172205

ECOFRIENDLY MANAGEMENT OF RHIZOCTONIA LEAF BLIGHT OF AMARANTHUS

PRIYADARSINI P.

Thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2003

Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

DECLARATION

I hereby declare that this thesis entitled "**Ecofriendly management of Rhizoctonia leaf blight of amaranthus**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
29-1 -2004.

PRIYADARSINI P.
(2001-11-24)

CERTIFICATE

Certified that this thesis entitled "**Ecofriendly management of Rhizoctonia leaf blight of amaranthus**" is a record of research work done independently by Ms. Priyadarsini P. (2001-11-24) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

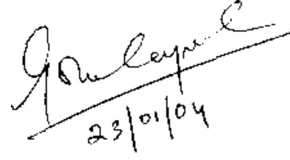
Vellayani,
23/11-2004.

Dr. C. GOKULAPALAN
(Chairman, Advisory Committee)
Associate Professor,
Department of Plant Pathology,
College of Agriculture, Vellayani
Thiruvananthapuram.

Approved by

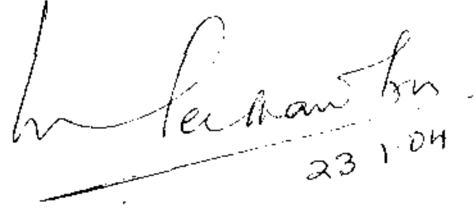
Chairman :

Dr. C. GOKULAPALAN
Associate Professor,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

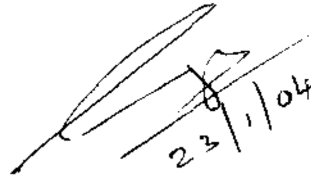


Members:

Dr. C.K. PEETHAMBARAN
Professor and Head,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.



Dr. V.K. GIRIJA
Associate Professor,
Department of Plant Pathology,
College of Agriculture, Vellayani.
Thiruvananthapuram-695522.



Dr. V.A. CELINE
Associate Professor,
Department of Olericulture,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

External Examiner :

Dr. VINAYAK HEGDE
Senior Scientist,
CTCRI, Sreekaryam,
Thiruvananthapuram

ACKNOWLEDGEMENT

I express my deep sense of gratitude and indebtedness to all those who did help me to complete my thesis.

I would like to express my heartfelt gratitude to Dr. C. Gokulapalan, Chairman of the Advisory Committee for his sincere guidance, critical suggestions, unfailing patience, constant encouragement and kind help through out the period of investigation and in the preparation of thesis.

My sincere gratitude to Dr. C.K. Peethambaran, Professor and Head of Plant Pathology for the constant advice and help during the course programme and research work and his scholarly suggestions and critical evaluation of the manuscript.

I wish to express my deep sense of gratitude to Dr. V.K. Girija, Associate Professor, Plant Pathology for her painstaking efforts, timely help, encouragement and valuable instructions given to me through out the period of study.

My immense gratitude to Dr. V.A. Celine, Associate Professor, Department of Olericulture for her constant support and kind co- operation during the study.

I am greatly indebted to Dr. P. Sivaprasad, Associate Professor of Plant Pathology for his kind co-operation, pertinent suggestions and constructive criticism through out the period of thesis work.

I convey my sincere thanks to Dr. Ajit Varma, Department of Life Sciences, Jawaharlal Nehru University, New Delhi for providing the cultures required for the study.

I wish to place on record my heartfelt gratitude to Dr. K. Umamaheshwaran, Assistant Professor of Plant Pathology for his timely advice, unstinting support and kind help during the biochemical analysis.

My sincere gratitude to Dr. K.K. Sulochana, Associate Professor of Plant Pathology and Dr. K.N. Anith, Assistant Professor of Microbiology for their whole hearted co-operation and valuable suggestions during the study.

I am grateful to Dr. Lulu Das, Associate Professor of Plant Pathology for her active support and help rendered during the study.

Dr. S.K. Nair, Professor of Microbiology and Dr. A. Naseema, Associate Professor of Plant Pathology for their valuable suggestions and whole hearted support during the entire period of study.

I wish to express my sincere gratitude to Dr. M. Suharban and Dr. D. Geetha Associate Professors, Instructional Farm, for their timely help and kind co-operation during the thesis work.

My heartfelt gratitude to C.E. Ajithkumar, Programmer, Agricultural Statistics for the help and assistance rendered during the analysis of the data.

I express my sincere thanks to Dr. M.S. Sheela, Associate Professor, Department of Agricultural Entomology for the help provided in preparing the photomicrographs of the fungal cultures used in the study.

My heartiest gratitude to Mr. Biju of ARDRA for the timely and neat preparation of the manuscript.

I acknowledge Kerala Agricultural University for awarding the Junior Research fellowship for the conduct of the study.

I am deeply obliged to all the students, teaching and non-teaching staff of the Department of Plant Pathology for their kind co-operation and immense help rendered during the entire course of study.

I am extremely grateful to all the labourers who have rendered their help during my thesis work.

I am greatly indebted to all my friends Dhanya chechi, Heera chechi, Anitha chechi, Rini chechi, Sreekala chechi, Santosh chettan, Pradeep, Vishnu, Arun, Reji, Pramod, Liza, Saritha, Giji, Renu, Resmi, Ayesha, Sonia and Simi for their self less help, moral support and encouragement through out the period of study.

My special thanks to Mr. Manoj who has helped me at times when I needed it the most.

I wish to place on record my heartiest gratitude to my classmates, Sherin, Beena, Senthil, Deepthi and Ancy for all their help and support.

I recall with love and gratitude the constant encouragement and unfailing support given to me by my parents, sister and other members of the family with out which the thesis would not have seen light.

And above all I am indebted to God Almighty for His generous blessings showered upon me for the successful completion of the thesis.

Priyadarsini P.

Dedicated to
My Family

CONTENTS

	Page No.
1. INTRODUCTION	1-2
2. REVIEW OF LITERATURE	3-15
3. MATERIALS AND METHODS	16-32
4. RESULTS	33-58
5. DISCUSSION	59-68
6. SUMMARY	69-70
7. REFERENCES	71-90
APPENDIX	
ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	*R. solani* isolates from different locations in Thiruvananthapuram district	34
2	Variation in the growth and sclerotial formation of *R. solani* isolates	34
3	Lesion development and disease intensity on amaranthus caused by *R. solani* isolates	37
4	Effect of *Trichoderma* spp. and *Piriformospora indica* on the mycelial growth of *R. solani*	37
5	Nature of reaction of the fungal antagonists on *R. solani*	38
6	Antagonism of bacterial isolates against *R. solani*	38
7	Influence of indigenous materials, biocontrol agents and mancozeb on the shoot length, root length, number of leaves and leaf area of amaranthus	41
8	Influence of indigenous materials, biocontrol agents and mancozeb on fresh and dry weight of shoots and roots of amaranthus	43
9	Effect of indigenous materials, biocontrol agents and mancozeb on the incidence and intensity of leaf blight of amaranthus	45
10	Percentage root colonization by *P. indica*	47
11	Effect of selected indigenous materials, biocontrol agents and mancozeb on the shoot length, root length, number of leaves and leaf area of amaranthus	47
12	Effect of selected indigenous materials, biocontrol agents and mancozeb on fresh and dry weight of shoots and roots of amaranthus	49

LIST OF TABLES CONTINUED

Table No.	Title	Page No.
13	Effect of selected indigenous materials. biocontrol agents and mancozeb on the incidence and intensity of amaranthus leaf blight of amaranthus	51
14	Effect of soil application of rice husk ash. *P. indica*, bacterial isolates and *T. harzianum* on total phenols, amino acids and carbohydrates in amaranthus leaves inoculated with *R. solani*	53
15	Effect of foliar spray of turmeric powder-baking soda, bacterial isolates, *T. harzianum* and mancozeb on total phenols, amino acids and carbohydrates in amaranthus leaves inoculated with *R. solani*	55
16	Effect of soil application of rice husk ash. *P. indica*, bacterial isolates and *T. harzianum* on changes in the activity of PAL, PO and PPO in amaranthus leaves inoculated with *R. solani*	57
17	Effect of foliar spray of turmeric powder-baking soda, bacterial isolates, *T. harzianum* and mancozeb on changes in the activity of PAL, PO and PPO in amaranthus leaves inoculated with *R. solani*	58

LIST OF FIGURES

Sl. No.	Title	Between pages
1	Percentage inhibition of fungal and bacterial antagonists against *Rhizoctonia solani*	61-62
2	Effect of Rice husk ash, *Pseudomonas fluorescens*, *Trichoderma harzianum* and mancozeb on shoot length, root length, number of leaves and leaf area of amaranthus	66-67
3	Effect of Rice husk ash, *Pseudomonas fluorescens*, *Trichoderma harzianum* and mancozeb on the intensity of foliar blight of amaranthus	67-68

LIST OF PLATES

Plate No.	Title	Between pages
1	0-9 scale for the scoring of foliar blight of amaranthus	24-25
2	Symptoms of foliar blight disease on amaranthus leaves	34-35
3	Hymenial layer of *Thanatephorus cucumeris* on the undersurface of the leaf	34-35
4	*In vitro* inhibition of *Rhizoctonia solani* by *Trichoderma virens*	37-38
5	*In vitro* inhibition of *R. solani* by *T. piluliferum*	37-38
6	*In vitro* inhibition of *R. solani* by *T. pseudokoningii*	37-38
7	*In vitro* inhibition of *R. solani* by *T. harzianum*	37-38
8	Interaction between *R. solani* and *Piriformospora indica*	38-39
9	*In vitro* inhibition of *R. solani* by *Pseudomonas fluorescens* (P_1)	38-39
10	*In vitro* inhibition of *R. solani* by *P. fluorescens* (P_5)	38-39
11	*In vitro* inhibition of *R. solani* by *P. fluorescens* (B_3)	39-40
12	*In vitro* inhibition of *R. solani* by *P. fluorescens* (KK_{16})	39-40
13	*In vitro* inhibition of *R. solani* by the culture filtrate of *P. fluorescens* (B_3)	39-40
14	Effect of turmeric powder-baking soda on the *in vitro* growth of *R. solani*	39-40

LIST OF PLATES CONTINUED

Plate No.	Title	Between pages
15	Effect of *P. fluorescens* (B_3) on the shoot length of amaranthus	41-42
16	Effect of Rice husk ash (1000 g) on the shoot length of amaranthus	41-42
17	Effect of turmeric powder-baking soda (10:1) on the shoot length of amaranthus	41-42
18	Effect of *P. fluorescens* (B_3) on the root length of amaranthus	41-42
19	Effect of Rice husk ash (1000g) on the root length of amaranthus	41-42
20	Effect of Rice husk ash (1000 g) on the incidence of foliar blight of amaranthus	45-46
21	Effect of *P. fluorescens* (B_3) on the incidence of foliar blight of amaranthus	45-46
22	Effect of *T. harzianum* on the incidence of foliar blight of amaranthus	45-46
23	Effect of *P. fluorescens* (KK_{16}) on the incidence of foliar blight of amaranthus	45-46
24	Root colonization of *P. indica* on amaranth roots showing the chlamydospores	47-48
25	Chlamydospores of *P. indica*	47-48

LIST OF APPENDICES

Sl. No.	Title	Appendix No.
1	Composition of different media	I
2	Preparation of stain	II
3	Buffers for enzyme analysis	III

LIST OF ABBREVIATIONS

%	Per cent
μl	Microlitre
μm	Micrometre
^{0}C	Degree Celsius
CD	Critical difference
cm	Centimetre
CRD	Completely Randomised Design
et al.	And others
Fig.	Figure
g	Gram
h	Hour
i.e.	That is
kg	Kilogram
l	Litre
m	Metre
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
mM	Millimolar
N	Normal
nm	Nanometre
rpm	Rotations per minute
sec	Second
spp.	Species
var	Variety
viz.	Namely
w/v	Weight/volume
w/w	Weight/weight

INTRODUCTION

1. INTRODUCTION

Amaranthus, widely known as "poor man's spinach" is one of the most popular and commercially cultivated leafy vegetable of Kerala. It is a nutritious vegetable, leaves being rich source of proteins, vitamins, minerals and dietary fibre. The crop can be easily raised even in nutritionally poor soils and yet give reasonable yields. The crop has relatively few insect and disease problems.

Recently, a serous disease has been reported in the crop in Kerala (Kamala *et al.*, 1996; Gokulapalan *et al.*, 2000). The fungus *Rhizoctonia solani* has been consistently isolated from the diseased tissues of amaranthus. The disease causes considerable economic loss due to low marketability of the produce.

Being a pathogen of great diversity with wide host range and lack of sharp differentiation among its strains, *R. solani* poses great difficulty in developing resistance in crop varieties. The red variety of amaranthus which is popular in Kerala is highly susceptible to the disease. The pathogen can be controlled by spraying mancozeb 0.40 per cent in cowdung supernatant at fortnightly intervals. However the use of chemicals, apart from being costly also pose severe threat to human health and environment due to the persistence of its residues on leaves. Mancozeb belongs to ethylene bis dithiocarbamate group whose degradation product is ethylene thiourea which is reported to be carcinogenic (Mah *et al.*, 1988).

The present trend in agriculture is towards organic farming by which the farmers can fetch a premium price for their produce. Organic farming envisages crop production without the use of synthetic chemicals including chemical fertilizers, pesticides, hormones etc. In this context, management of the disease using biocontrol agents and indigenous

materials gains momentum. Biological control of plant pathogens is gaining importance in recent years as this will reduce the pesticidal hazards and cost of cultivation in environmentally safe agricultural systems. Therefore the present investigation was carried out with the objective of evolving an ecofriendly practice for the management of leaf blight of amaranthus using biocontrol agents and indigenous materials. The study made use of biocontrol agents like *Trichoderma harzianum* and *Pseudomonas fluorescens* along with a newly discovered plant growth promoting root endophyte, *Piriformospora indica* and materials like turmeric powder-baking soda and rice husk ash. The main objectives of the study are:

- To find out the efficacy of *T. harzianum*, *P. fluorescens*, *P. indica*, turmeric powder–baking soda and rice husk ash in inhibiting the mycelial growth of *R. solani* under *in vitro* conditions.
- To find out the effect of culture filtrates of *T. harzianum*, *P. fluorescens* and *P. indica* on the growth of *R. solani*.
- To evolve a management practice for the disease using the biocontrol agents along with indigenous materials like turmeric powder-baking soda and rice husk ash under *in vivo* conditions.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Foliar blight caused by *Rhizoctonia solani* is a serious disease of amaranthus (*Amaranthus tricolor* L.) in Kerala (Kamala *et al.*, 1996). Gokulapalan *et al.* (2000) reported that the symptoms of the disease were manifested as small irregular whitish cream spots on leaves which enlarged under high humidity to cause extensive translucent and light green lesions and shot-hole symptoms. Aerial blights caused by *R. solani* have been reported on other vegetables such as radish, cabbage spinach and sugarbeet (Baker,1970; Galindo *et al.*, 1983; Jana *et al.*, 1990).

2.1 PATHOGEN

The perfect stage of *R. solani* was reported to be *Thanatephorus cucumeris* (Frank) Donk (Talbot. 1970). This stage was first reported from India in rice by Saksena and Chaubey (1972). In amaranthus, *T. cucumeris* observed as white powdery mass on underside of leaves was responsible for aerial spread and secondary infection (Gokulapalan *et al.*, 2000). Duggar (1915) observed that young hyphal branches of *R. solani* were inclined to the direction of growth and constricted at the point of union of main hyphae. Branching of young hyphae at right angles, later bending towards the direction of growth was observed by Palo (1926). Parmeter and Whitney (1970) stipulated that isolates of *R. solani* possessed the following characters: some shade of brown hyphal pigmentation. branching near distal septum of cells in young hyphae, constriction of hyphae and formation of septa a short distance from the point of origin of hyphal branches, dolipore septum and multinucleate cells in young hyphae.

2.2 BIOLOGICAL CONTROL OF *R. solani*

Although chemical control of the disease through the use of fungicides was found to reduce the severity of the disease (Jana *et al.*, 1990; Gokulapalan *et al.*, 1999) continuous application of chemicals cause long term damage to the

environment. In 1987, large quantities of leafy vegetables exported from Malaysia to Singapore were rejected due to high levels of ethylene bis dithiocarbamate residues in the produce as a result of continuous application of mancozeb (Mah *et al.*, 1988). Biological control has been reported to provide protection against many foliar diseases in crop plants (Blakeman and Fokkema, 1982). Several fungal and bacterial antagonists like *Trichoderma* spp, *Bacillus subtilis* and *Pseudomonas fluorescens* have been found to be effective in checking diseases caused by *R. solani* on crops such as rice, pea and cotton (Elad *et al.*, 1982). In the recent years, direct application of antagonistic micro organisms to control foliar and root infecting pathogens has gained momentum (Whipps, 1992).

2.2.1 Biocontrol by *Trichoderma* spp.

The most exhaustively researched microorganism as a biocontrol agent is *Trichoderma* spp. Weindling (1932) first demonstrated that *T. viride* was parasitic on and antagonistic to *R. solani*. Hadar *et al.* (1979) observed that *T. harzianum* could directly attack *R. solani* and that a wheat bran culture of the fungus could control damping off of bean, tomato and eggplant seedlings caused by *R. solani*. Wu (1980) used *T.pseudokoningii* Rifai and *T. harzianum* for seed treatment of soybean to control pre emergence damping off caused by *R. solani*. Harman *et al.* (1980) found that *T.hamatum* reduced *R. solani* incited seedling disease of radish and pea under field conditions. Elad *et al.* (1981a) used *T. harzianum* for controlling *R. solani* causing black root rot of strawberries under field conditions. Disease severity in *Trichoderma* treated plants was reduced by 18-46 per cent. Biocontrol of *R.solani* affecting carnations was achieved by using *T. harzianum* (Elad *et al.*, 1981b). Chet and Elad (1982) successfully controlled the soil and bulb borne pathogens of Iris viz., *R. solani* and *Sclerotium rolfsii*, using *T. harzianum* along with soil solarisation. Marshall (1982) reported that there was reduction in incidence of damping off of snap beans caused by *R. solani* by seed treatment with *T. harzianum*. Venkatasubbiah *et al.* (1984) found that *T. harzianum* was an effective biocontrol agent for *R. solani*, the incitant of collar rot of coffee

seedlings. Gokulapalan and Nair (1984) reported that *T. harzianum*, *T. viride*, *Aspergillus niger* and *A. flavus* exhibited inhibitory action on *R. solani* infecting rice. Lifshitz *et al.* (1985) reported decrease in incidence of pre emergence damping off of radish caused by *R. solani* by using *T. harzianum*. Lewis and Papavizas (1985) reported the inhibitory effect of mycelial preparation of *Trichoderma* and *Gliocladium* on the population of *R. solani* and incidence of damping off in sugarbeet. Padmakumary (1989) found *T. harzianum* and *T. viride* to be antagonistic to *R. solani* under *in vitro* conditions. Viswakumar (1989) reported that *T. viride* was the most efficient antagonist against *R. solani*, the causal agent of sheath blight of rice. Among the various substrates tested, application of wheat bran and rice bran cultures gave the best control of the disease.

Among several fungi tested against *R. solani*, *Trichoderma* sp, *Rhizopus oryzae* and *A. niger* were found to be superior in inhibiting the growth of the pathogen. In pot culture studies, *T. harzianum* showed least intensity of sheath blight followed by *T. koningii* (Zacharia, 1990). An isolate of *T. harzianum* was capable of controlling rice sheath blight pathogen, *R. solani* (Kumaresan and Manibhushan Rao,1991). Mukherjee and Mukhopadhyay (1995) suggested mycoparasitism as one of the important mechanisms through which *T. virens* suppressed *R. solani*.

