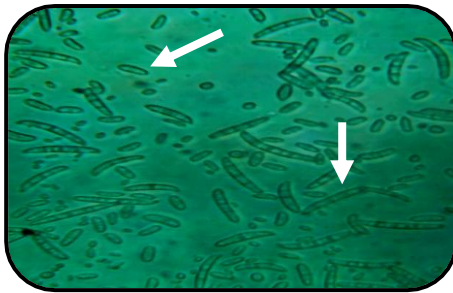
Plate 4.4a Proving pathogenicity (rhizome rot) by *Pythium ultimum*



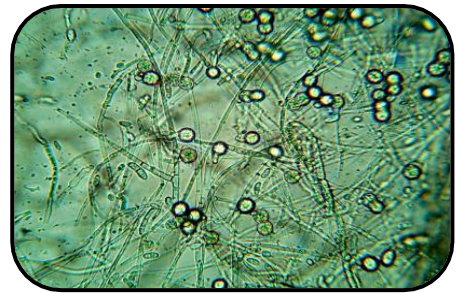
Plate 4.4b Proving pathogenicity (rhizome rot) by *Fusarium solani*



Fusarium solani growth on PDA medium



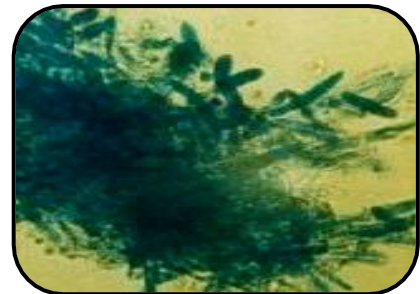
Macro and microconidia



Chlamydospores produced by secondary mycelium



Chlamydospores produced by microconidia



Sporodochium

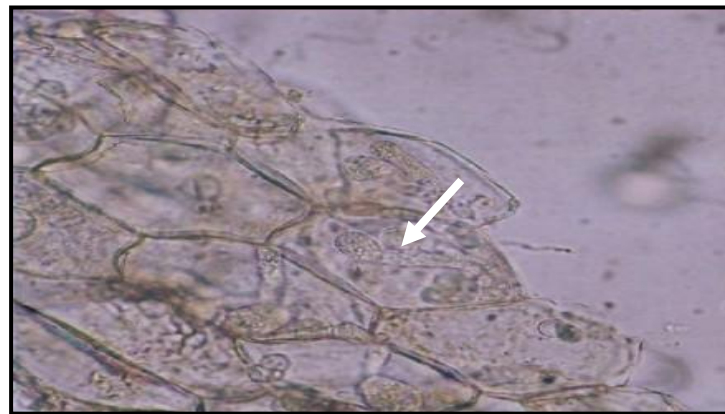
Plate 4.6 Microscopic (400 X) and morphological characters of *Fusarium oxysporum*