Out of the seven biocontrol agents tested against *R. solani* causing root rot of French bean, *T. virens* and *T. harzianum* reduced the disease to 6.7 and 13.30 per cent as compared to 36.70 per cent in control (Abraham Mathew and Gupta, 1998). Smitha (2000) reported that among the fungal antagonists isolated from rhizosphere, *T. longibrachiatum* was found to be superior in inhibiting *R. solani*, the causal agent of foliar blight of amaranthus. An appreciable reduction in disease intensity was obtained when talc based formulation of *T. longibrachiatum* was given as soil application followed by foliar spray under field conditions. Prasad and Rangeshwaran (2000) found that a wheat bran kaolin formulation of *T. harzianum* controlled seed rot and damping off of chickpea caused by *R. solani*

up to 85.00 per cent resulting in high plant stand. Dubey (2000) reported that among the biocontrol agents, *T. viride*, *T. harzianum* and *T. virens* evaluated for their antagonism against *R. solani in vitro*, *T. virens* was found to produce maximum growth inhibition (59.80 per cent) and sclerotial production (70.00 per cent). Rama *et al.* (2000) tested the efficacy of different isolates of *Trichoderma viz.*, *T. harzianum*, *T. viride*, *T. reeisei* and *T. koningii* against *R. solani* causing black scurf of potato and sheath blight of rice in field. *T. viride* gave maximum inhibition of both diseases and the combinations of wheat straw, wheat bran and used tea leaves with two per cent molasses gave fast growth and maximum biomass production of *T. harzianum* and *T. viride*. Charitha Devi and Reddy (2002) reported *T. harzianum* to be the most potential antagonist among five isolates of *Trichoderma* spp, *P. fluorescens* and *Bacillus* sp against *R. solani*. causing damping off of groundnut. Bunker and Mathur (2001) reported that among the five local isolates of *Trichoderma* spp. *viz.*, *T. harzianum*, *T. viride* (two isolates), *T. aureoviride*, and *T. virens* isolated from ginger rhizosphere evaluated to assess their antagonism against chilli dry root rot pathogen. *T. harzianum* was most effective in suppressing the growth and sclerotial formation of *R. solani*.

Khan and Hussain (1991) tested the inhibitory effects of culture filtrates of nine test fungi isolated from the rhizosphere of cowpea against *R. solani*. The maximum inhibition of *R. solani* was obtained with the filtrate of *T. viride* and reduction in the mycelial weight was directly correlated with the concentration of the filtrate.

2.2.2 Biocontrol by *Pseudomonas fluorescens*

Fluorescent pseudomonads have emerged as the largest and potentially the most promising group of plant growth promoting rhizobacteria which can effectively control many soil borne plant pathogens (Cook and Rovira, 1976; Defago *et al.*, 1990; Kloepper and Schroth, 1978). This unique group can suppress many plant diseases due to their biological activities such as competition for space and nutrients, production of antibiotics, volatile and

antimicrobial substance and compounds like iron chelating siderophores and HCN (Dowling and O'Gara, 1994; Rosales *et al.*, 1995; Dave and Dube, 2000; Mondal *et al.*, 2000)

Fluorescent pseudomonads inhibit or displace *R. solani* from the soil or plant and provide protection to several annual and perennial crops such as cotton, tobacco, potato, flax, radish, cucumber, wheat and rice (Howell and Stipanovic, 1979; Kloepper and Schroth, 1981; Mew and Rosales, 1986). Accordingly, spraying of *P. fluorescens* suspension on rice plants caused reduction in the incidence and lesion size of sheath blight caused by *R. solani* (Mew and Rosales, 1986). The use of fluorescent pseudomonads for the management of *R. solani* infecting rice has been recorded (Sakthivel *et al.*, 1986; Podile *et al.*, 1990). They also reported increased growth of rice plants when fluorescent pseudomonads were applied. Efficient strains of *P. fluorescens* isolated from rice rhizosphere protected the rice varieties, IR 20 and TKM9 seedlings from infection by *R. solani* in green house tests. (Devi *et al.*, 1989) Seed treatment with *Pseudomonas* spp. reduced the incidence of *R. solani* on cotton (Qui *et al.*, 1990). Sarkar *et al.* (1992) observed that seed bacterization with *P. fluorescens* IS-241 reduced the incidence of sheath blight of rice. Ninety nine isolates of fluorescent pseudomonads from roots of beans (*Phaseolus vulgaris*) showed antagonism against *R. solani* (Wolk and Sarkar, 1993).

Seed bacterization by fluorescent pseudomonads suppressed root rot and damping off of cotton, sheath blight of rice (Laha and Verma, 1998; Sivakumar and Narayanaswami, 1998) and reduced incidence of *R. solani* in cowpea (Barbosa *et al.*, 1995). Several pathogens of tomatoes including *R. solani* were reported to be inhibited by fluorescent pseudomonads (Varshney and Chaube, 1999). Velazhahan *et al.* (1999) observed the inhibition of mycelial growth of *R.solani* by *P. fluorescens* isolated from the rhizosphere of rice plants. Smitha (2000) identified a fluorescent pseudomonad (P_1) as the best bacterial antagonist against *R. solani* causing foliar blight of amaranthus. Nandakumar *et al.* (2001) reported that two strains of *Pseudomonas fluorescens*, Pf-1 and FP-7 which

inhibited mycelial growth of *R. solani* and increased seedling vigour of rice *in vitro*, were found to induce systemic resistance against the pathogen.

The culture filtrate of a strain of *P. fluorescens*. PfALR2 completely inhibited the germination and reduced the virulence of sclerotia of *R. solani*. causing sheath blight of rice (Rabindran. 1994).

2.3 USE OF INDIGENOUS MATERIALS FOR CONTROLLING PATHOGENS

Williams and Williams (1985) reported that researchers in Japan obtained control of midew on cucumbers, eggplants and straw berries by weekly sprays of one- fourth ounce of baking soda per gallon of water. Ziv and Zitter (1992) found that a single spray of 0.50 per cent baking soda and horticultural oil almost completely inhibited powdery mildew on heavily infected pumpkin foliage. Williams and Williams (1993) found that spraying of one ounce of baking soda per gallon of water controlled powdery mildew on climbing roses. It also provided good control of Urocladium leaf spot in cucumber. Alternaria leaf blight and stem blight in musk melon.

The use of turmeric -baking soda combination for the management of soil borne diseases in rice has been reported by Gangopadhyay (1998). Turmeric-baking soda was used at a ratio of 10:1 as a seed treatment and foliar spray. Joshi (2002) reported the use of rice husk ash as an indigenous material for the management of blast of rice. The plants were more erect and resistant to infection which was attributed to increased silicification.

2.4 GROWTH PROMOTION BY BACTERIA AND FUNGI

Some plant growth promoting rhizobacteria like *P. fluorescens* and *P.aeruginosa* may promote plant growth by secreting hormones such as gibberellic acid (Katznelson and Cole, 1965; Suslow, 1982: Schippers *et al.*, 1987; Weller, 1988). Kloepper *et al.* (1980) attributed the enhancement of plant growth to yellow green siderophores produced by fluorescent pseudomonads.

Seed treatment with fluorescent pseudomonads are reported to improve growth in potato, sugarbeet and radish. (Burr *et al.*, 1978; Kloepper and Schroth.

1978; Suslow and Schroth, 1982) Kaiser *et al.* (1989) reported increased emergence and yield of chickpea in soils naturally infected with *Pythium ultimum* when fluorescent pseudomonads were applied as seed treatment. Howie and Suslow (1991) reported 70.00 per cent reduction of Pythium infection in cotton and about 50.00 per cent increase in emergence of seedlings by an antibiotic producing *P. fluorescens*. Hofte *et al.* (1991) reported that plant growth promoting strains of *P. fluorescens* ANP 15 and *P. aeruginosa* 7NSK 2 increased germination of maize seeds. Seed treatment of rice seeds with fluorescent pseudomonads reduced the intensity of sheath blight and promoted seedling growth. (Lin *et al.*, 1992) Gnanamanickam and Mew (1992) obtained slight increase in grain yield due to seed treatment with strains of *P. fluorescens*. Muthamilan (1994) observed increased growth rate of rice plants by seed treatment with *P. fluorescens*. Gupta *et al.* (1995) reported growth promotion of tomato by rhizobacteria, *P. fluorescens*. Inoculation of *P. fluorescens* increased seedling emergence rate, total dry weight and length of root and shoot. Izhar *et al.* (1999) observed that seed treatment with *P. fluorescens* enhanced the growth of cotton and reduced infection by *R. solani*, Seed treatment with Pf1 reduced infection of sheath blight and increased crop yield in field trials (Vidhyasekaran and Muthamilan, 1999). A strain of *P. fluorescens*, RRLJ 130 isolated from pigeon pea rhizosphere has enhanced the percentage of seed germination, shoot height, root length fresh and dry weight in chickpea and pigeon pea (Dileepkumar, 2002). Raji and Lekha (2003) reported that seed bacterization with talc based formulation of *P. fluorescens* enhanced the root length and height of rice plants. Application of the formulation by different methods increased the yield and reduced sheath blight severity.

Ganesan *et al.* (2000) found that application of *Trichoderma* significantly increased root dry weight in pepper cuttings when compared to untreated control. Anith and Manomohandas (2001) reported that the combined application of *T. harzianum* and *Alcaligenes* also resulted in enhanced shoot weight in pepper. Dubey (2002) reported that foliar spray of *T. virens* and *T. viride* was found to be efficient in increasing the grain yield in urd and mung beans. A significant

increase in root length and number of root nodules over control were also observed. Anandaraj *et al.* (2003) found that application of two strains of *P.fluorescens*, a strain of *Bacillus* and *T. harzianum* resulted in significantly enhanced growth of black pepper which resulted in increased number of nodes and consequently cuttings. The increase was 44.70 per cent over control for *P. fluorescens* and 38.90 per cent for treatment with a consortium of *P. fluorescens* and *T. harzianum*.

Piriformospora indica

Piriformospora indica Verma, Varma, Kost, Rexer and Franken sp. nov. is a newly described axenically cultivable plant growth promoting endo symbiont which simulates the capabilities of AMF.

Cytological analysis revealed that *P. indica* belongs to Hymenomycetes of Basidiomycota and molecular data based on 18srRNA sequence indicated that it is related to *R. solani* (Verma *et al.*, 1998). Similar to *R. solani*, *P. indica* penetrates rhizoids of protocorm of terrestrial orchids and develops morphological structures as seen in orchid mycorrhizae (Blechert, 1999). Varma *et al.* (1999) found that the fungus colonizes the cortex of plant roots and develop inter and intra cellular vesicles, hyphal coils and pear shaped chlamydospores.

Varma *et al.* (1999) observed that *P. indica* tremendously improved the growth and over all biomass production of different plants like herbaceous monocots and dicots, trees including medicinal plants like *Bacopa monnieri*, *Artemesia anua* and several economically important crops. Sahay and Varma (1999) reported that regenerated plantlets of tobacco inoculated with *P. indica* when subjected to two different biological hardening techniques showed 88-94.00 per cent survival as compared to 62.00 per cent survival of un inoculated controls under similar conditions. Rai *et al.* (2001) reported positive growth response in medicinal plants, *Spilanthus calva* and *Withania somnifera* to inoculation with *P. indica* under field condition.

Culture filtrate of the mycelium containing fungal exudates (hormones, enzymes proteins etc.) also exerted growth promotional effects in plants. Treatment with culture filtrate showed an increase in root length, shoot length and plant biomass in maize, *Bacopa monnieri* and tobacco. *P. indica* when co-inoculated with the soil borne plant pathogen, *Gaeumannomyces graminis* completely inhibited the growth of the pathogen. The culture filtrate of *P. indica* was also found inhibitory to the pathogen (Varma *et al.*, 2001).

2.5 DEVELOPMENT OF FORMULATION OF FUNGAL ANTAGONISTS

For the biological control of soil borne pathogens it is necessary to mass produce the promising antagonists rapidly in the form of spores, mycelia or mixtures (Papavizas *et al.*, 1984). To improve the efficacy of microbial antagonists several formulations of biocontrol agents have been developed, which are being produced commercially in many countries (Papavizas, 1985).

The production of biomass containing the most effective conidia, conidiophore or mycelium of *T. harzianum* by solid state fermentation using rice bran and wheat bran was reported by Mehta *et al.* (1995). Jeyarajan and Ramakrishnan (1995) developed a talc based formulation of *T. viride* for dry seed treatment of oil seeds and pulses using fermented grown biomass in molasses–yeast medium. Prasad *et al.* (1997) highlighted the superiority of PDA as a medium for biomass production of *T. harzianum* when compared to V-8 juice and molasses and brewer's yeast medium.

2.6 DEVELOPMENT OF FORMULATION OF BACTERIAL ANTAGONIST

Kloepper and Schroth (1981) observed that population of PGPR did not decline in the talc mixture with 20.00 per cent xanthan gum after storage for two months at 4°C. Capper and Higgins (1993) developed a peat based formulation of *P. fluorescens* for the control of take all of wheat. Muthamilan (1994) found that a strain of *P. fluorescens*, Pf 1 reduced sheath blight incidence when seeds were treated with peat based formulation at the rate of 10g/kg.

A talc based formulation of *P. fluorescens* was prepared by mixing 48 hour old growth of bacteria in King's broth with sterilized talc @ 400ml/kg talc along with five gram of the sticker carboxy methyl cellulose (Vidhyasekaran and Muthamilan 1995; Kamala, 1996). *Bacillus* cereus and *P. fluorescens* survived in peat or vermiculite/clay formulation for at least 150 days and effectively controlled Rhizoctonia damping off in greenhouse trials (Gasoni *et al.*, 1998). A powder formulation of the bacteria, *P. fluorescens* with shelf life of more than eight months has been developed by Vidhyasekharan and Muthamilan (1999). Smitha (2000) reported that a fluorescent pseudomonad (P_1) was found to survive well in talc even after five months of storage. There was a significant reduction in the disease intensity when talc based formulation of P_1 was given as soil application followed by foliar spray. Heera (2002) found that application of talc based formulation of a strain of *P. fluorescens*, P_{II} was effective in controlling sheath blight of rice.

2.7 BIOCHEMISTRY OF DEFENSE TO PLANT DISEASES

2.7.1 Phenol Content

Walker and Link (1935) first reported that the resistance of onion varieties to *Colletotrichum circinans* was due to accumulation of flavones, anthocyanins and simple phenolics like protocatechuic acid and catechol in dead outer scales. Tohamy *et al.* (1993) observed that the amount of free phenols increased in plants protected with biocontrol agents like *T. harzianum* and *T. longibrachiatum* challenged with *Fusarium oxysporum* f.sp *cepae* or *Sclerotium cepivorum* as compared to un inoculated control. However, the amount of total phenols tended to decrease in the above treatments.

High amount of chlorogenic acid was reported by Lee and Le Tourneau (1958) in potato roots resistant to Verticillium wilt. Similar observation was made by Patil *et al.* (1962) who showed that young potato roots resistant to Verticillium wilt had high levels of phenolics till five weeks after sprouting. Mahadevan (1966) recorded higher amounts of phenols in cotton varieties resistant to wilt disease than in susceptible ones. The accumulation of phenolic

compounds due to infection by pathogens was reported in crops like ragi (Vidhyasekaran, 1974) mung (Arora and Bajaj, 1978; Arora, 1983) tea (Borah *et al.*, 1978) and rice (Chattopadhyay and Bera, 1980). Higher quantity of phenols and its increase in resistant genotypes than that in susceptible ones were recorded due to infection of groundnut with *Cercospora arachidicola* and *Phaeoisariopsis personata* (Sindhan and Jaglan, 1988) and also with *Puccinia arachidis* (Reddy and Ravindranath, 1988) Beckman (2002) related the physiological aspect of disease resistance and phenol as due to rapid oxidation of phenolic compounds which resulted in lignification and suberization of cells and cell death that sealed off further infection at the site of cellular penetration by the pathogen.

2.7.2 Amino Acids

In the early stages of pathogenesis of *R. solani* in mung bean, there appeared to be a drastic increase in the amount of amino acids (Wu, 1973). Mohan (1976) found a reduction in aromatic amino acids in a moderately resistant variety of rice infected with *Acrocylindrium oryzae*. Vijayakumar and Rao (1980) found that the quantity of free amino acids increased in both resistant and susceptible variety of wheat as a result of infection by *Alternaria tritici*. Sundaram (1980) reported that amino nitrogen content in rice plants decreased due to *R. solani* inoculation and potassium application. Girija (1993) reported that the content of amino acid showed a progressive decrease with increase in lesion development in rice plants inoculated with *R. solani*.

2.7.3 Carbohydrates

Sridhar (1970) reported higher amount of reducing and non reducing sugars in blast susceptible varieties. Wu (1972) noticed a drastic increase of reducing sugars in mung bean seedlings as a response of infection to *R. solani*. He suggested that this resulted due to inhibition of aldolase and glucose-6 phosphate dehydrogenase activities accompanied by enhanced activities of phosphorylase and amylase.

Dhanapal (1975) reported an increase in reducing and non reducing sugars immediately after inoculation with *Helminthosporium oryzae*. Sundaram (1980)

reported that inoculation of *R. solani* in rice depleted the reducing sugar content and decrease in reducing sugars was more marked in inoculated than healthy plants. Padhi and Chakrabarti (1984) observed that susceptible rice cultivars on infection with *Pyricularia oryzae* had higher levels of reducing sugars but lower level of non reducing sugars than resistant ones.

2.8 DEFENSE RELATED ENZYMES

2.8.1 Phenyl alanine ammonia lyase

Dixon and Fuller (1976) got a positive correlation between increased PAL activity and biosynthesis of phaseollin, the phytoalexin of bean. Treatment with *P. fluorescens* caused increase in activities of PAL and PO isozyme in tobacco while chitinase activity was limited (Schneider and Ullrich, 1994). Meena *et al.* (1999) measured changes in the activities of Phenyl alanine ammonia lyase (PAL), chitinase and B-1,3 glucanase on rice leaves at different times after treatment with *P. fluorescens*. Spraying of *P. fluorescens* increased PAL activity one day after treatment, chitinase after three days and B-1,3 glucanase after one day. Jebakumar (2001) studied the activity of PAL in both leaf and root tissues of three pepper varieties (tolerant P24 and susceptible, Panniyoor and Subhakara) in healthy and *P. capsici* infected tissues. It was found that infection by *Phytophthora* enhanced the enzyme activity and the variety P24 expressed maximum PAL activity. Sivakumar and Sharma (2003) observed a higher activity of PAL, peroxidase, poly phenol oxidase and phenols in maize plants raised from seeds bacterized with *P. fluorescens* after inoculation with *R. solani*.

2.8.2 Peroxidase

Bonner (1950) reported that peroxidase was a key enzyme involved in disease resistance as it played an important role in the biosynthesis of lignin and oxidation of mono and diphenolic compounds and aromatic amines to highly toxic quinones in the presence of hydrogen peroxide.

Chitinase and peroxidase activities were increased in both root and leaf tissue of cucumbers grown in the presence of *T. harzianum* strain 203 (Yedidia *et al.*,

1999). Howell *et al.* (2000) studied the biochemistry of biocontrol of damping off of cotton by *T. virens* and observed an increase in host resistance due to enhanced peroxidase activity and terpenoid synthesis.

The level of peroxidase activity in potato tubers before infection was positively correlated with resistance to *Phytophthora infestans* (Fehrman and Dimond, 1967). Kasuge (1969) observed that peroxidase activity was frequently increased in plants infected by pathogens which was correlated with disease resistance. Hammerschmidt *et al.* (1982) found enhanced peroxidase activity to be associated with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Wasfy *et al.* (1984) reported that the number and concentration of peroxidase isoenzymes in bean hypocotyls increased in response to fungal infection by *R. solani.* Arora and Bajaj (1985) observed a fluctuating activity of peroxidase in the hypocotyls of mung bean inoculated with *R. solani.* Shamina and Sarma (2000) reported that the activity of peroxidase, polyphenol oxidase and B-1.3 glucanase showed an earlier and greater increase in the roots and leaves of resistant selection of pepper than the susceptible one due to root infection caused by *Phytophthora capsici*.

2.8.3 Polyphenol oxidase

The role of polyphenol oxidase, a copper containing enzyme which oxidized phenolics to highly toxic quinones in plant disease resistance was reported by Umaerus (1959), Kasuge (1969) Yamamoto *et al.* (1978). Maxwell and Bateman (1967) reported that polyphenol activity in tissues adjacent to infection loci in bean hypocotyls infected by *R. solani* increased when compared to the uninoculated. Increased polyphenol activity in rice tissues following infection with *Helminthosporium oryzae* has been reported by Oku (1967), Mukherjee and Ghosh (1975) Chattopadhyay and Sammadar (1980). Rao *et al.* (1988) explained that the increase in polyphenol oxidase activity was due to activation of latent host enzyme. Avdiushko *et al.* (1993) also obtained enhanced PPO activity in cucumber leaves in the vicinity of lesions caused either by *Colletotrichum lagenarium* or Tobacco Necrosis Virus.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation on "Ecofriendly management of Rhizoctonia leaf blight of amaranthus" was carried out at College of Agriculture, Vellayani during the year 2002-2003. Twenty five day old amaranthus seedlings of var. Arun were used in the study.

3.1 ISOLATION OF THE PATHOGEN

Rhizoctonia solani, causing foliar blight of amaranthus was isolated from the infected amaranthus plants collected from ten different locations in Thiruvananthapuram district. For isolation of the pathogen, leaves showing typical leaf blight symptoms were cut into small bits along with some healthy portions. The bits were then surface sterilized with 0.10 per cent mercuric chloride and then washed in two changes of sterile water. The bits were then placed on Potato Dextrose Agar (PDA) (Appendix IA) in sterile petri dishes and incubated at room temperature. The fungal growth was noticed 24 h after inoculation. The growth was purified by hyphal tip method and transferred to PDA slants. The different isolates were then maintained by periodic subculturing on PDA.

3.2 IDENTIFICATION OF THE PATHOGEN

The identification of *R. solani* was done by observing the characters of mycelium, sclerotium, basidium and basidiospores.

3.3 PATHOGENICITY TEST

Koch's postulates were proved for confirming the pathogenicity of the different isolates of *R. solani*. For this, 25 day old amaranthus seedlings were raised in pots and the seedlings were mildly injured by giving pin pricks on the leaves. The leaves were then inoculated on the lower surface with seven day old fungal mycelia of the different isolates

grown on PDA. A thin layer of moistened cotton was placed over it in order to provide humidity. Re-isolation of the pathogen was done from the leaves showing the typical blight symptoms and the identity of the pathogen was established. The isolates were graded based on the time taken for symptom development, size of the lesions they produced and disease intensity. From among the ten isolates, the isolate which produced maximum disease symptoms was used for further studies.

3.4 *IN VITRO* STUDIES ON PATHOGEN SUPPRESSION

3.4.1 Fungal Antagonists

3.4.1.1 Trichoderma spp.

Four fungal antagonists- *Trichoderma harzianum, T. pseudokoningii, T.piluliferum* an *T. virens* used in the study were obtained from Indian Agricultural Research Institute. New Delhi. To test the effect of these antagonists on *R. solani*, modified dual plate method by Skidmore and Dickinson (1976) was followed. Agar blocks of five mm diameter containing seven day old mycelial growth of *R. solani* and the fungal antagonists were placed five cm apart on PDA in a petri dish and incubated at room temperature. Three replications were maintained. Plates inoculated with *R.solani* alone served as control. The nature of reaction of the fungal antagonists on *R. solani* was grouped as :

A: Homogenous- Free intermingling between pairing organisms

B: Overgrowth- The pathogen overgrown by the test organism

C: Cessation- Cessation of growth at the line of contact of cultures

D: Aversion- A clear zone of inhibition between two organisms.

Inhibition of mycelial growth of the pathogen by each antagonist was studied using the formula :

$$I = \frac{100\,(C-T)}{C}$$

I - inhibition of mycelial growth of the pathogen

C- radial growth of pathogen in control plates (cm)

T - radial growth of pathogen in treated plates (cm)

(Vincent, 1927)

3.4.1.2 Piriformospora indica

The culture of *P. indica* was obtained from the Department of Life Sciences, Jawaharlal Nehru University, New Delhi .To test the effect of *P. indica* on *R. solani*. modified dual plate method devised by Skidmore and Dickinson (1976) as described under 3.4.1.1 was followed.

3.4.2 Bacterial Antagonists

Four proven native isolates of *P. fluorescens* with high biocontrol efficiency *viz*., P_1, P_5, B_3 and KK_{16} maintained in the Department of Plant Pathology. College of Agriculture, Vellayani were tested for their antagonism against *R. solani*.

Molten PDA was poured into petri dishes. Five mm discs of seven day old mycelial growth of *R. solani* were cut out with a cork borer. A triangle was marked on the bottom of the petri dish leaving two cm on each side from the centre of the dish. The mycelial discs of the pathogen were placed at the center of the triangle and isolates of *P. fluorescens* grown on King's B medium (Appendix IB) were then streaked on the sides of the triangle, length of the streak being 4.5 cm. Three replications were maintained for each isolate. Plates containing the pathogen alone served as control. The plates were incubated at room temperature and observations on the mycelial growth of *R. solani* was taken for a period of seven days.

3.5 EXTRACTION OF CULTURE FILTRATES OF THE ANTAGONISTS

To test the effect of culture filtrates of the four selected isolates of *P. fluorescens* on *R. solani*, single colonies of these bacterial isolates were inoculated into 100 ml King's broth taken in 250 ml conical flasks and incubated for seven days. The cells were then separated from the broth by centrifugation at 10,000 rpm for 15 min. The supernatant was collected and passed through bacteriological filter (0.2 µm Fisher brand. Cat. No. 09-719 C) and this was used for the studies.

To prepare the culture filtrate of *T. harzianum*, the fungus was grown in potato dextrose broth for 15 days. The mycelial mats of the fungus were then removed from the broth and it was centrifuged at 10,000 rpm for 15 min. The supernatant was then passed through bacteriological filter.

The filtrate of *P. indica* was prepared as was done for *T. harzianum*. However, the filtrate of *P. indica* was not centrifuged.

Sterile PDA was melted and poured into sterile petri dishes. Ten mm diameter wells were made at the centre of PDA plates by removing agar disc from the medium using a cork borer. The wells were then partially sealed with soft agar (0.30 per cent). After solidification of the agar, 100 µl of the filtrates of four isolates of *P. fluorescens* and isolate of *T. harzianum* and *P. indica* were put into the wells and allowed to percolate. Bioassay against *R. solani* was performed by co- inoculating agar discs of *R. solani* at a distance of two cm from the well at three points surrounding the well. Growth of *R. solani* was observed after incubation for two days. Control plates containing molten potato dextrose broth in the wells were also maintained.

3.6 INDIGENOUS MATERIALS

3.6.1 Turmeric powder-baking soda

Three different ratios of turmeric powder- baking soda, as per the quantities mentioned below, were added to 200ml Potato dextrose agar

taken in 250 ml conical flasks before autoclaving. After sterilization the media containing the different concentrations of turmeric powder - baking soda were poured into sterile petri dishes. Five mm discs were cut out from the edges of seven day old mycelial growth of *R. solani* with a cork borer of 0.5 cm diameter. The discs were then placed in the centre of each dish. For each combination, three replications were maintained. Plates containing PDA with *R. solani* inoculated at the centre served as control. Observations on the mycelial growth of the pathogen was taken for a period of seven days.

Ratios of turmeric powder-baking soda	Quantity of turmeric powder in 200ml PDA	Quantity of baking soda in 200ml PDA
6:4	0.48g	0.32g
8:2	0.64g	0.16g
10:1	0.727g	0.073g

3. 6.2 Rice husk ash

The effect of three different concentrations of rice husk ash (RHA) *viz*., 0.50, 0.75 and 1.00 per cent on *R. solani* were tested by growing the fungus on PDA incorporated with RHA. The experiment was conducted as described under 3.6.1

3.7 *IN VIVO* STUDIES ON PATHOGEN SUPPRESSION AND PLANT GROWTH PROMOTION

3.7.1 Effect of biocontrol agents and indigenous materials on plant growth and disease incidence

3.7.1.1 Treatments

T_0 - Untreated control

T_1 - Soil application of RHA (500g/m^2)

T_2 - Soil application of RHA ($750g/m^2$)

T_3 - Soil application of RHA ($1000g/m^2$)

T_4 - Soil application of talc based formulation of *T. harzianum* (20g/kg soil) + 1.00 per cent foliar spray

T_5 - Soil application of talc based formulation of bacterial isolate P_1 (20g/kg soil) + 2.00 per cent foliar spray

T_6 - Soil application of bacterial isolate P_5 (20g/kg soil) +2.00 per cent foliar spray

T_7 - Soil application of bacterial isolate B_3 (20g/kg soil) +2.00 per cent foliar spray

T_8 - Soil application of bacterial isolate KK_{16} (20g/kg soil) +2.00 per cent foliar spray

T_9 - Foliar spray of mancozeb 0.40 per cent in cowdung supernatant

T_{10} - Foliar spray of turmeric powder -baking soda 6:4 (4g/l)

T_{11} - Foliar spray of turmeric powder- baking soda 8:2 (4g/l)

T_{12} - Foliar spray of turmeric powder- baking soda 10:1(4g/l)

T_{13} - Soil application of *P. indica* (0.50 per cent w/v)

T_{14} - Soil application of *P. indica* (1.00 per cent w/v)

T_{15} - Soil application of *P. indica* (2.00 per cent w/v)

The details of the experiment are as follows

Design- CRD

Replications –4

Variety- Arun

3.7.1.2 Preparation of Pathogen Inoculum

Seven day old mycelial growth of *R. solani* grown in petri dishes was used for inoculation. In all pots including control, the pathogen was inoculated five days after transplanting amaranthus seedlings.

3.7.1.3 Preparation of Talc Based Formulation of Fungal Antagonist

The biomass of fungal antagonist was prepared by a modified liquid fermentation process of Papavizas *et al.* (1984). Five mm diameter disc of *T. harzianum* was inoculated into 500 ml of potato dextrose broth in one litre flasks and incubated at room temperature for 15 days. The mycelial mat was then separated from the broth by filtering through muslin cloth. Fungal mats were then pressed, air dried and powdered. The resulting powder was then mixed with sterilized talc in polypropylene bags @ 10 per cent w/w to which one per cent carboxy methyl cellulose was added.

3.7.1.4 Preparation of Talc based Formulations of Bacterial Antagonists

Talc based formulations of selected bacterial isolates were prepared following the method of Vidhyasekaran and Muthamilan (1995). Four isolates of *P. fluorescens* were multiplied in King's broth. A loopful of each isolate was inoculated into the broth and incubated for 48 h at room temperature. Hundred grams of talc was taken in polypropylene bags. One gram of carboxy methyl cellulose was added to this, sealed and autoclaved for two hours at 1.05 kg cm^{-2}. To this, 40 ml of 48 h grown inoculum was added, mixed thoroughly and then stored at room temperature.

3.7. 1.5 Mass Multiplication **of *P. indica***

P. indica was multiplied in potato dextrose broth. After two weeks of incubation at room temperature, the mycelial mats of the fungus were separated from the aqueous broth by passing through Sartorius filter paper grade 292. The separated mycelial mats were used for inoculation.

3.7.1.6 Application of Indigenous Materials and Biocontrol Agents

Twenty day old seedlings of amaranthus of variety Arun were transplanted in 20 cm diameter pots after mixing different quantities of rice husk ash *i.e.*, 15.7g, 23.55g and 31.4g respectively for 500g/m^2, 750g/m^2 and 1000g/m^2 with topsoil.

Three ratios of turmeric powder-baking soda (6:4, 8:2 and 10:1) were prepared by dissolving them in water and this was sprayed two weeks after transplanting amaranthus seedlings.

Talc based formulation of the fungal and bacterial antagonists were applied to soil at the rate of 20g per kg soil in pots of diameter 20 cm. The formulations were thoroughly mixed with soil before transplanting amaranthus seedlings

One percent aqueous suspension of the formulated product of the fungal antagonist was sprayed two weeks after its soil application. For the bacterial antagonists, two percent aqueous suspension of the formulations were sprayed after two weeks of their soil application.

The mycelium of *P. indica* mixed with a small amount of sterile soil was placed as a layer at the root zone. Above this, one layer of soil was added to keep it in position. The inoculum was added at 0.50, 1.00 and 2.00 per cent (w/v) of the total soil content in the pot. The seedlings of Arun were then planted in such a way that their roots came in constant touch with the inoculum (Sahay and Varma, 1999).

3.7.1.7 Observations

Observations of amaranthus plants were taken 45 days after transplanting

3.7.1.7.1 Shoot Length (cm)

The length of the shoot from the ground level to the growing tip of each plant was measured after depotting.

3.7.1.7.2 Root Length (cm)

The length of the longest root of each plant was measured after depotting.

3.7.1.7.3 Number of Leaves

The number of leaves in each plant was counted.

3.7.1.7.4 Leaf Area (cm^2)

Leaf area was calculated by plotting the leaves on a graph paper and counting the number of unit squares.

3.7.1.7.5 Fresh Weight of Shoot and Root (g)

Fresh weight was taken in an electronic balance immediately after depotting.

3.7.1.7.6 Dry Weight of Shoot and Root (g)

Dry weight was taken after drying the samples to a constant weight in a drying oven at 60°C.

3.7.1.7.7 Disease Incidence (%)

Disease incidence was calculated as

$$\frac{\text{Number of plants diseased}}{\text{Total number of plants assessed}} \times 100$$

3.7.1.7.8 Disease Intensity

Disease severity was graded using a 0-9 scale (KAU, 1996) (Plate1).

Grade	Description
0	No infection
1	1-10 per cent of leaf area infected
3	11-25 per cent of leaf area infected
5	26-50 per cent of leaf area infected
7	51-75 per cent of leaf area infected
9	>76 per cent of leaf area infected

Plate 1   0-9 scale for the scoring of foliar blight of amaranthus

Plate 1

Percentage disease index was calculated using the formula:

$$PDI = \frac{\text{Sum of grades of each leaf}}{\text{Number of leaves assessed}} \times \frac{100}{\text{Maximum grade used}}$$

(Mayee and Datar, 1986)

3.7.1.7.9 Root Colonization and Characterization of *P. indica*

The plants treated with *P. indica* were uprooted 45 days after transplanting and the roots were examined for the extend of colonization following the staining procedure of Phillip and Hayman (1970). After uprooting, the roots were washed in tap water to remove soil and was then cut into one cm bits and transferred into test tubes containing 10 per cent potassium hydroxide solution for clearing. This was kept at room temperature for 24 h. After clearing the root bits were washed with two per cent hydrochloric acid to neutralize the alkali present in it. The root bits were stained by boiling it in 0.05 per cent trypan blue solution (Appendix II A) for 1-5 min and destained by keeping in fresh lactophenol (Appendix II B) overnight at room temperature.

The root bits were then arranged side by side on a glass slide, pressed gently with another slide so that the mycelium became visible. Root bits with blue stained mycelium and chlamydospores were scored positive.

Percentage root colonization was calculated using the formula :

$$\text{Percentage of } P.\ indica \text{ infection} = \frac{\text{Number of root bits scored positive}}{\text{Total number of root bits}} \times 100$$

The mycelium from ten day old culture of *P. indica* was transferred to lactophenol cotton blue and microscopic mounts were prepared and observed to study the morphological characters of the fungus.

3.7.2 Management of Rhizoctonia Leaf Blight Using Biocontrol Agents and Indigenous Materials

The treatments in 3.7.1.1 that showed potential for disease control and plant growth promotion were further assayed on another pot trial.

3.7.2.1 Treatments

T_0 - Untreated control

T_3 - Soil application of RHA (1000 g/m^2)

T_4 - Soil application of *T. harzianum* (20 g/kg of soil) + 1.00 per cent foliar spray

T_7 - Soil application of bacterial isolate B_3 (20 g/kg of soil) + 2.00 per cent foliar spray

T_8 - Soil application of bacterial isolate KK_{16} (20 g/kg of soil) + 2.00 per cent foliar spray

T_9 - Foliar spray of mancozeb (0.40 per cent) in cowdung supernatant

T_{12} - Foliar spray of turmeric powder- baking soda 10:1 (4g/l)

T_{15} - Soil application of *P. indica* (2.00 per cent w/v)

Design- CRD

Replications-4

Variety- Arun

The inoculation of pathogen, application of formulation of the antagonists, foliar spray of turmeric powder- baking soda and soil application of *P. indica* were similar to that described under 3.7.1.2 and 3.7.1.6

3.7.2.2 Observations

3.7.2.2.1 Shoot Length (cm)

The length of the shoot from the ground level to the growing tip of each plant was measured after depotting.

3.7.2.2.2 Root Length (cm)

The length of the longest root of each plant was measured after depotting.

3.7.2.2.3 Number of Leaves

The number of leaves in each plant was counted.

3.7.2.2.4 Leaf Area (cm^2)

Leaf area was calculated by plotting the leaves on a graph paper and counting the number of unit squares.

3.7.2.2.5 Fresh Weight of Shoot and Root (g)

Fresh weight was taken in an electronic balance immediately after depotting.

3.7.2.2.6 Dry Weight of Shoot and Root (g)

Dry weight was taken after drying the samples to a constant weight in a drying oven at 60°C

3.7.2.2.7 Disease Incidence (%)

Disease incidence was calculated as

$$\frac{\text{Number of plants diseased}}{\text{Total number of plants assessed}} \times 100$$

3.7.2.2.8 Disease Intensity

Disease severity was graded using a 0-9 scale (KAU, 1996) (Plate1).

Grade	Description
0	No infection
1	1-10 per cent of leaf area infected
3	11-25 per cent of leaf area infected
5	26-50 per cent of leaf area infected
7	51-75 per cent of leaf area infected
9	>76 per cent of leaf area infected

Percentage disease index was calculated using the formula :

$$\text{PDI} = \frac{\text{Sum of grades of each leaf}}{\text{Number of leaves assessed}} \times \frac{100}{\text{Maximum grade used}}$$

(Mayee and Datar, 1986)

3.7.2.2.9 Root Colonization and Characterisation of *P.indica*

The staining procedure using trypan blue used to estimate the extend of root colonization was studied as described under 3.7.1.7.9. Percentage root colonization was calculated using the formula

$$\text{Percentage of } P.\ indica \text{ infection} = \frac{\text{Number of root bits scored positive}}{\text{Total number of root bits}} \times 100$$

3.7.2.2.10 Biochemical Studies

Leaf samples of the different treatments were collected for estimating changes in activity of phenols, amino acids, carbohydrates and defense related enzymes such as phenyl alanine ammonia lyase, peroxidase and poly phenol oxidase. Where ever the treatments were incorporated in soil. leaf samples were taken ten days after application while in treatments involving foliar spray the samples were taken one week after their application.

3.7.2.2 10.1 Total Phenols

Total phenol content was estimated following the procedure described by Bray and Thorpe (1954).

One gram of leaf sample was ground in 10 ml of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min, supernatant was saved and residue was extracted with five times the volume of 80 per cent ethanol and centrifuged. The supernatant was evaporated to dryness and the residue was dissolved in a known volume of distilled water (five ml). An aliquot of 0.3ml was pipetted out and made up to three ml with distilled water. Folin–Ciocalteau reagent (0.5 µl) was added and 2.0 µl of 20 per cent sodium carbonate solution was added to each tube after three min. This was mixed thoroughly and kept in boiling water for one min. This was cooled and absorbance was measured at 650 nm against reagent blank. Standard curve was prepared using different concentrations of catechol and expressed in catechol equivalents as microgram per gram leaf tissue on fresh weight basis.

3.7.2.2.10.2 Total Free Amino acids

Total free amino acids was estimated by Spectrophotometry (Sadasivam and Manickam, 1992).

Five hundred milligram leaf material was ground in a pestle and mortar with a small quantity of acid washed sand. To this homogenate five ml of 80 per cent ethanol was added and centrifuged. The supernatant was saved and re- extracted twice and supernatant was pooled. To 0.1 ml of this extract, one ml of ninhydrin solution was added and the volume made up to 2.0 ml with distilled water. The tubes were kept in boiling water bath for 20 min. Five ml of the diluent was added and contents were mixed well. The intensity of purple colour was read after 15 min against reagent blank in a colorimeter at 570nm. The reagent blank was prepared as above by taking 0.1ml of 80 per cent ethanol instead of the extract.