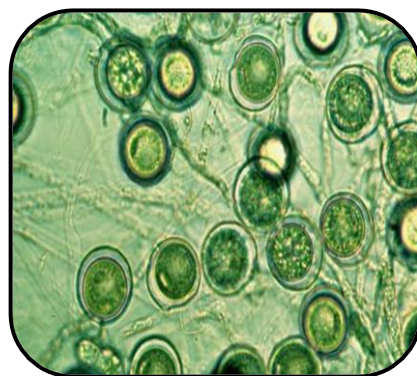
Growth on PDA



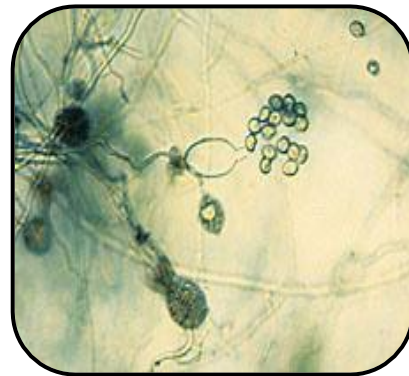
Pure culture of *P. ultimum*



Perennating oospores in rhizomes

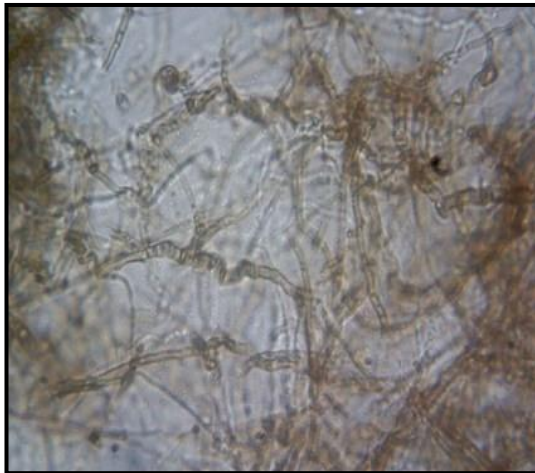


Oospores



Liberated zoospores from sporangia

Plate 4.5 Microscopic (400 X) and morphological characters of *Pythium ultimum*



Trichoderma viride (Coiling)

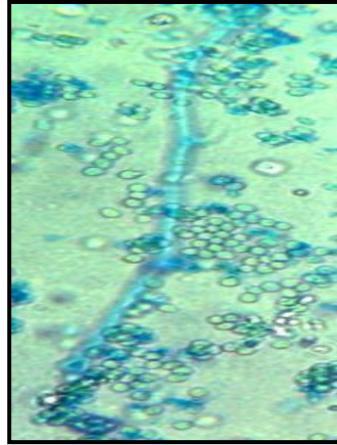


Trichoderma harzianum
(Hyphal disintegration)

Plate 4.10 Mechanism of action of fungal antagonists with *Pythium ultimum* of turmeric



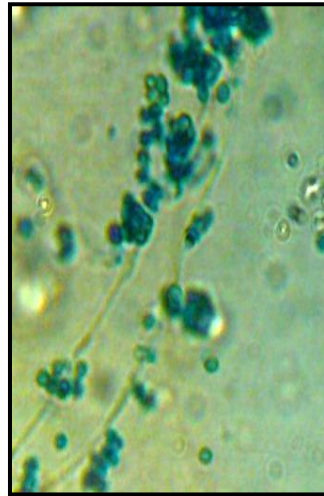
Pure culture of
Trichoderma harzianum



Trichoderma harzianum

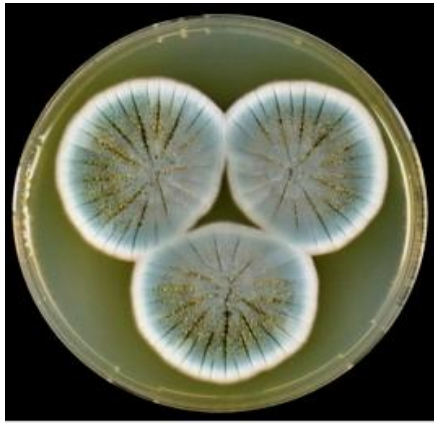


Pure culture of *Trichoderma viride*

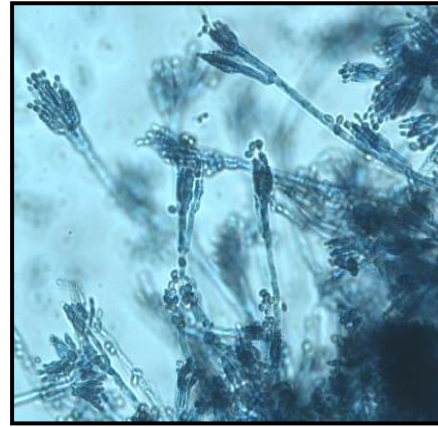


Trichoderma viride

Plate 4.16 Identification of fungal antagonists



Pure culture of
Penicillium chrysogenum



Penicillium chrysogenum



Pure culture of *Aspergillus*
oryzae



Aspergillus oryzae



Pseudomonas sp.

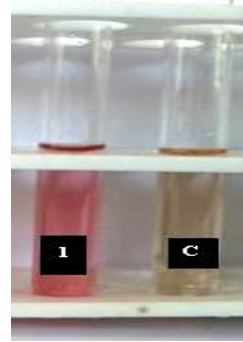


Bacillus sp.