For preparing the standards, 50mg leucine was dissolved in 50ml distilled water in a volumetric flask. Ten ml of this was diluted to 100ml in another volumetric flask. A series of volume from 0.1-1 ml of this standard solution were taken and proceeded as that of sample and the intensity of color was read. Using the standard curve the concentration of total free amino acids in the sample were found out and expressed as percentage equivalent of leucine

3.7.2.2.10.3 Carbohydrates

Total carbohydrates was estimated by Anthrone method (Hedge and Hofreiter, 1962.)

Hundred milligram sample was taken in boiling tubes and hydrolysed by keeping it in boiling water bath for three hours with 5.0 ml of 2.5 N hydrochloric acid and cooled to room temperature. Sodium carbonate was added to neutralize it until the effervescence ceased and the volume made up to 100 ml and centrifuged. 0.5ml aliquot of supernatant was taken for analysis. The standards were prepared by taking 0, 0.2 0. 4, 0.6, 0 .8 and 1.0 ml of working standards from which the standard zero served as blank. The volume was made up to one ml in all tubes including sample tubes by adding distilled water. Four ml anthrone reagent was then added and heated for eight min in a boiling water bath, cooled rapidly and read at 630nm. Standard graph was prepared and from the graph the amount of carbohydrate was calculated.

3.7.2.2.10.4 Phenyl alanine ammonia lyase (PAL)

PAL activity was analysed using the procedure described by Dickerson *et al.* (1984).

The enzyme extract was prepared by homogenizing one g leaf sample in five ml of 0.1M sodium borate buffer (pH 8.7) (Appendix II B) containing 0.05g of poly vinyl pyrrolidone using chilled pestle and mortar. The homogenate was centrifuged at 10,000rpm for 20 min at 4°C and the

supernatant was used for the assay. The reaction mixture contained 3 ml of 0.1M sodium borate buffer, 0.2ml enzyme extract and 0.1ml of 12mM L-phenyl alanine prepared in the same buffer. The blank contained three ml of 0.1M sodium borate buffer and 0.2 ml enzyme extract. The reaction mixture and blank were incubated at 40°C for 30 min and reaction was stopped by adding 0 .2ml of 3N hydrochloric acid. The absorbance was read at 290 nm in a specrophotometer.

PAL activity was expressed as microgram of cinnamic acid produced per minute per gram on fresh weight basis.

3.7.2.2.10.5 Peroxidase (PO)

Peroxidase activity was determined according to procedure described by Srivastava (1987.

Leaf samples of 200mg was homogenized in 1.0 ml of 0.1M sodium phosphate buffer (pH 6.5)(Appendix II A) to which 0.05g of polyvinyl pyrrolidone was added. Homogenisation was done at 4°C using pestle and mortar. The homogenate was filtered through a muslin cloth and centrifuged at 5,000rpm for 15 min at 4°C. The supernatant was used as enzyme extract. The reaction mixture consisting of one ml of 0.05M pyrogallol and 50 μl enzyme extract was taken in both reference and sample cuvettes mixed and placed in the spectrophotometer with reading adjusted to zero at 420 nm. The enzyme reaction was started by adding one ml of one per cent hydrogen peroxide into sample cuvettes and change in absorbance was measured at 30 sec interval.

3.7.2.2.10.6 Estimation of Polyphenol oxidase (PPO)

PPO was determined as per the procedure given by Mayer *et al.* (1965).

Leaf samples of 200mg was homogenized in one ml of 0.1M sodium phosphate buffer (pH 6.5) to which 0.05g poly vinyl pyrrolidone was

added. Homogenisation was done at 4°C using chilled pestle and mortar. The homogenate was filtered through a muslin cloth and centrifuged at 5,000rpm for 15 min at 4°C and the supernatant was used as enzyme extract. The reaction mixture contained one ml of 0.1 M sodium phosphate buffer and 1.0 ml of 0.01 M catechol. Cuvettes were placed in spectrophotometer and absorbance was set at zero. The reaction was started after adding 50 µl of enzyme extract. The change in absorbance was recorded at 495 nm and PPO activity expressed as change in absorbance of reaction mixture per milliliter per gram on fresh weight basis.

3.8 STATISTICAL ANALYSIS

Statistical analysis of the data was done by the method described by Snedecor and Cochran (1967). The data generated from the experiments were subjected to analysis of variance (ANOVA), after appropriate transformations wherever needed.

RESULTS

4. RESULTS

4.1 ISOLATION OF THE PATHOGEN

The pathogen causing foliar blight of amaranthus was isolated from infected amaranthus plants collected from ten different locations in Thiruvananthapuram district (Table1). After isolation the different isolates were purified and maintained on PDA slants. When inoculated into healthy host plants, symptoms of the disease began as small irregular whitish cream spots on the foliage which enlarged under high humidity. Gradually the spots became translucent with irregular brown margins (Plate 2). Severely infected leaves showed shot-hole symptoms leading to defoliation. On the undersurface of the leaves powdery coating made up of the hymenial layer of *Thanatephorus cucumeris*, the teleomorph of *R.solani* was observed (Plate 3).

4.2 IDENTIFICATION OF THE PATHOGEN

The pathogen had light brown mycelium which produced few or no sclerotia. Hyphal thickness measured 6.2-15.5 µm. The average number of sclerotia produced per plate (90mm) was 120. The length and breadth of sclerotia ranged from 153-272 µm and 148-221 µm respectively. The basidia of the teleophase, *T. cucumeris* measured 9.3-15.5 x 6.2-9.3 µm and the basidiospores measured 6.2-9.3 µ x 6.2-7.8 µ in size. The spores were borne on sterigmata measuring 3.1-7.08 µ x 2.3-3.1 µ. Three to four sterigmata were produced on each basidium. Based on these characters, the pathogen was identified as *Rhizoctonia solani* following microscopic examination of the morphological characters (Parmeter and Whitney, 1970).

The different isolates of *R. solani* varied in their growth rate and time of sclerotial formation when grown on PDA (Table 2). The average

Table 1 *R. solani* isolates from different locations in Thiruvananthapuram district

Isolates of *R. solani*	Locations
R_1	Pappanchani
R_2	Kalliyoor
R_3	Vellayani
R_4	Palappoor
R_5	Pallichal
R_6	Vandithadom
R_7	Pothencode
R_8	Nellivila
R_9	Nedumangad
R_{10}	Parassala

Table 2 Variation in the growth and sclerotial formation of *R. solani* isolates

Isolate of *R. solani*	Average radial growth per day (mm)	Time of sclerotia formation (Days after inoculation)
R_1	9.00	8
R_2	11.25	6
R_3	11.25	6
R_4	7.50	9
R_5	7.50	9
R_6	9.00	8
R_7	9.00	8
R_8	6.45	10
R_9	6.45	10
R_{10}	6.45	10

Plate 2 Symptoms of foliar blight disease on amaranthus leaves

Plate 3 Hymenial layer of *Thanatephorus cucumeris* on the undersurface of the leaf

Plate 2

Plate 3

growth rate per day ranged from 6.45 –11.25 mm. The isolates R_2 and R_3 recorded the highest growth rate (>10mm/day). The least growth rate was observed for isolates R_8, R_9 and R_{10} (<7mm/day). All the other isolates showed growth rate between 7-10 mm/day. The isolates R_2 and R_3 produced sclerotia six days after inoculation whereas the isolates R_8, R_9 and R_{10} took ten days for sclerotial formation.

4.3 PATHOGENICITY TEST

Pathogenicity of the different isolates of *R. solani* was proved following Koch's postulates. The different isolates varied with respect to the time taken for symptom development, size of the lesions and intensity of disease (Table 3). The isolate R_3 produced lesions of five mm three days after inoculation. The isolates R_2 and R_4 produced lesions of size three mm and took four days for the development of symptoms whereas isolate R_9 produced lesions of only two mm size within the same period. Isolates R_1, R_6 and R_8 took five days for the development of symptoms and produced lesions of size two mm. The remaining isolates R_5, R_7 and R_{10} took six days for symptom development and produced the smallest lesions of one mm. The isolate R_3 also recorded the highest disease intensity of 2.77 where as the isolate R_5 produced the least intensity of disease (0.72). From among the ten isolates, isolate R_3 (Vellayani) was selected for further studies since it incited the development of lesions three days after inoculation and produced the highest intensity of disease with maximum lesion size of five mm.

4.4 *IN VITRO* STUDIES ON PATHOGEN SUPPRESSION

4.4.1 Fungal Antagonists

4.4.1.1 Trichoderma spp.

Four isolates of *Trichoderma*- *T. harzianum*, *T. pseudokoningii*, *T. piluliferum* and *T. virens* were tested for their antagonism against

R. solani. The percentage inhibition of these isolates on the mycelial growth of *R. solani* was found out (Table 4). Among the four isolates, *T. harzianum* showed the maximum inhibition of 47.41 per cent followed by *T. virens* (45.18 per cent). Regarding the nature of reaction, three isolates – *T. virens* (Plate 4), *T. piluliferum* (Plate 5) and *T. pseudokoningii* (Plate 6) were found to overgrow the pathogen when they were subjected to dual culture. *T. harzianum* which emerged as the most potential antagonist initially caused a clear zone of inhibition between the paired cultures and later was found to over grow the pathogen (Plate 7). All the isolates completely overgrew the pathogen after seven days (Table 5).

4.4.1.2 Piriformospora indica

P. indica did not cause significant inhibition on the growth of *R. solani*. Initially it caused 5.92 per cent inhibition of the pathogen under *in vitro* conditions (Table 4 and Plate 8). How ever, the pathogen was found to overgrow the fungus after four days.

4.4.2 Bacterial Antagonists

Four proven isolates of *P. fluorescens* – P_1, P_5, B_3 and KK_{16} were tested for their antagonism against *R. solani*. All the bacterial isolates exerted significant inhibition on *R. solani* (Plates 9, 10, 11, 12). The maximum inhibition was shown by the isolate B_3 (85.55 per cent) followed by KK_{16} (84.07 per cent). The effect of B_3 was significantly superior to that of isolate P_5 (80.37 per cent) and isolate P_1 (81.85 per cent) (Table 6).

4.5 EXTRACTION OF CULTURE FILTRATES OF ANTAGONISTS

The culture filtrate of an isolate of *P. fluorescens viz.*, B_3 alone produced inhibitory effects on the growth of the pathogen (Plate 13). The culture filtrates of the other isolates of *P. fluorescens*, *T. harzianum* and *P. indica* did not exert any inhibitory effect on the mycelial growth of

Table 3 Lesion development and disease intensity on amaranthus caused by *R. solani* isolates

Isolate of *R. solani*	Time of development of symptoms (Days after inoculation)	Size of the lesions after 8 days (mm)	Disease intensity
R_1	5	2	1.68
R_2	4	3	2.05
R_3	3	5	2.77
R_4	4	3	1.98
R_5	6	1	0.72
R_6	5	2	1.56
R_7	6	1	0.83
R_8	5	2	1.59
R_9	4	2	1.42
R_{10}	6	1	0.89

Table 4 Effect of *Trichoderma* spp. and *Piriformospora indica* on the mycelial growth of *R. solani*

Treatments	*Percentage inhibition
T. harzianum	47.41
T. pseudokoningi	44.44
T. piluliferum	42.96
T. virens	45.18
Piriformospora indica	5.92
CD (0.05)	3.78

*Mean of three replications

Plate 4 ***In vitro*** **inhibition of** ***Rhizoctonia solani*** **by** ***Trichoderma virens***

T_2 : ***Trichoderma virens***

Plate 5 ***In vitro*** **inhibition of** ***R. solani*** **by** ***T. piluliferum***

T_4 : ***T. piluliferum***

Plate 6 ***In vitro*** **inhibition of** ***R. solani*** **by** ***T. pseudokoningii***

T_6 : ***T. pseudokoningii***

Plate 7 ***In vitro*** **inhibition of** ***R. solani*** **by** ***T. harzianum***

T_8 : ***T. harzianum***

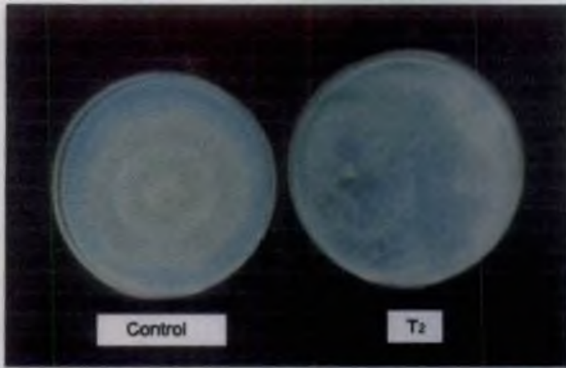


Plate 4

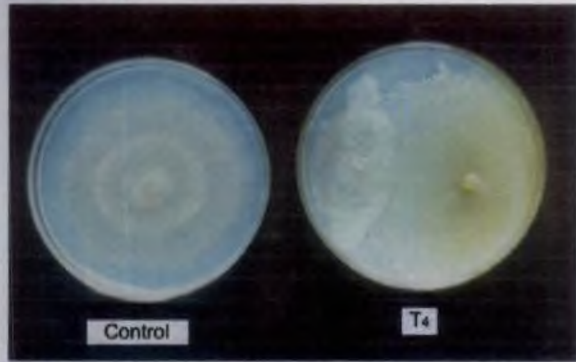


Plate 5

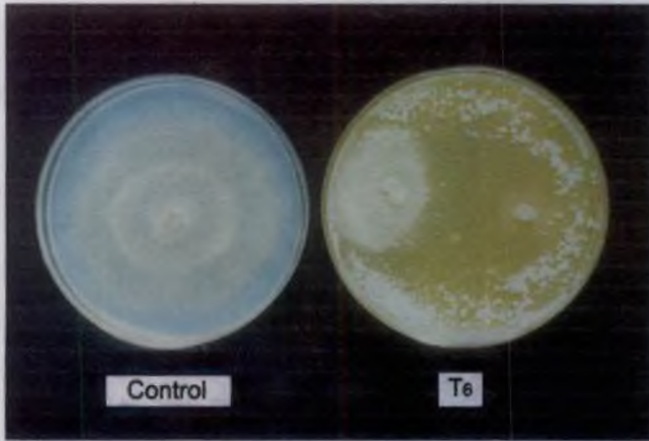


Plate 6

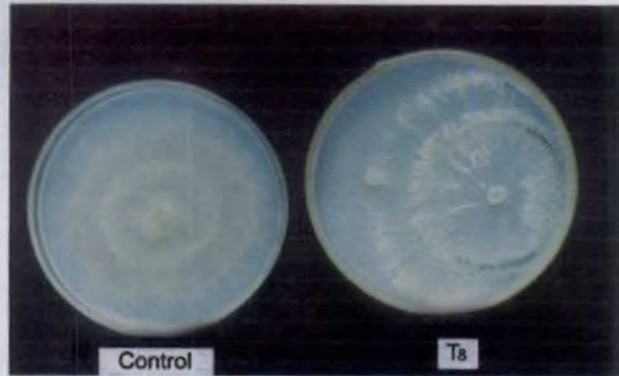


Plate 7

Table 5 Nature of reaction of the fungal antagonists on *R. solani*

Antagonist used for pairing	Type of reaction
T. harzianum	D
T. pseudokoningii	B
T. piluliferum	B
T. virens	B

A – Free intermingling between pairing organisms
B – The pathogen overgrown by the test organism
C – Cessation of growth at the line of contact of cultures
D – A clear zone of inhibition between two organisms

Table 6 Antagonism of bacterial isolates against *R. solani*

Bacterial isolates	*Percentage inhibition
P_1	81.85
P_5	80.37
B_3	85.55
KK_{16}	84.07
CD (0.05)	2.328

*Mean of three replications

Plate 8 Interaction between *R. solani* and *Piriformospora indica*

1. *P. indica*
2. *R. solani* x *P. indica*
3. *R. solani*

Plate 9 *In vitro* inhibition of *R. solani* by *Pseudomonas fluorescens* (P_1)

Plate 10 *In vitro* inhibition of *R. solani* by *P. fluorescens* (P_5)

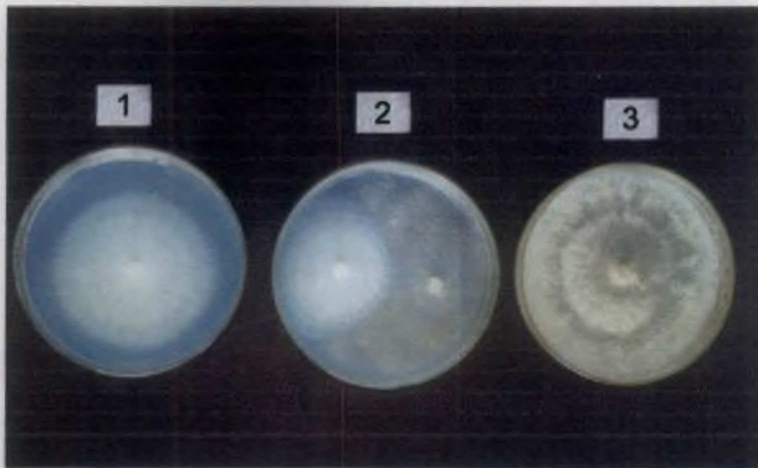


Plate 8

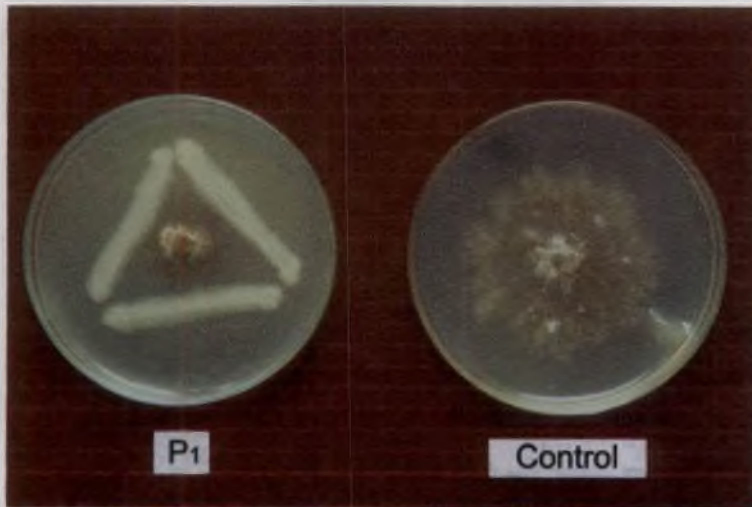


Plate 9

Plate 10

R. solani. The pathogen was found to overgrow the wells on PDA containing the filtrates of these antagonists.

4.6 INDIGENOUS MATERIALS

4.6.1 Turmeric powder-baking soda

Three different ratios of turmeric powder-baking soda were prepared as described under 3.6.1 and tested for their effects on *R. solani* (Plate 14). None of the combinations exerted any inhibitory effect on the growth of *R. solani.*

4.6.2 Rice husk ash

Three concentrations of RHA- 0.50, 0.75 and 1.00 per cent were tested for their effects on *R. solani*. None of the concentrations inhibited the growth of the pathogen.

4.7 *IN VIVO* STUDIES ON PATHOGEN SUPPRESSION AND PLANT GROWTH PROMOTION

4.7.1 Effect of biocontrol agents and indigenous materials on plant growth and disease incidence

4.7.1.7 Observations

4.7.1.7.1 Shoot length

The maximum shoot length was observed for the treatment with bacterial isolate, B_3 (36.5 cm) (Table 7). This was followed by treatment with the highest concentration of rice husk ash (34.33cm) (Plates15 and 16) whose shoot length was statistically on par with that of isolate B_3. A significant increase in shoot length over control was observed for rice husk ash 750g and 10:1 combination of turmeric powder- baking soda (Plate17). A reduction in shoot length was observed for treatments with *P. indica.*

Plate 11 *In vitro* **inhibition of** ***R. solani*** **by** ***P. fluorescens*** **(B_3)**

Plate 12 *In vitro* **inhibition of** ***R. solani*** **by** ***P. fluorescens*** **(KK_{16})**

Plate 13 *In vitro* **inhibition of** ***R. solani*** **by the culture filtrate of** ***P. fluorescens*** **(B_3)**

Plate 14 Effect of turmeric powder-baking soda on the *in vitro* **growth of** ***R. solani***

1. **10 : 1 combination**
2. **8 : 2 combination**
3. **6 : 4 combination**

Plate 11

Plate 12

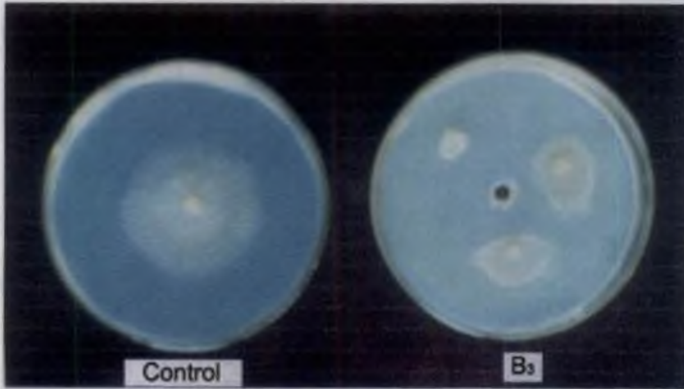


Plate 13

Plate 14

The treatment with *T. harzianum* also increased the shoot length significantly over control (31.5 cm). A significant increase in shoot length over control was also observed for plants treated with 8:2 combination of turmeric powder- baking soda, mancozeb and bacterial isolate KK_{16}.Shoot length of treatments with RHA 500g, bacterial isolates P_1 and P_5, 6:4 combination of turmeric powder-baking soda was statistically on par with the control.

4.7.1.7.2 Root length

The elongation of roots was observed to be maximum for treatment with the bacterial isolate B_3 when compared to control (44.23 cm) (Table 7). This was followed by treatment with rice husk ash 1000g (41.58 cm) (Plates 18 and 19) whose root length was found to be statistically on par with that of bacterial isolate. A significant increase in root length over control was observed for *T. harzianum* (37.83 cm) and rice husk ash 750 g (34.5 cm).

As the quantity of rice husk ash increased there was a corresponding increase in root length also. 10 : 1 combination of turmeric powder - baking soda showed a significant increase in root length (32.3 cm) over control. Treatment with two per cent concentration of *P. indica* caused a significant increase in root length over control (36.22 cm). There was a reduction in root length for treatments with RHA 500 g, bacterial isolates P_1 and P_5 and *P.indica* 0.05 per cent when compared to control. The root length of all other treatments including mancozeb was found to be statistically on par with the control .

4.7.1.7.3 Number of leaves

There was a significant increase in the number of leaves due to treatments with RHA, bacterial isolates B_3 and KK_{16}, turmeric powder-baking soda and *T.harzianum* (Table 7). Among the three concentrations of rice husk ash, number of leaves increased significantly for 750 g

Table 7 *Influence of indigenous materials, biocontrol agents and mancozeb on the shoot length, root length, number of leaves and leaf area of amaranthus

Treatments	Shoot length (cm)	Percentage variation over control	Root length (cm)	Percentage variation over control	Number of leaves	Percentage variation over control	Leaf area (cm^2)	Percentage variation over control
Rice huskash								
500g	29.0	13.77	24.16	-3.78	27.50	21.04	13.38	1.98
750 g	32.83	28.79	34.50	37.4	50.83	123.72	20.36	55.18
1000 g	34.33	34.68	41.58	65.59	52.66	131.78	29.39	124.01
Pseudomonas fluorescens P_1	23.16	-9.14	14.08	-43.92	28.00	23.24	23.72	80.79
P. fluorescens P_5	23.25	-8.79	14.83	-40.94	24.50	7.83	25.24	92.38
P. fluorescens B_3	36.5	43.19	44.23	76.14	40.66	78.96	32.94	151.07
P. fluorescens KK_{16}	30.50	19.65	24.50	-2.43	30.50	34.24	25.33	93.06
Turmeric powder – baking soda								
6 : 4	30.25	10.63	25.83	2.86	33.16	45.95	12.71	-3.125
8 : 2	28.20	18.67	25.16	0.19	29.66	30.54	10.77	-17.91
10 : 1	32.33	26.83	32.3	28.63	36.66	61.35	8.11	-38.19
P. indica								
0.5 %	18.10	-28.99	18.36	-26.88	11.16	-50.88	8.21	-37.42
1 %	19.10	-25.07	23.20	-7.61	18.4	-19.01	11.53	-12.12
2 %	18.66	-26.79	36.22	44.25	24.36	7.22	12.70	-3.20
T. harzianum	31.5	23.58	37.83	50.66	47.66	109.77	28.66	118.45
Mancozeb	30.33	18.99	29.00	15.49	28.00	23.24	22.39	70.66
Control	25.49		25.11		22.72		13.12	
CD (0.05)	3.787		4.621		5.141		3.287	

* Mean of three replications

Plate 15 Effect of *P. fluorescens* (B_3) on the shoot length of amaranthus

T_7 : *P. fluorescens* (B_3)
T_0 : Control

Plate 16 Effect of Rice husk ash (1000 g) on the shoot length of amaranthus

T_3 : Rice husk ash (1000 g)
T_0 : Control

Plate 17 Effect of turmeric powder-baking soda (10:1) on the shoot length of amaranthus

T_{12} : Turmeric powder-baking soda (10:1)
T_0 : Control

Plate 15

Plate 16

Plate 17

Plate 18 Effect of *P. fluorescens* (B_3) on the root length of amaranthus

T_7 : *P. fluorescens* (B_3)
T_0 : Control

Plate 19 Effect of Rice husk ash (1000g) on the root length of amaranthus

T_3 : Rice husk ash (1000g)
T_0 : Control

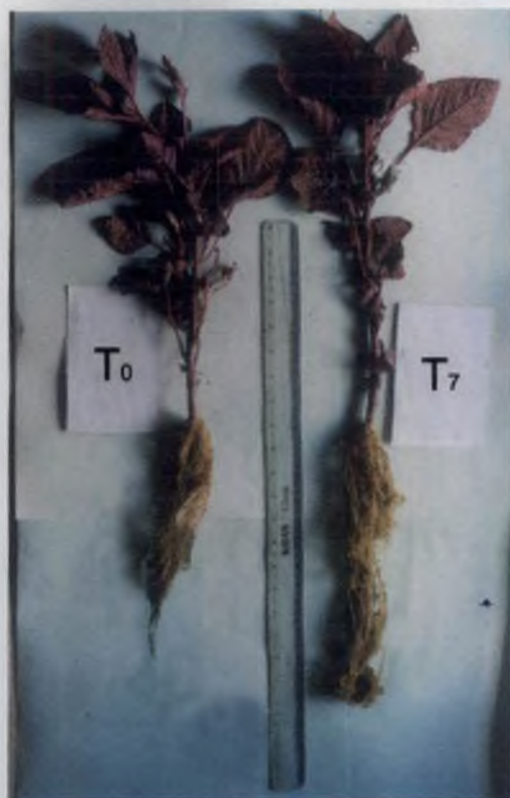


Plate 18

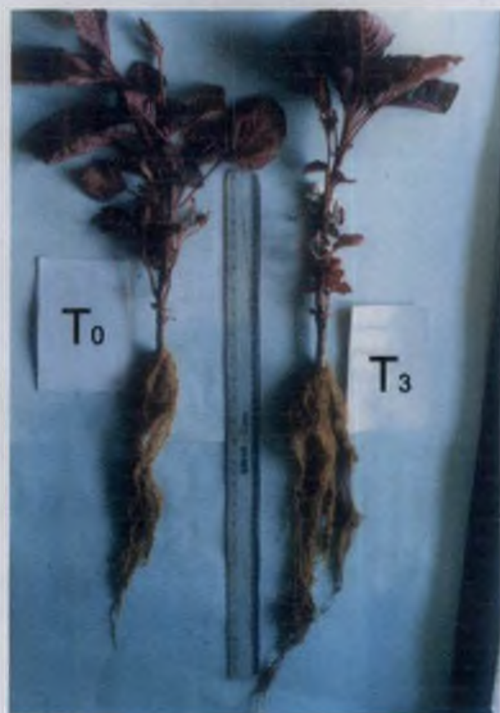


Plate 19

(50.83) and 1000g (52.66) when compared to the control plants. Treatments with bacterial isolates B_3 (40.66) and KK_{16} (30.50) and fungal antagonist *T. harzianum* (47.66) had significant effect on the number of leaves. All the three combinations of turmeric powder- baking soda recorded significant increase in the number of leaves when compared to control. A reduction in leaf number was observed for treatment with *P. indica* 0.50 per cent. The number of leaves of all other treatments including mancozeb was statistically on par with the control.

4.7.1.7.4 Leaf area

A significant increase in leaf area was observed for treatments with rice husk ash, bacterial isolates, *T. harzianum* and mancozeb (Table 7). Two concentrations of rice husk ash, 1000 g (29.390 cm^2) and 750 g (20.36 cm^2) showed significant increase in leaf area over control. All the bacterial isolates significantly increased the leaf area, isolate B_3 showing the maximum (32.94 cm^2). Treatments with *T. harzianum* (28.66 cm^2) and mancozeb (22.39 cm^2) also showed significant increase in leaf area over control. There was a reduction in leaf area due to treatment with *P. indica* 0.50 per cent. Leaf area of all other treatments was statistically on par with the control.

4.7.1.7.5 Fresh weight (shoot and root)

There was a significant increase in the fresh weight of shoot over control for treatments with RHA, bacterial isolates B_3 and KK_{16} and *T. harzianum*. The highest concentration of RHA and bacterial isolate B_3 recorded the maximum fresh weight of 30g. This was followed by treatment with *T. harzianum* (26.66g) whose fresh weight was statistically on par with that of above treatments (Table 8).

As the quantity of rice husk ash increased, there was a corresponding increase in the fresh weight also. A significant increase in fresh weight was observed for treatment with bacterial isolate KK_{16} (21.66 g)

Table 8 *Influence of indigenous materials, biocontrol agents and mancozeb on fresh and dry weight of shoots and roots of amaranthus

Treatments	Fresh weight shoot (g)	Percentage variation over control	Fresh weight root (g)	Percentage variation over control	Dry weight shoot (g)	Percentage variation over control	Dry weight root (g)	Percentage variation over control
Rice husk ash								
500g	20.00	250.88	8.33	143.56	3.38	144.92	2.3	389.36
750 g	23.33	309.29	10.00	192.39	4.09	196.37	2.3	389.36
1000 g	30.00	426.32	13.33	289.76	4.26	208.69	3.75	697.87
Pseudomonas fluorescens P_1	11.66	104.56	4.00	16.95	1.75	26.81	1.10	134.04
P. fluorescens P_5	13.33	133.85	6.66	94.73	1.74	26.08	1.17	148.93
P. fluorescens B_3	30.00	426.32	16.66	387.13	4.73	242.75	3.37	617.02
P. fluorescens KK_{16}	21.66	280.00	13.33	289.76	2.76	100.00	1.85	293.61
Turmeric powder – baking soda								
6 : 4	2.83	-50.35	1.25	-63.45	1.33	-3.62	0.20	-57.44
8 : 2	2.58	-54.73	1.00	-70.76	1.26	-8.69	0.32	-31.91
10 : 1	3.10	-45.61	1.25	-63.45	1.37	-0.72	0.34	-27.65
P. indica 0.5 %	3.96	-30.52	1.55	-54.67	1.01	-26.81	0.39	-17.02
1 %	5.40	-5.26	2.19	-35.96	1.40	1.44	0.43	-8.51
2 %	5.87	2.98	3.47	1.46	1.67	21.01	1.56	234.91
T. harzianum	26.66	367.71	11.66	240.93	4.2	204.34	2.68	470.21
Mancozeb	15.00	163.15	8.33	143.56	2.15	55.79	1.42	202.12
Control	5.70		3.42		1.38		0.47	
CD (0.05)	9.250		5.309		0.783		0.645	

*Mean of three replications

when compared to control. There was a reduction in the fresh weight for treatments with turmeric powder- baking soda and *P. indica*. Fresh weight of shoot for all other treatments including mancozeb was statistically on par with the control.

The increase in fresh weight of root followed a similar trend for all treatments except the treatment with RHA (500 g). The fresh weight of roots of this treatment was found to be statistically on par with the control (Table 8).

4.7.1.7.6 Dry weight (shoot and root)

Dry weight of shoot increased significantly over control for the treatments with RHA, bacterial isolates B_3 and KK_{16} and *T. harzianum* (Table 8). The highest concentration of rice husk ash (1000 g) recorded the maximum dry weight (4.26 g) among its three concentrations. The bacterial isolates B_3 and KK_{16} caused a significant increase in the dry weight over control. Treatment with *T. harzianum* also enhanced the dry weight significantly. The shoot dry weight of all other treatments including mancozeb was statistically on par with the control.

A similar trend was observed for root dry weight also except for treatment with *P. indica* two per cent and mancozeb. Treatments with *P. indica* two per cent and mancozeb significantly increased the root dry weight when compared to the control plants (Table 8).

4.7.1.7.7 Disease incidence

Significantly lower incidence was observed for treatments with RHA, bacterial isolates B_3 and KK_{16}. *T. harzianum*and mancozeb. There was no disease incidence in plants treated with rice husk ash (1000 g) and bacterial isolate B_3 (Plates 20 and 21). These two treatments were found to most effective in suppressing the disease (Table 9). Treatments with *T. harzianum* and bacterial isolate KK_{16} recorded lower incidence of 5.56

Table 9 Effect of indigenous materials, biocontrol agents and mancozeb on the incidence and intensity of leaf blight of amaranthus

Treatments	Disease incidence ❖	Disease intensity*	Percentage variation over control
Rice huskash			
500g	61.11(51.49)	1.82 (1.67)	-15.92
750 g	44.44(41.78)	2.73 (2.73)	73.88
1000 g	0(1.65)	0 (1)	100.00
Pseudomonas fluorescens P_1	55.56(48.24)	2.73 (1.93)	73.88
P. fluorescens P_5	61.11(51.49)	1.82 (1.67)	15.92
P. fluorescens B_3	0(1.65)	0 (1)	100.00
P. fluorescens KK_{16}	27.77(31.54)	0.91 (1.38)	-42.03
Turmeric powder –baking soda			
6 : 4	61.11(51.49)	2.73 (1.93)	73.88
8 : 2	55.56(48.24)	1.82 (1.67)	15.92
10 : 1	22.22(27.82)	1.82 (1.67)	15.92
P. indica			
0.5 %	55.56(48.24)	2.73 (1.93)	73.88
1 %	55.56(48.24)	2.73 (1.93)	73.88
2 %	44.44(41.78)	1.82 (1.67)	15.92
T. harzianum	5.56(24.1)	0.91 (1.38)	-42.03
Mancozeb	27.78(31.06)	1.19 (1.48)	-24.20
Control	44.44(41.78)	1.57 (1.60)	
CD (0.05)	12.22	0.076	

❖ Values in parenthesis after angular transformation

*Values in parenthesis after $\sqrt{x+1}$ transformation

Plate 20 Effect of Rice husk ash (1000 g) on the incidence of foliar blight of amaranthus

T_3 : Rice husk ash (1000 g)
T_0 : Control

Plate 21 Effect of *P. fluorescens* (B_3) on the incidence of foliar blight of amaranthus

T_7 : *P. fluorescens* (B_3)
T_0 : Control

Plate 22 Effect of *T. harzianum* on the incidence of foliar blight of amaranthus

T_4 : *T. harzianum*
T_0 : Control

Plate 23 Effect of *P. fluorescens* (KK_{16}) on the incidence of foliar blight of amaranthus

T_8 : *P. fluorescens* (KK_{16})
T_0 : Control

Plate 20

Plate 21

Plate 22

Plate 23

and 27.77 per cent respectively (Plates 22 and 23). Plants sprayed with 10:1 combination of turmeric powder-baking soda and mancozeb also showed reduction in the disease incidence. All the other treatments showed higher incidence over control.

4.7.1.7.8 Disease intensity

Significantly lower intensity of disease was observed for treatments with *T. harzianum* bacterial isolate KK_{16} and mancozeb when compared to control. Treatments with rice husk ash (1000g) and bacterial isolate B_3 were found to be most effective in suppressing the disease. All other treatments recorded a higher intensity of disease over control (Table 9).

4.7.1.7.9 Root Colonization and Characterization of *P. indica*

The root colonization increased with increased concentration of *P.indica*. The highest concentration (2.00 per cent) gave 52.00 per cent colonization when compared to 26.00 per cent for the lower concentration of 0.50 per cent (Table 10). The root bits were scored positive based on the presence of mycelium and chlamydospores (Plate 24). The spores appeared pear shaped and measured 16-25 μm in length and 10-17 μm in width (Plate 25).

4.7.2 Management of Rhizoctonia leaf blight using biocontrol agents and indigenous materials

4.7.2.2 Observations

4.7.2.2.1 Shoot length

The maximum increase in shoot length over control was observed for treatment with rice husk ash (33.16 cm) followed by *T. harzianum* (30.33 cm) whose shoot length was statistically on par with that of rice husk ash. Shoot length was found to enhance significantly for treatments with bacterial isolates B_3 (27.66 cm), KK_{16} (27 cm) and 10:1 combination

Table 10. Percentage root colonization by *P. indica*

Concentration of *P. indica*	Percentage root colonization
0.50 per cent	26.00
1.00 per cent	44.00
2.00 per cent	52..00

Table 11. *Effect of selected indigenous materials, biocontrol agents and mancozeb on the shoot length, root length, number of leaves and leaf area of amaranthus

Treatments	Shoot length (cm)	Percentage variation over control	Root length (cm)	Percentage variation over control	Number of leaves	Percentage variation over control	Leaf area (cm^2)	Percentage variation over control
Rice husk ash 1000g	33.16	49.43	54.91	138.01	38.5	79.06	27.61	89.62
Turmeric powder-baking soda 10 : 1	26.97	21.54	26.16	13.39	26.66	19.35	22.16	52.19
P. indica 2%	13.33	-39.92	26.08	13.04	17.0	-20.93	10.37	-28.77
P. fluorescens B_3	27.66	24.65	36.41	57.82	27.66	28.66	21.39	46.90
P. fluorescens KK_{16}	27.00	21.67	24.33	5.46	27.16	26.32	18.36	26.09
T. harzianum	30.33	35.19	45.0	95.05	35.16	63.53	26.64	82.96
Mancozeb	24.33	9.64	29.5	27.87	25.66	19.34	18.44	26.64
Control	22.19		23.07		21.5		14.56	
CD (0.05)	3.351		4.063		4.493		5.099	

*Mean of three replications

Plate 24 Root colonization of *P. indica* on amaranth roots showing the chlamydospores (300 x)

Plate 25 Chlamydospores of *P. indica* (480 x)

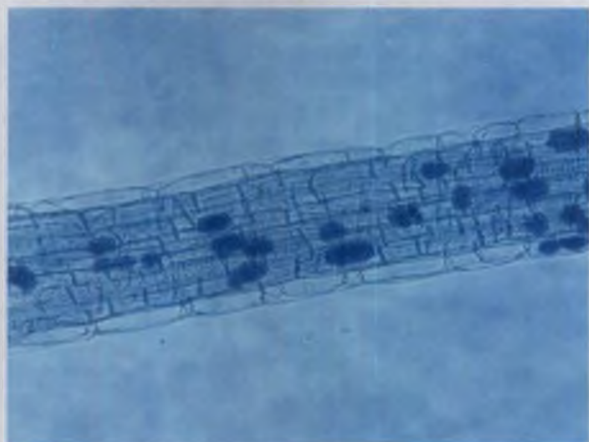


Plate 24

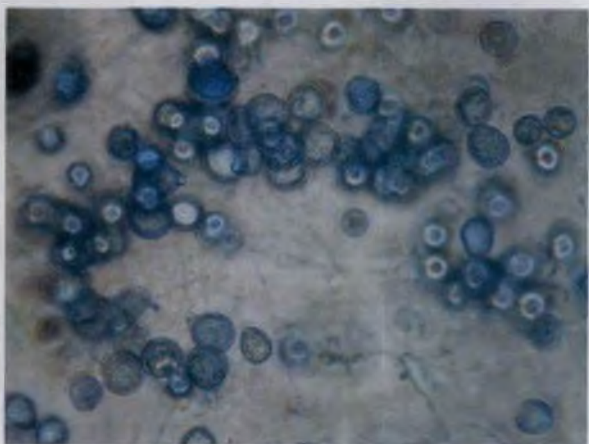


Plate 25

of turmeric powder- baking soda (26.97cm). Shoot length of the treatment with mancozeb was found to be on par with the control. A reduction in shoot length was obtained for treatment with *P. indica* (Table 11).