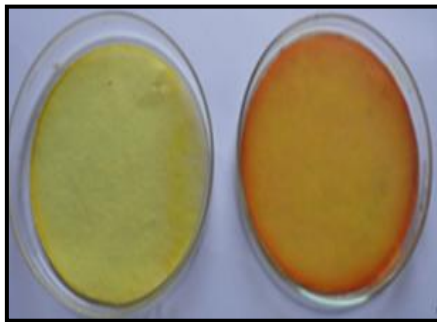
Plate 4.16 Identification of fungal and bacterial antagonists



Ammonia production



IAA production



HCN



Siderophore production

Plate 4.11 Mechanism of action of selected bacterial antagonists



Plate 3.1 Media used for isolation of endophytes



Plate 3.2 Mass multiplication of rhizome rot pathogens



Resistant (Dindigam)



Moderately resistant (TCP-70)



Moderately Susceptible (NDS-18)



Susceptible (Battiguda)



Highly susceptible (Mega turmeric)

Plate 4.14 Screening of turmeric germplasm for resistance against rhizome rot disease under field condition



Turmeric rhizome rot disease scale (0-4)



Resistant lines (Salem and Prathibha)



Susceptible lines (CL-12 and RH-9/90)

Plate 4.15 Screening of turmeric germplasm for resistance against rhizome rot disease under pot culture condition



Plate 3.4 Field view of the pot culture experiment



Plate 4.7a Inhibitory effect of fungal endophytes on mycelial growth of *Fusarium solani* in dual culture test
Top: left to right Control, Tc ed f1, Tc ed f2, Tc ed f3, Tc ed f4



Plate 4.7b Inhibitory effect of fungal endophytes on mycelial growth of *Pythium ultimum* in dual culture test
Top: left to right Tc ed f1, Tc ed f2, Tc ed f3, Tc ed f4, Control.



Plate 4.8a Inhibitory effect of different bacterial endophytes on mycelial growth of *Fusarium solani* in dual culture test
Top: left to right Tc ed f1, Tc ed f2, Tc ed f3, Tc ed f4, Control.



Plate 4.8b Inhibitory effect of different bacterial endophytes on mycelial growth of *Pythium ultimum* in dual culture test
Top: left to right Tc ed f1, Tc ed f2, Tc ed f3, Tc ed f4, Control.



Plate 4.9a Inhibitory effect of different fluorescent pseudomonads isolates on mycelial growth of *Fusarium solani* in dual culture test
Top: left to right Tc ed f1, Tc ed f2, Tc ed f3, Tc ed f4, Control.



Plate 4.9b Inhibitory effect of different fluorescent pseudomonads isolates on mycelial growth of *Pythium ultimum* in dual culture test
Top: left to right Tc ed f1, Tc ed f2, Tc ed f3, Tc ed f4, Control.