4.7.2.2.2 Root length

Maximum elongation of roots was observed for plants treated with RHA (54.91 cm). Treatments with *T. harzianum* (45.00 cm), bacterial isolate B_3 (36.41) and mancozeb (29.50 cm) also showed significant increase in root length over control. Root length of all other treatments was statistically on par with the control (Table 11).

4.7.2.2.3 Number of leaves

Leaf number increased significantly over control for treatments with rice husk ash (38.50), *T. harzianum* (35.16) bacterial isolates B_3 (27.66) and KK_{16} (27.16) and turmeric powder- baking soda (26.66). Number of leaves for treatment with *P. indica* and mancozeb was statistically on par with the control (Table 11).

4.7.2.2.4 Leaf area

A significant increase in leaf area over control was observed for treatments with rice husk ash (27.61 cm^2), *T. harzianum* (26.64 cm^2) turmeric powder- baking soda (22.16 cm^2) and bacterial isolate B_3 (21.39 cm^2). There was a reduction in leaf area for plants treated with *P. indica*. The leaf area of other treatments was statistically on par with the control (Table 11).

4.7.2.2.5. Fresh weight (shoot and root)

A significant increase in the fresh weight of shoot was observed for treatments with *T. harzianum* (32.5g), RHA (31.66 g), bacterial isolates B_3 (22.16 g) and KK_{16} (22.00 g). There was a reduction in the fresh weight

Table 12. *Effect of selected indigenous materials, biocontrol agents and mancozeb on fresh and dry weight of shoots and roots of amaranthus

Treatments	Fresh weight (Shoot)	Percentage variation over control	Fresh weight (root)	Percentage variation over control	Dry weight (Shoot)	Percentage variation over control	Dry weight (root)	Percentage variation over control
Rice husk ash 1000g	31.66	91.87	18.33	107.58	5.06	101.59	2.96	108.45
Turmeric powder - baking soda 10 : 1	16.66	0.96	11.58	31.14	2.90	15.53	1.88	32.39
P. indica 2%	9.33	-43.45	6.66	-24.57	1.59	-36.65	0.99	-30.28
P. fluorescens B_3	22.16	34.30	13.66	54.69	3.96	57.76	2.20	49.29
P. fluorescens KK_{16}	22.00	33.33	10.33	16.98	3.36	25.29	1.84	29.57
T. harzianum	32.5	96.96	12.50	41.56	5.36	113.54	2.23	57.04
Mancozeb	18.0	9.09	12.66	43.37	2.57	2.39	2.12	54.92
Control	16.50		8.83		2.51		1.42	
CD (0.05)	3.585		3.173		0.671		0.564	

*Mean of three replications

of shoot for treatment with *P. indica* when compared to control. The fresh weight of shoot of all other treatments was statistically on par with the control (Table 12).

Fresh weight of roots increased significantly over control for treatments with RHA (18.33 g) bacterial isolate B_3 (13.66 g). *T. harzianum* (12.5) and mancozeb (12.66 g). The fresh weight of roots of all the other treatments was on par with the control (Table 12).

4.7.2.2.6 Dry weight (shoot and root)

A significant increase in the dry weight of shoot over control was observed for treatments with *T. harzianum* (5.36 g), rice husk ash (5.06 g). bacterial isolates B_3 (3.96 g) and KK_{16} (3.36 g). There was a reduction in the dry weight of shoot for *P. indica* when compared to control. The dry weight of shoot of all other treatments including mancozeb was statistically on par with the control (Table 12).

Dry weight of roots also showed significant increase for rice husk ash (2.96 g). *T. harzianum* (2.23 g), bacterial isolate B_3 (2.20 g) and mancozeb (2.12 g). Dry weight of all other treatments was statistically on par with the control (Table 12).

4.7.2.2.7 Disease incidence

Among the treatments, bacterial isolate B_3 was found to be the most effective in reducing the disease incidence. All treatments except *P. indica* showed significantly lower incidence over control. Treatments with RHA (5.56 per cent), *T. harzianum* (11.11 per cent), bacterial isolate KK_{16} (27.77 per cent), mancozeb (44.44 per cent) and turmeric powder-baking soda (44.44 per cent) showed reduction in disease incidence. Disease incidence was found to be higher for plants treated with *P. indica* when compared to control (Table 13).

Table 13. Effect of selected indigenous materials, biocontrol agents and mancozeb on the incidence and intensity of leaf blight of amaranthus

Treatments	+ Disease incidence (%)	*Disease intensity	Percentage variation over control
RHA 1000g	5.56 (24.1)	1.13 (1.45)	-33.52
Turmeric powder – baking soda 10 : 1	44.44 (38.5)	1.67 (1.63)	-1.76
P. indica 2%	55.56 (51.49)	2.59(1.89)	52.35
P. fluorescens B_3	0 (1.65)	0 (1.00)	100.00
P. fluorescens KK_{16}	27.77(27.8)	1.31 (1.52)	-22.94
T. harzianum	11.11 (35.26)	0.93 (1.39)	-45.29
Mancozeb	44.44 (31.61)	1.53 (1.59)	-10.00
Control	55.56 (51.49)	1.70 (1.64)	
CD (0.05)	8.451	0.375	

+ Value in parenthesis after angular transformation *Value in parenthesis after $\sqrt{x+1}$ transformation

172205

4.7.2.2.8 Disease intensity

The bacterial isolate B_3 was found to be the best treatment in suppressing the disease. Significantly lower intensity was observed for treatments with *T. harzianum* (0.93), rice husk ash (1.13), bacterial isolate KK_{16} (1.31) and mancozeb (1.53) when compared to control plants. Disease intensity of the treatment with turmeric powder baking soda was statistically on par with the control and treatment with *P. indica* recorded higher intensity over control (Table 13).

4.7.2.2.9 Root Colonization and Characterization of *P. indica*

The extend of root colonization in two per cent *P.indica* treated plants was estimated by staining the root bits using 0.05 per cent trypan blue after clearing. A total of 50 root bits were examined and the percentage of root colonization was found to be 32.00. The root bits were scored positive based on the presence of mycelium and pear shaped chlamydospores.

4.7.2.2.10 Biochemical estimation

4.7.2.2.10.1 Total Phenol

Soil application of *T. harzianum* alone had significant effect on the total phenol content. The phenol content of all other treatments was found to be statistically on par with the control (Table 14).

The phenol content was significantly increased due to foliar application of *T. harzianum*. Spraying of turmeric powder- baking soda was found to reduce the phenol content when compared to control. The phenol content of all other treatments due to foliar application was statistically on par with the control (Table 15).

Table 14. *Effect of soil application of rice husk ash, *P. indica*, bacterial isolates and *T. harzianum* on total phenols, amino acids and carbohydrates in amaranthus leaves inoculated with *R. solani*

Treatments	Total phenols (µg catechol eqvt./g fresh weight)	Percentage variation over control	Amino acid % equvt. of leucine	Percentage variation over control	Carbohydrates (per gram leaf tissue)	Percentage variation over control
Rice husk ash 1000g	756.66	-1.30	0.23	-54.90	90.66	-24.02
P. indica 2%	536.66	-30.00	0.28	-45.09	95.33	-20.11
P. fluorescens B_3	913.33	19.13	0.46	-9.80	116.00	-2.79
P. fluorescens KK_{16}	873.33	13.91	0.28	-45.09	104.66	12.29
T. harzianum	973.33	26.95	0.42	-17.64	123.33	3.35
Control	766.66		0.51		119.33	
CD (0.05)	198.250		0.188		23.943	

*Mean of three replications

4.7.2.2.10.2 Total Free Amino Acids

Soil application of rice husk ash, *P. indica* and bacterial isolate KK_{16} were found to reduce the amino acid content when compared to control. The amino acid content of all other treatments due to soil application was statistically on par with the control (Table 14).

There was a reduction in the free amino acid content due to foliar application of turmeric powder-baking soda, bacterial isolate KK_{16} and mancozeb when compared to control plants. The amino acid content of allother treatments was statistically on par with the control (Table 15).

4.7.2.2.10.3 Carbohydrates

A reduction in carbohydrate content was observed due to soil application of rice husk ash and *P. indica*. The carbohydrate content due to soil application of all other treatments was statistically on par with the control (Table 14).

There was a reduction in the carbohydrate content due to foliar application of turmeric powder- baking soda. The carbohydrate content of all other treatments was statistically on par with the control (Table 15).

4.7.2.2.10.4 Phenyl alanine ammonia lyase (PAL)

There was a significant increase in PAL activity over control due to soil application of bacterial isolates B_3 and KK_{16} and *T. harzianum*. Changes in PAL activity was observed five days after inoculation with *R. solani*. The highest PAL activity was shown by *T. harzianum* (50.97) followed by the bacterial isolate B_3 (48.47). PAL activity due to soil application of RHA and *P. indica* was statistically on par with the control (Table 16).

Table 15. *Effect of foliar spray of turmeric powder-baking soda, bacterial isolates, *T. harzianum* and mancozeb on total phenols, amino acids and carbohydrates in amaranthus leaves inoculated with *R. solani*

Treatments	Total Phenols	Percentage variation over control	Amino acids	Percentage variation over control	Carbohydrates	Percentage variation over control
Turmeric powder - baking soda 10 : 1	1000.00	-18.91	0.28	-50.00	96.66	-21.19
P. fluorescens B_3	1310.00	6.21	0.45	-19.60	117.33	-4.34
P. fluorescens KK_{16}	1150.00	-6.75	0.31	-44.64	104.66	-14.67
T. harzianum	1443.33	17.02	0.47	-16.07	123.33	0.54
Mancozeb	1120.00	-9.18	0.30	-46.42	112.00	−8.69
Control	1233.33		0.56		122.66	
CD (0.05)	136.978		0.136		22.535	

*Mean of three replications

PAL activity was significantly enhanced due to foliar spray of *T. harzianum* (47.41), bacterial isolates B_3 (47.72) and KK_{16} (47.66) when compared to control. The activity was found to be lower due to the foliar application of turmeric powder- baking soda. PAL activity of plants sprayed with mancozeb was found to be statistically on par with the control (Table 17)

4.7.2.2.10.5 Peroxidase (PO)

Peroxidase activity was significantly enhanced due to soil application of *T. harzianum* (5.63) and bacterial isolate B_3 (5.33) when compared to control. The activity of peroxidase of all other treatments was found to be statistically on par with the control (Table 16).

Significantly higher activity of PO was observed due to foliar application of bacterial isolates B_3 (5.39) and KK_{16} (4.00) and fungal antagonist *T. harzianum* (4.06). PO activity due to foliar application of turmeric powder-baking soda and mancozeb was found to be statistically on par with the control (Table 17).

4.7.2.2.10.6 Poly phenol oxidase (PPO)

A significant increase in PPO activity over control was shown by *T.harzianum* (1.62) and bacterial isolates B_3 (1.63) and KK_{16} (1.25). A reduction in PPO activity was observed due to soil application of rice husk ash whereas the PPO activity for treatment with *P. indica* was found to be statistically on par with the control (Table 16).

Foliar application of *T. harzianum* and bacterial isolates, B_3 and KK_{16} significantly increased PPO activity. The activity of all other treatments was statistically on par with the control (Table 17).

Table 16. *Effect of soil application of rice husk ash, *P. indica*, bacterial isolates and *T. harzianum* on changes in the activity of PAL, PO and PPO in amaranthus leaves inoculated with *R. solani*

Treatments	PAL (n mol trans cinnamic acid/min/ g fresh weight)	Percentage variation over control	Peroxidase in units (1unit = change in absorbance of .001 / minute / g fresh weight	Percentage variation over control	Polyphenol oxidase	Percentage variation over control
RHA 1000g	39.16	-3.42	3.63	17.47	0.38	-51.28
P. indica 2%	39.44	-2.73	2.56	-17.15	0.47	-39.74
P. fluorescens B_3	48.47	19.53	5.33	72.49	1.63	108.97
P. fluorescens KK_{16}	47.78	17.82	4.37	41.42	1.25	60.25
T. harzianum	50.97	25.69	5.69	84.14	1.62	107.69
Control	40.53		3.09		0.78	
CD (0.05)	5.535		1.606		0.375	

*Mean of three replications

Table 17. *Effect of foliar spray of turmeric powder-baking soda, bacterial isolates, *T. harzianum* and mancozeb on changes in the activity of PAL, PO and PPO in amaranthus leaves inoculated with *R. solani*

Treatments	PAL	Percentage variation over control	PO	Percentage variation over control	PPO	Percentage variation over control
Turmeric powder-baking soda 10 : 1	35.08	-11.99	1.9	-19.49	0.57	5.55
P. fluorescens B_3	47.72	19.71	5.39	128.38	1.79	231.48
P. fluorescens KK_{16}	47.66	19.56	4.00	69.49	0.77	42.59
T. harzianum	47.41	18.94	4.06	72.03	1.06	96.29
Mancozeb	38.60	-3.16	2.19	-7.20	0.44	-18.51
Control	39.86		2.36		0.54	
CD (0.05)	4.348		1.386		0.151	

*Mean of three replications

DISCUSSION

5. DISCUSSION

In the present study, the pathogen causing foliar blight of amaranthus was isolated from infected plants collected from ten different locations in Thiruvananthapuram district. The different isolates of *Rhizoctonia solani* varied in their growth rate and time of sclerotia formation when grown on PDA. The isolates R_2 and R_3 recorded the highest growth rate and produced sclerotia six days after inoculation. Such variations among the different isolates of *R. solani* in characters like hyphal thickness, colour of mycelium, sclerotial size and distribution has been reported earlier (Gokulapalan, 1981). Vijayan (1986) identified four morphological groups – MG1, MG2, MG3 and MG4 from the 41 isolates of *R. solani* obtained from various plants/habitats and each group had its own distinctive morphological and cultural characters.

R. solani is an ubiquitous soil-borne pathogen having a wide host range. Teleomorph of this pathogen (*Thanatephorus cucumeris*) causes foliar blight of numerous crops (Galindo *et al.*, 1983; Jana *et al.*, 1990; Koike and Subbarao, 1999). The foliar blight of amaranthus caused by *R. solani* was reported recently from Kerala (Kamala *et al.*, 1996; Gokulapalan *et al.*, 2000).

When the different isolates of *R. solani* were tested for pathogenicity, isolate R_3 (Vellayani) was found to produce maximum lesion size (five mm) and the highest intensity of disease (2.77) and so it was selected for further studies. Meena *et al.* (2001) reported that from among the five isolates of *R. solani* obtained from paddy growing areas in Tamil Nadu tested for their virulence, the Madurai isolate was found to be most virulent which exerted maximum lesion height of 66.40 % on rice by artificial inoculation and Trichy isolate was the least virulent. Variation

in the virulence of isolates of *R. solani* was earlier reported by several workers (Shajahan *et al.*, 1987; Singh *et al.*, 1990; Girija, 1993).

In the present investigation, biocontrol agents such as *Trichoderma harzianum, Pseudomonas fluorescens* and a newly discovered plant growth promoting root endophyte *viz., Piriformospora indica* were tested for their effects on the growth of *R. solani* by dual culture studies under *in vitro* conditions. Among the four isolates of *Trichoderma* - *T. harzianum, T. virens, T. pseudokoningii* and *T. piluliferum* tested against *R. solani, T. harzianum* caused maximum inhibition of the mycelial growth of *R. solani* (47.41 per cent). *T. harzianum* when subjected to dual culture with *R. solani*, produced a clear zone of inhibition between the cultures. *T. piluliferum, T. pseudokoningii* and *T. virens* inhibited the growth of *R. solani* to 42.96, 44.44 and 45.18 per cent respectively. All the isolates completely overgrew the pathogen after seven days. The antagonistic effect of *Trichoderma* spp. against *R. solani* have been recorded in numerous studies (Hadar *et al.*, 1979; Harman *et al.*, 1980; Elad *et al.*, 1982; Gokulapalan and Nair, 1984; Padmakumary, 1989; Kumaresan and ManibhushanRao, 1991; Abraham Mathew and Gupta, 1998; CharithaDevi and Reddy, 2002).

Bunker and Mathur (2001) studied the antagonistic potential of *T. harzianum, T. viride, T. virens* and *T. aureoviride* against *R. solani* causing dry root rot of chilli under *in vitro* conditions. Among the various isolates, *T. harzianum* was most effective in causing significant suppression of mycelial growth and sclerotial formation through production of volatile and non-volatile antibiotics followed by *T. virens* and *T. aureoviride* which overgrew the pathogen. The inhibition of mycelial growth of *R. solani* by *T. harzianum* could be due to the production of lytic enzymes like B-1,3 glucanase and chitinase (Hadar *et al.*, 1979) or production of volatile and non-volatile antibiotics (Dubey and Patel, 2001).

When *P. indica* was subjected to dual culture with *R. solani*. there was no inhibition on the mycelial growth of the pathogen. The pathogen was found to overgrow the fungus in four days. The lack of *in vitro* suppression of pathogen by antagonistic fungi has been reported by Bhuvaneswari and Subbarao (2001) who reported that when *Trichoderma viride* interacted with several post harvest pathogens of mango. it was found ineffective in inhibiting the growth of *Penicillium purpurogenum* and *Phoma* sp.

Antagonism of four selected isolates of *P. fluorescens viz.*, P_1, P_5, B_3 and KK_{16} against *R. solani* were tested. Of the four isolates, isolate B_3 showed the highest *in vitro* antagonism (85.55 per cent) followed by the isolate KK_{16} (84.07 per cent). Howell and Stipanovic (1979) reported that a strain of *P. fluorescens* isolated from the rhizosphere of cotton seedlings inhibited the mycelial growth of *R. solani*. The antagonism exhibited by the bacterium is attributed to the production of an antifungal antibiotic pyrrolnitrin. High degree of inhibition by fluorescent pseudomonads against *in vitro* growth of foliar pathogens affecting various crops have been reported by many earlier workers (Kloepper and Schroth, 1981; Mew and Rosales, 1986; Devi *et al.*, 1989; Podile *et al.*, 1990; Velazhahan *et al.*, 1999). The percentage inhibition of bacterial and fungal antagonists against *R. solani* is given in Fig. 1.