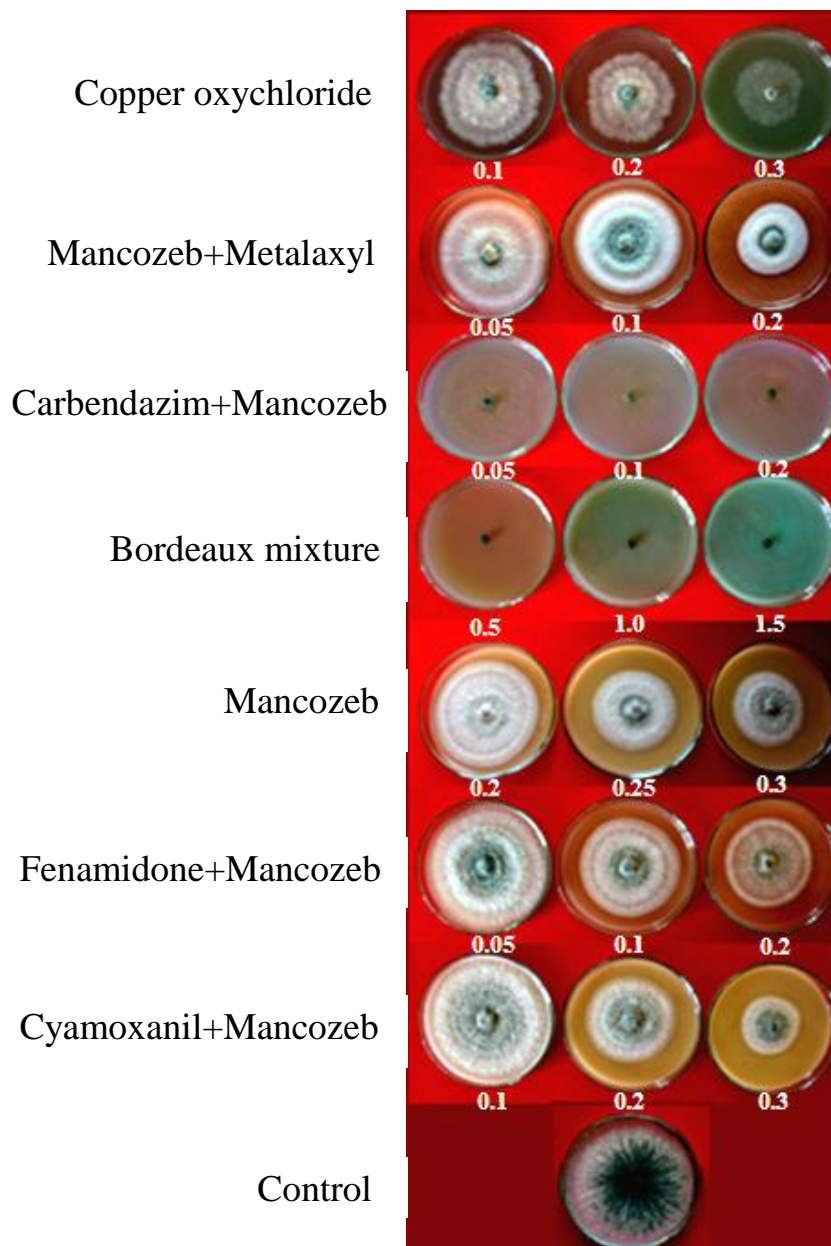


Plate 4.12 Compatibility of *Trichoderma harzianum* (Tc ed f 4) with fungicides in *in vitro* conditions

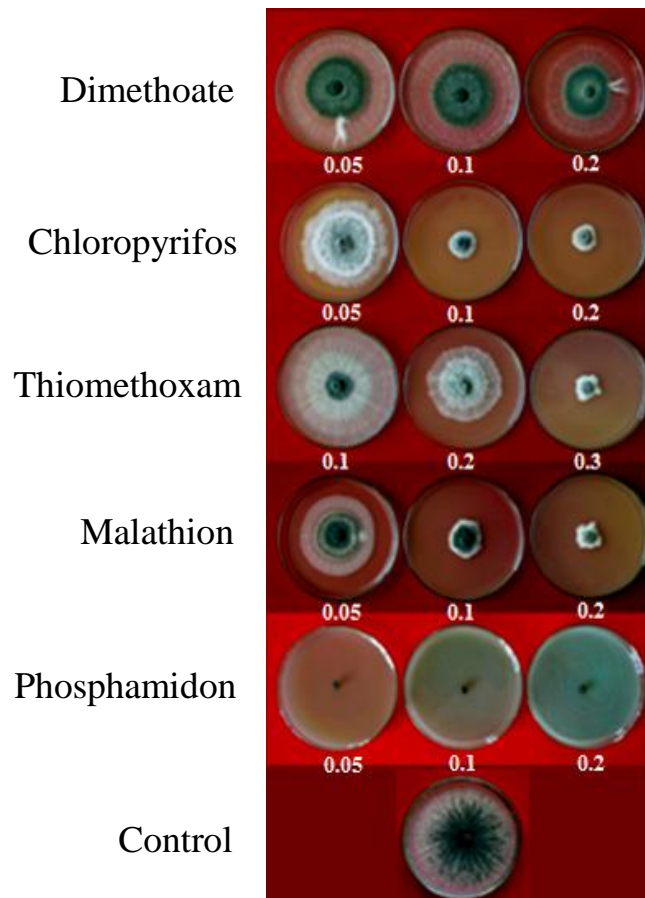


Plate 4.13 Compatibility of *Trichoderma harzianum* (Tc ed f 4) with insecticides in *in vitro* conditions



Plate 3.4 Field view of the pot culture experiment



Plate 3.3 Field view of experimental plot