The culture filtrates of *P. fluorescens*, *T. harzianum* and *P. indica* were tested for their effects on the growth of the pathogen. The culture filtrate of the isolate of *P. fluorescens viz.* B_3 alone exerted inhibitory effects on the growth of *R. solani*. For all other filtrates, the pathogen was found to overgrow the wells on PDA which contained them. Rabindran (1994) reported that the culture filtrate of a strain of *P. fluorescens*, PfALR2 completely inhibited the germination and reduced the virulence of sclerotia of *R.solani*, the incitant of sheath blight of rice. Borowicz and Omer (2000) reported that the production of various metabolites by the

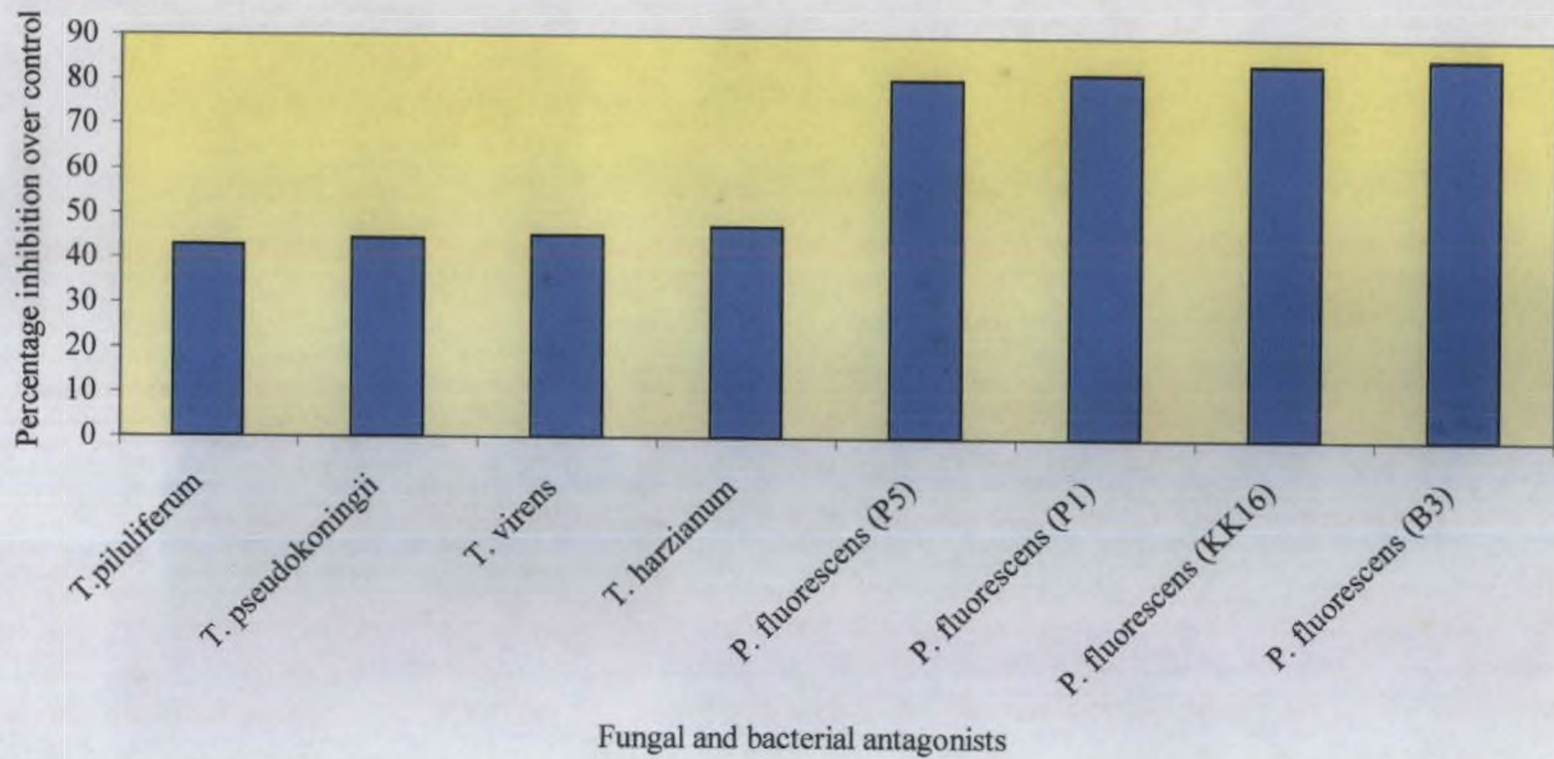


Fig. 1 Percentage inhibition of fungal and bacterial antagonists against *Rhizoctonia solani*

bacterial isolates are induced in the presence of various media components and this will vary for different media. So the non-inhibitory effects of the culture filtrates of *P. fluorescens* may be attributed to lesser production of metabolites in King's broth in which it was grown. Dubey and Patel (2001) reported that the culture filtrates of *T. viride*, *T. virens* and *T. harzianum* varied greatly in their ability to inhibit the growth of *R. solani* and this may due to the quantity and quality of inhibitory substances present in the filtrates. So there is a possibility that the quantity and quality of metabolites present in the culture filtrate of *T. harzianum* must have exerted the non-inhibitory effects on *R. solani*. The culture filtrate of *P. indica* did not produce any inhibitory effects on the growth of *R. solani*.

The three combinations of turmeric powder-baking soda tested against *R. solani* did not inhibit the growth of the pathogen. Dhanya (2000) reported that there was only slight inhibition of *Xanthomonas axonopodis* pv. *dieffenbachiae*, causal agent of bacterial blight of anthurium, when three levels of turmeric powder-baking soda 0.05, 0.10 and 0.15 per cent were tested against it.

The three concentrations of rice husk ash also did not exert inhibitory effects on the growth of the pathogen. This can be attributed to the fact that the mode of action of rice husk ash is silicification which leads to increased resistance in plants thus reducing the disease intensity and not the direct action against the pathogen. Joshi (2002) reported that seed treatment with rice husk ash was effective in managing the blast disease of rice and that the increased resistance can be attributed to increased silicification and not due to the direct inhibitory action on *Magnaporthe grisea*.

Pot culture experiments were conducted to study the effects of rice husk ash, turmeric powder -baking soda, bacterial and fungal antagonists and *P. indica* in enhancing the growth and checking the disease in amaranthus. The presently recommended control measure of 0.40 per cent

mancozeb in cowdung supernatant was kept as standard check. The treatment with rice husk ash tremendously improved the growth of amaranthus plants. The treatment with the highest concentration of rice husk ash (1000 g) recorded an increase of 35.00 per cent in shoot length and 50.00 per cent increase in root length over absolute control. Rice husk ash also exerted a significant effect on the number of leaves, leaf area, fresh weight and dry weight of amaranthus. The treatment when put on a second trial also showed enhancement of growth. Savant and Savant (1995) reported that application of black-grey ash of rice hulls (0.5-2 kg/m^2) made rice seedlings healthy and strong and increased their biomass. The weight of straw and roots and the plant height were also greater for rice plants treated with ash.

The highest concentration of rice husk ash was also effective in checking the disease. In the first trial, plants treated with ash remained completely free from the disease. When the trial was repeated there was a reduction in the incidence of disease and the intensity was reduced to 34.00 per cent. Silica, the main constituent of rice husk ash gets deposited at the external surface of cell wall of plants and forms a mechanical barrier to the penetration of pathogen. Joshi (2002) reported that seed treatment with rice husk ash was effective in managing blast disease of rice. Similar results were also observed by Laha *et al.* (2000) in rice infected by sheath blight. Significantly more number of tillers showed resistant reaction in soils amended with rice husk ash whereas in unamended soils, maximum tillers showed susceptible reaction.

Among the four selected isolates of *P. fluorescens*, two isolates B_3 and KK_{16} were found to enhance the growth significantly. The shoot length and root length for plants treated with B_3 isolate recorded and increase of 43.00 per cent and 77.00 per cent respectively whereas treatment with KK_{16} showed an increase of 20.00 per cent in shoot length over control. Other growth parameters like leaf area, number of leaves,

fresh weight and dry weight also showed significant increase over control with these treatments. The growth promoting effect of fluorescent pseudomonads can be attributed to the production of hormones and yellow green fluorescent siderophores. The siderophores complex Fe^{3+} in the root zone and makes it unavailable to deleterious microorganisms (Leong, 1986). Many workers have attributed the plant growth promotion by rhizobacteria such as *P. fluorescens* and *P.aeruginosa* to the secretion of hormones like gibberellic acid (Katznelson and Cole, 1965; Suslow, 1982; Schippers *et al.*, 1987; Weller, 1988). Gupta *et al.* (1995) reported that treatment with *P. fluorescens* increased the length of shoot and root, dry weight and seedling emergence in tomato. Dileepkumar (2002) also recorded that a strain of *P. fluorescens* RRLJ130 has enhanced percentage of seed germination, shoot height, root length, fresh and dry weight in chick pea and pigeon pea.

A lower incidence of disease was recorded in plants treated with bacterial isolates – B_3 and KK_{16}. Plants treated with B_3 isolate remained completely free from disease. Treatment with isolate KK_{16} reduced the disease intensity to 42.00 per cent. Smitha (2000) reported that soil application followed by foliar spray of the talc based formulation of *P. fluorescens* was effective in reducing the intensity of foliar blight. Many workers have reported that suppression of plant diseases by fluorescent pseudomonads can be due to their activities like competition for space and nutrients, production of antibiotics, volatile and antimicrobial substances, siderophores and HCN (Dowling and O' Gara,1994; Rosales *et al.*, 1995, Dave and Dube, 2000; Mondal *et al.*, 2000). Nandakumar *et al.* (2001) reported that *P. fluorescens* application as bacterial suspension or through seeds, roots, soil and foliage either alone or in combination reduced the intensity of sheath blight of rice and promoted plant growth.

The other two isolates of *P. fluorescen,* P_1 and P_5 though effective *in vitro* did not reduce the disease intensity under *in vivo* conditions. Mew and Rosales (1986) reported that a fluorescent pseudomonad, In-b-24 was not effective aganist *R. solani,* causing sheath blight under *in vitro* conditions but effective *in vivo.*

A significant increase in root length and root dry weight was recorded for plants treated with two per cent *P. indica.* The growth promoting effect of *P. indica* can be due to the production of growth promoting substances and translocation of phosphorus and other mineral elements to the host roots. Varma *et al.* (1999) observed that *P. indica* tremendously improved the growth and biomass production of herbaceous monocots and dicots, trees including medicinal plants like *Bacopa monnieri, Artmesia anua* and other economically important plants.

Among the different combinations of turmeric baking soda, 10:1 combination had significant effects on the growth of amaranthus plants. . Though this combination was not effective against the pathogen under *in vitro* conditions, there was a reduction in the disease incidence due to foliar application of 10:1 combination in pot culture trials. Gangopadhyay (1998) reported that turmeric powder-baking soda when used at a ratio of 10:1 as seed treatment and foliar spray could effectively control soil borne diseases in rice. Dhanya (2000) reported that there was maximum reduction of bacterial blight of anthurium when four rounds of spraying of turmeric powder-baking soda (0.15 per cent) was given. The disease was not observed in plants when five rounds of turmeric powder-baking soda (0.15 per cent) was sprayed.

Treatment with *T. harzianum* significantly improved the shoot length, root length, number of leaves, leaf area, fresh weight and dry weight of amaranthus. Shoot length increased up to 24.00 per cent and root length up to 66.00 per cent due to the treatment. Ganesan *et al.* (2000) found that application of *Trichoderma* significantly increased root

dry weight in pepper cuttings when compared to untreated control. Dubey (2000) reported that foliar spray of *T. virens* and *T. viride* significantly increased the root length, number of root nodules and grain yield in urd and mung beans. The increased growth parameters by application of *Trichoderma* spp. may be due to the biological control of minor plant pathogens or by production of growth regulatory metabolites (Chang *et al.*, 1986; Windham and Baker, 1986). The effect of rice husk ash (1000 g), *P. fluorescens* B_3 and *T. harzianum* on the shoot length, root length, number of leaves and leaf area of amaranthus is given in Fig. 2.

Soil application followed by one per cent foliar spray of the talc based formulation of *T. harzianum* was found effective in checking the disease. Treatment with *T. harzianum* significantly reduced the disease intensity to 42.00 per cent when compared to 24.00 per cent with mancozeb application. Smitha (2000) found that combining soil application followed by foliar spray was the most effective method in the delivery of formulation of *T. longibrachiatum* for checking foliar blight of amaranthus. Saikia and Gandhi (2003) reported that among the three antagonists – *T. viride, T. virens* and *T. harzianum* tested for their biocontrol ability against *R. solani* causing stem rot of cauliflower, *T. viride* protected 37.50 per cent seedlings from infection when applied at the rate of 10 g/kg soil whereas *T. harzianum* and *T. virens* protected 15.00 per cent and 22.50 per cent seedlings. The effect of rice husk ash (1000g), *P. fluorescens* B_3 and *T. harzianum* on the intensity of foliar blight of amaranthus in the first and second trials is given in Fig. 3.

Changes in the activity of defense related enzymes – PAL, PO and PPO, total phenols, amino acids and carbohydrates due to various treatments were estimated. There was an increase in total phenols due to soil application followed by foliar spray of *T. harzianum* after inoculation with the pathogen. There was no increase in carbohydrate and amino acid content due to these treatments when compared to control.

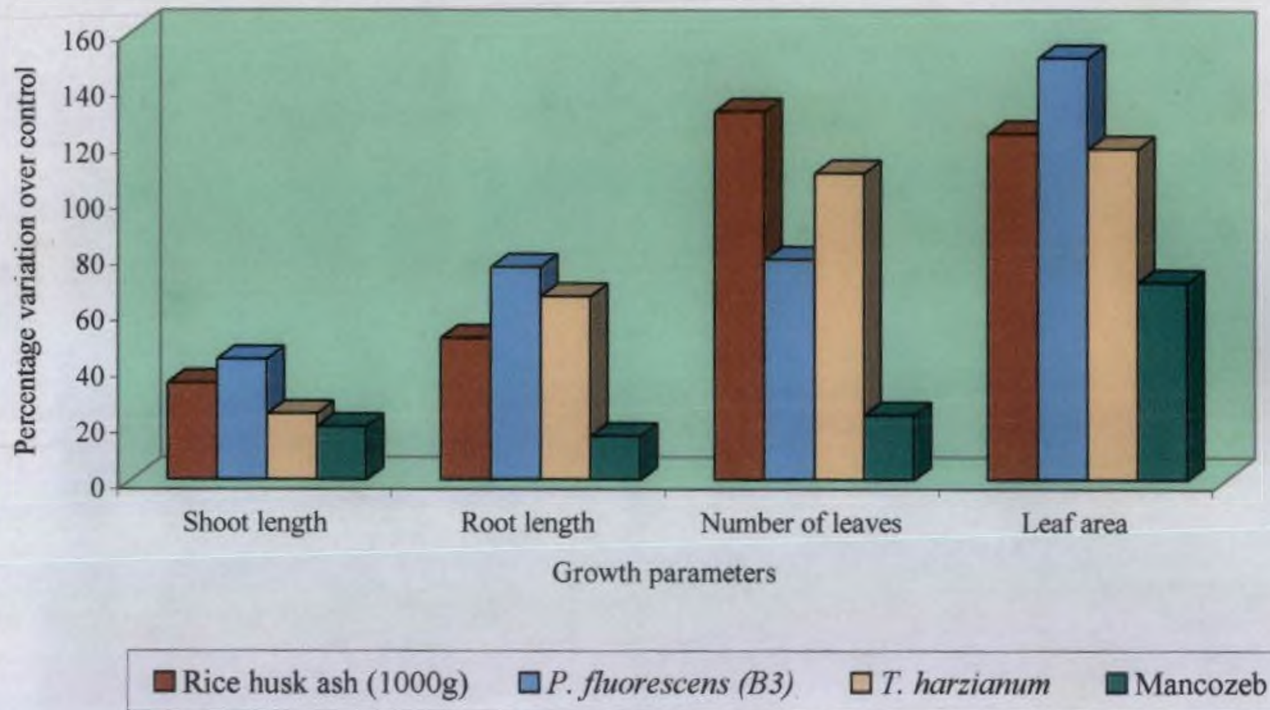


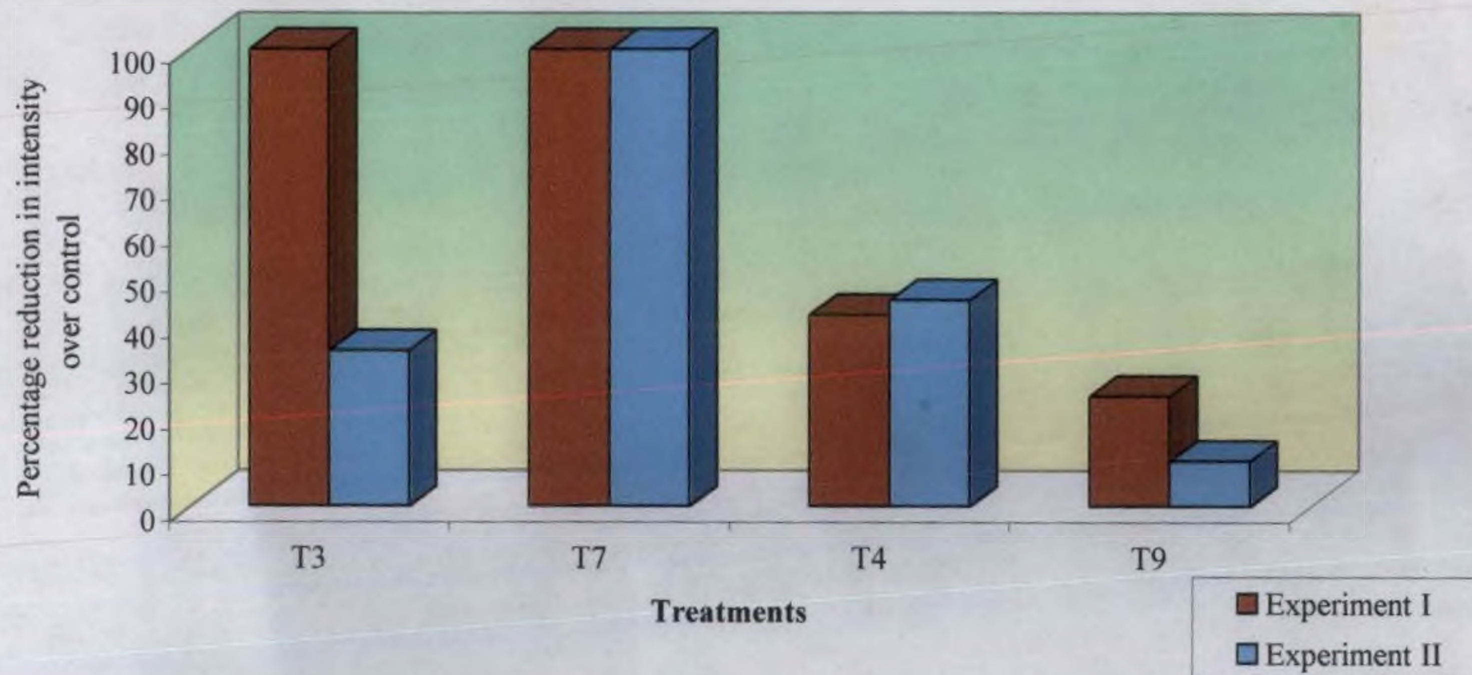
Fig. 2 Effect of Rice husk ash, *Pseudomonas fluorescens*, *Trichoderma harzianum* and mancozeb on shoot length, root length, number of leaves and leaf area of amaranthus

There was a significant increase in PAL activity due to treatments with *T. harzianum* and bacterial isolates – B_3 and KK_{16}. The increase in PAL activity can be correlated with the increased resistance in plants. PAL is the first enzyme of the phenol propanoid pathway which leads to the synthesis of phenolics and is the most important enzyme in inducing disease resistance in crop plants. Meena *et al.* (1999) found that spraying rice leaves with *P. fluorescens* increased PAL activity one day after treatment.

Peroxidase activity was significantly enhanced due to treatments with *T. harzianum* and bacterial isolates. Yedidia *et al.* (1999) reported that chitinase and peroxidase activities were increased in both root and leaf tissues of cucumbers grown in the presence of *T. harzianum* strain 203. Sivakumar and Sharma (2003) reported that maize plants raised from *P. fluorescens* treated seeds showed higher activity of PO, PPO and PAL. Peroxidase enzyme oxidises phenolics to highly toxic quinones resulting in disease resistance (Vidhyasekaran, 1988). A similar increase in PPO activity was also observed due to treatments with bacterial and fungal antagonists.

The newly discovered root endophyte, *P. indica* has successfully improved the root growth and root dry weight of amaranthus. This fungus has been isolated from desert soil and therefore is a promising candidate for an attempt to improve the drought resistance in crop plants. This would be a milestone in the development of agriculturally important plants for the arid regions. The ability of *P. indica* to detoxify xenobiotics or heavy metals could be used to grow plants in symbiosis with *P. indica* in areas where the contamination of the soil leads to environmental problems.

In this study, effective control of the disease and enhancement of growth could be obtained with the highest concentration of rice husk ash. The biocontrol agents like *T. harzianum* and *P. fluorescens* were highly



T3 - Rice husk ash (1000 g)
T7 - *P. fluorescens* (B3)
T4 - *T. harzianum*
T9 - Mancozeb (0.4 %)

Fig. 3 Effect of Rice husk ash, *Pseudomonas fluorescens* , *Trichoderma harzianum* and mancozeb on the intensity of foliar blight of amaranthus

effective in managing the disease and improving the growth of amaranthus. The results of this study indicate that the efficient strains of *T. harzianum* and *P. fluorescens* (soil or foliar application) can be used in tandem with rice husk ash to evolve a suitable ecofriendly disease management strategy to combat foliar blight of amaranthus.

SUMMARY

6. SUMMARY

Foliar blight caused by *Rhizoctonia solani* has recently emerged as a serious disease affecting amaranthus in Kerala. The high variability of the pathogen has made it difficult to develop resistance in the crop varieties. The chemical control measures though effective is costly and poses severe threat to human health and environment. Therefore the present investigation was carried out with the objective of evolving an ecofriendly practice for the management of the disease using biocontrol agents like *Trichoderma harzianum*, *Pseudomonas fluorescens*, a newly discovered root endophyte. *Piriformospora indica* and indigenous materials like turmeric powder-baking soda and rice husk ash.

The pathogen was isolated from ten different locations in Thiruvananthapuram district and from among these isolates, isolate R_3 (Vellayani) was selected for the study since it incited maximum disease symptoms. *In vitro* studies were carried out to test the effect of biocontrol agents and indigenous materials on *R. solani*. Among the four isolates of *Trichoderma* tested against *R. solani*. *T. harzianum* emerged as the most potential antagonist causing maximum inhibition of the pathogen. The fungus, *P. indica* when subjected to dual culture with *R. solani* did not exert any inhibitory effect on its growth.

The four selected isolates of *P. fluorescens viz.*, P_1, P_5, B_3 and KK_{16} were found to exert significant inhibition on the growth of *R. solani*. Among the four isolates, maximum inhibition was shown by the isolate B_3 followed by the isolate KK_{16}.

The culture filtrate of the isolate of *P. fluorescens viz.*, B_3 alone exerted inhibitory effect on the growth of the pathogen.

The different combinations of turmeric powder-baking soda did not inhibit the growth of the pathogen. The three concentrations of rice husk ash also did not exert any inhibitory effect on the growth of the pathogen.

Pot culture experiments were conducted to assess the efficacy of biocontrol agents and indigenous materials in managing the disease and enhancing the growth of amaranthus. The efficient biocontrol agents, *T. harzianum* and *P. fluorescens* were formulated in talc and used for the experiment. Among the three concentrations of rice husk ash tested, the highest concentration of 1000 g was found to be superior in enhancing growth and suppressing disease. The bacterial isolates, B_3 and KK_{16} were found effective in checking the disease and improving growth. The fungal antagonist, *T. harzianum* was also found successful in managing the disease and enhancing the growth. Among the three combinations of turmeric powder-baking soda, 10:1 combination was effective in improving the growth and reducing the disease incidence in amaranthus. The fungus *P. indica* though not found effective in controlling the disease exerted significant effects on root length and root dry weight of amaranthus. The recommended control measure of spraying 0.40 per cent mancozeb in cowdung supernatant was effective in reducing the disease intensity but the reduction was less when compared to the efficient biocontrol agents and highest concentration of rice husk ash.

Changes in the levels of total phenols, amino acids, carbohydrates and activities of defense related enzymes – PAL, PO and PPO due to the above treatments were estimated. Soil application followed by foliar spray of fungal and bacterial antagonists enhanced the activity of enzymes after inoculation with the pathogen. The level of total phenols was found to be higher for plants treated with *T. harzianum*. However, there was no significant increase in the total amino acid and carbohydrate content due to these treatments.

REFERENCES

7. REFERENCES

Abraham Mathew. K. and Gupta. S.K. 1998. Biological Control of Root Rot of French bean caused by *Rhizoctonia solani*. *J. Mycol. Pl. Pathol.* 28: 202-203

Anandaraj, M., Diby, P., Jisha, P.J., Kumar, A., Saju, K.A., Thankam, C.K. and Sarma, Y.R. 2003. Potential of a consortium of Plant Growth Promoting Rhizobacteria and *Trichoderma* for effective nursery management in Black pepper (*Piper nigrum* L.) Proceedings of the Sixth International Workshop on Plant Growth Promoting Rhizobacteria. Oct 5-10. 2003 (eds. Reddy.M.S.. Anandaraj, M., Eapen,S.J., Sarma, Y.R Kloepper, J.W.). Indian Institute of Spice Research, Calicut pp. 8-11

Anith, K.N. and Manomohandas. T.P. 2001. Combined application of *Trichoderma harzianum* and *Alcaligenes* sp strain AMB8 for controlling nursery root disease of black pepper. *Indian Phytopath.* 54: 335-339

Arora, Y.K. 1983. Metabolic changes in mungbean (*Vigna radiata*) due to *Rhizoctonia solani* Kuhn infection. *Plant Physiol. Biochem.* 10 : 40-45

*Arora, Y.K. and Bajaj. K.L. 1978. Phenolic changes in mung infected by *Rhizoctonia solani*. *Acta Phytopath. Acad. Sci. Hung.* 13 : 337-341

Arora, Y.K. and Bajaj. K.L. 1985. Peroxidase and Polyphenol oxidase associated with induced resistance of mungbean to *Rhizoctonia solani* Kuhn. *Phytopath. Z.* 114 : 325-331

Avdiushko, S.A., Ye, X.S. and Kuc, J. 1993. Detection of several enzymatic activities in leaf prints of cucumber plants. *Physiol. Mol. Plant Pathol.* 42 : 441-454

Baker, K.F. 1970. Types of Rhizoctonia diseases and their occurrence. *Rhizoctonia solani. Biology and Pathology* (ed. Parmeter. J.R). University of California Press, Berkeley, p. 255

*Barbosa, M.A.G.. Michereff, S.J., Mariano, R.L.R. and Maranchao. E. 1995. Biological control of *Rhizoctonia solani* in cowpea by seed treatment with fluorescent *Pseudomonas* spp. *Summa Phytopathologica* 21 : 151-157

Beckman, G.H. 2002. Phenolic storing cells. Key to programmed cell death and periderm formation in wilt disease resistance in defense responses in plants. *Physiol. Mol. Plant Pathol.* 57 : 101-110

Bhuvaneswari.V and Subbarao,M. 2001. Evaluation of *Trichoderma viride* antagonistic to post harvest pathogens on mango. *Indian Phytopath.* 54 : 493-494

Blakeman. J.P. and Fokkema, N.J. 1982. Potential for biological control of plant disease on the phylloplane. *Annu. Rev. Phytopathol.* 20 : 167-192

*Blechert, O.. Kost, G., Hassel, A., Rexer, K.H. and Varma, A. 1999. First remarks on the symbiotic interactions between *Piriformospora indica* and terrestrial orchids. *Mycorrhizae: Structure, function molecular biology and biotechnology* (eds. Varma, A. and Hock, B). Second edition. Springer, Berlin Heidelberg, New York, pp. 683-688

Bonner, J. 1950. *Plant Biochemistry.* Academic Press, New York, p. 537

Borah. R.C.. Bajaj, K.L. and Bhatia, I.S. 1978. Biochemical changes in tea leaves after infection with red rust, *Cephaleuros parasiticus. Phytopath. Z.* 93 : 208-217

Borowicz, J.J. and Omer,S. Z. 2000. Influence of rhizobacterial culture media on plant growth and on inhibition of fungal pathogens. *BioControl* 45 : 355-371

Bray, G.G. and Thorpe, W.V. 1954. Analysis of phenolic compounds of interest in metabolism. *Methods Biochem. Anal.* 1 : 27-52

Bunker, R.N. and Mathur, K. 2001. Antagonism of local biocontrol agents to *Rhizoctonia solani* inciting dry root rot of chilli. *J. Mycol. Pl. Pathol.* 31: 50-52

Burr, T.J., Schroth, M.N. and Suslow, T. 1978. Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *Pseudomonas putida*. *Phytopathology* 68 : 1377-1383

Capper, A.L. and Higgins, K.P. 1993. Application of *Pseudomonas fluorescens* isolates to wheat as potential biocontrol agents against take all. *Plant Pathology* 42 : 560-67

Chang,Y., Baker,O and Chet,I 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. *Plant Dis.* 70 :145-148

CharithaDevi, M. and Reddy, M.N. 2002. *In vitro* screening of effective biocontrol agents against *Rhizoctonia solani*. *J. Mycol. Pl. Pathol.* 32 : 399

Chattopadhyay, S.B. and Bera, A.K. 1980. Phenols and Polyphenol oxidase activity in rice leaves infected with *Helminthosporium oryzae Phytopath. Z.* 98 : 59-63

Chattopadhyay, A.K. and Sammadar, K.R. 1980. Effects of *Helminthosporium oryzae* infection and ophiobolin on the phenol metabolism of host tissues. *Phytopath. Z.* 98 : 193-202

Chet, I. and Elad, Y. 1982. Prevention of plant infection by biological means. Le Calloquesde I' INRA, Bordeaux, France 21-26 March :195-204

Cook, R.J. and Rovira, A.D. 1976. The role of bacteria in the biological control of *Gaeumannomyces gramines* by suppressive soils. *Soil Biol. Biochem.* 8 : 569-571

Dave, B.P. and Dube, H.C. 2000. Detection and chemical characterisation of siderophores of rhizobacterial fluorescent pseudomonads *Indian Phytopath.* 53 : 97-98

Defago, G., Berling, C.H., Burger, U., Hass, D., Kahr, G., Keel, C., Voisard, C., Wirthner, P. and Wuthrich, B. 1990. Suppression of black root rot of tobacco and other root diseases by strains of *Pseudomonas fluorescens*: Potential applications and mechanisms. *Biological Control of Soil Borne Plant Pathogens* (ed. Hornby, D.) Wallingford, Oxon, U.K, CAB International : 93-108

Devi, T.V., Malarvizhi, R., Sakthivel, N. and Gnanamanickam, S.S. 1989. Biological control of sheath blight of rice in India with antagonistic bacteria. *Plant and Soil.* 119 : 325-330

Dhanapal, N. 1975. Studies on certain aspects of sesame leaf spot of rice caused by *Helminthosporium oryzae.* Breda de Haan. M.Sc thesis, Annamalai University, Annamalai Nagar, Tamil Nadu, p.82

Dhanya, M.K. 2000. Etiology and management of bacterial blight of Anthurium (*Anthurium andreanum* Linden). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p. 72

Dickerson, D.P., Pascholati, S. F., Hagerman, A.E., Butler, L.G. and Nicholson, R.L. 1984. Phenyl alanine ammonia lyase and hydroxy cinnamate : COA ligase in maize mesocotyl inoculated with *Helminthosporium maydis* or *Helminthosporium carborum. Physiol. Plant Pathol.* 25 : 111-123

Dileepkumar, B.S. 2002. Disease suppression and growth promotion in leguminous crop plants by a plant growth promoting rhizobacteria and its bioactive metabolites in presence of a rhizobial strain. *J. Mycol. Pl. Pathol.* 32 :404

Dixon, R.A. and Fuller, K.W. 1976. Effects of synthetic auxin levels on phaseollin production and phenylalanine ammonia lyase activity in tissue cultures of *Phaseolus vulgaris. Physiol. Plant Pathol.* 9 : 299-301

Dowling,D.N. and O'Gara.F.1994. Metabolites of Pseudomonas involved in biological control of plant diseases. *TIBTECH* 12:133-141

Dubey, S.C. 2000. Biological management of Web blight of groundnut (*Rhizoctonia solani*). *J. Mycol. Pl. Pathol.* 30 : 89-90

Dubey, S.C. 2002. Evaluation of *Trichoderma virens* and *Trichoderma viride* as foliar spray against Web blight of Urd and Mung. *J. Mycol. Pl. Pathol.*32 : 236-237

Dubey, S.C. and Patel, B. 2001. Evaluation of fungal antagonists against *Thantephorus cucumeris* causing web blight of urd and mung bean. *Indian Phytopath.* 54 : 206-209

*Duggar, B.M. 1915. *Rhizoctonia crocorum* (Pers) DC and *Rhizoctonia solani* Kuhn (*Corticium vagum* B and C) with notes on other species. *Ann. Mo. Bot. Gd.* 2 : 403-458

Elad, Y., Chet, I. and Henis, Y. 1981 a. Biological control of *Rhizoctonia solani* in straw berry fields by *Trichoderma harzianum*. *Plant and Soil* 60 : 245-254

Elad, Y., Hadar, Y., Chet, I. and Henis, Y. 1981 b. Biological control of *Rhizoctonia solani* by *Trichoderma harzianum* in carnation. *Plant Dis.* 65 : 675-671

Elad, Y., Kalfon, A. and Chet, I. 1982. Control of *Rhizoctonia solani* in cotton by seed coating with *Trichoderma* species. *Plant and Soil* 66 : 279-281

Fehrman, H. and Dimond, A.E. 1967. Peroxidase activity and Phytophthora resistance in different organs of the potato plant *Phytopathology* 57 : 69-72

Galindo, J.J., Abawi, G.S., Thurston, H.D. and Galvez, G. 1983. Source of inoculum and development of bean web blight in Costa Rica. *Plant Dis.* 67 : 1016-1021

Ganesan, G., Ravishankar, H and Bhargava, B.S. 2000. Mass multiplication of *Trichoderma harzianum.Proceedings of the International Conference on Integrated Plant Disease Management for Sustainable Agriculture.Vol I Nov.11-15* (ed. Jain, R.K.) Indian Phytopathological Society, New Delhi pp. 333-334

Gangopadhyay, S. 1998. *Curcuma longa* -an ecofriendly ingredient to control soil borne diseases. *Curr. Sci.* 75 : 16-17

*Gasoni, L. Cozzi, J and Kobayashi, K. 1998. Survival of potential biocontrol bacteria in various formulations and their ability to reduce radish damping off caused by *Rhizoctonia solani*. *Zeitschrift fur pflanzen krankheiten und pflanzenschutz* 105: 41-48

Girija,V.K.1993.Rhizoctonia solani Kuhn: Infection process in Rice.Ph.D thesis, Indian Agricultural Research Institute, New Delhi, p.139

*Gnanamanickam, S.S. and Mew, T.W. 1992. Biological control of blast disease of rice (*Oryza sativa* L.) with antagonistic bacteria and its mediation by a *Pseudomonas* antibiotic *Ann. Phytopath. Soc. Jpn.* 58 : 380-385

Gokulapalan, C. and Nair, M.C. 1984. Antagonism of a few fungi and bacteria against *Rhizoctonia solani* Kuhn. *Indian J. Microbiol.* 24 : 57-58

Gokulapalan, C. Reghunath, P., Celine, V.A. and Ramachandran Nair, S. 1999. Managing leaf blight on amaranth. *Indian Hort.* 44 : 33

Gokulapalan, C., Kamala Nayar and Umamaheswaran, K. 2000. Foliar blight of Amaranthus caused by *Rhizoctonia solani* Kuhn. *J. Mycol. Pl. Pathol.* 30: 101-102

Gokulapalan,C. 1981.Role of the Rice Root Nematode (*Hirschmaniella oryzae*) in the incidence of Sheath blight disease of Rice in Kerala. M.Sc (Ag) thesis, Kerala Agricultural University, Thrissur. p.88

Gupta, S., Arora, D.K. and Srivastava, A.K.1995. Growth promotion of tomato plants by rhizobacteria and imposition of energy stress in *Rhizoctonia solani*. *Soil. Biol. Biochem.* 27 : 1051-1058

Hadar, Y., Chet, I. and Henis, Y. 1979. Biocontrol of damping off with wheat bran culture of *Trichoderma harzianum*. *Phytopathology* 69 : 64-68

Hammerschmidt, R., Nuckles, E.M. and Kuc, J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol. Pl. Pathol.* 20 : 73-82

Harman, G.E., Chet, I. and Baker, R. 1980. *Trichoderma hamatum* effects on seedling disease induced in radish and pea by *Pythium* sp. or *Rhizoctonia solani*. *Phytopathology* 70 : 1167-1172

Hedge, J.E. and Hofreiter, B.T. 1962. *Carbohydrate Chemistry 17* (eds. Whistler, R.L. and Bemiller, J.N) Academic Press, New York, p.125

Heera, G. 2002. Evaluation of fluorescent pseudomonads for the management of sheath blight and bacterial blight of rice (*Oryza sativa* L.). M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, p. 53

Hofte,M., Boelens, J. and Verstrete, W. 1991. Seed protection and promotion of seedling emergence by plant growth beneficial *Pseudomonas* strains 7NSK 2 and ANP 15. *Soil. Biol. Biochem.* 23 : 407-410

Howell, C.R. and Stipanovic, R.D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. *Phytopathology* 69 : 480-482

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

Howie, W.J. and Suslow, T.V. 1991. Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in the cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. *Mol. Plant Microbe Interact.* 4 : 393-399

*Izhar, I., Siddiqui, I., Syed Ehteshamul- Haque and Ghaffar,A. 1999. Multiplication of PGPR for the control of root rot diseases of crop plants. *Pakist. J. Bot.* 31 : 397-405

Jana, B.K., Laha, S.K. and Khatua, D.C. 1990. Leaf blight disease of vegetables and ornamental crops under high rainfall zone of West Bengal and its control possibilities. *J. Mycopathol. Res.* 28 : 51-55

Jebakumar, R.S., Anandaraj, M. Sharma, Y.R. 2001. Induction of pathogenesis related proteins and defense related enzymes in black pepper due to inoculation with *Phytophthora capsici*. *Indian Phytopath.* 54 : 23-28

Jeyarajan, R. and Ramakrishnan, G. 1995. Mass production of *Trichoderma viride* for biological control. *Indian J. Mycol. Pl. Pathol.* 25 : 125

Joshi, A.B. 2002. Greener revolution without chemical fertilizers. *Asian. Agri. History* 6 : 43-62

Kaiser, W.J., Hannan, R.M. and Weller, D.M. 1989. Biological control of seed rot and pre emergence damping- off of chickpea with *fluorescent pseudomonads*. *Soil. Biol. Biochem.* 21 : 269-273

Kamala Nayar, Gokulapalan, C. and Nair, M.C. 1996. A new foliar blight of Amaranthus caused by *Rhizoctonia solani*. *Indian Phytopath.* 49 : 407

Kamala Nayar,1996. Development and evaluation of a bio pesticide formulation for control of foliar pathogens of rice. Ph.D thesis, Tamil Nadu Agricultural University, Coimbatore, p:160

Kasugé, T. 1969. The role of phenolics in host response to infection. *Annu. Rev. Phytopathol.* 7 : 195

Katznelson, H. and Cole, S.E. 1965. Production of gibberellin substances by bacteria and actinomycetes. *Can. J. Microbiol.* 11: 733-741

KAU. 1996. Management of important pests and diseases of amaranthus. *Research Report.* Kerala Horticulture Development Programme (R&D) Vellanikkara, Thrissur, Kerala, India, p.33

Khan, T.A. and Hussain, S.I. 1991. Studies on the toxicity of culture filtrates of different fungi on the growth of *Rhizoctonia solani*. *New Agriculturist* (2) : 107-110

Kloepper, J.W. and Schroth, M.N. 1978. Plant growth promoting rhizobacteria on radish. Proceedings of seventh International Conference on Plant Pathogenic Bacteria Vol, 11 pp. 879-882

Kloepper, J.W. Schroth, M.N. and Miller, T.D. 1980. Effect of rhizosphere colonisation by plant growth promoting rhizobacteria to plant growth and displacement of root microflora. *Phytopathology* 70 : 1078-1082

Kloepper,J.W. and Schroth, M.N. 1981. Relationship of *in vitro* antibiosis of plant growth promoting rhizobacteria to plant growth and displacement of root microflora. *Phytopathology* 71 : 1020-1024

Koike, S.T. and Subbarao, K.V. 1999. Leaf blight of Endive and Escarole, caused by *Rhizoctonia solani* in California. *Plant Dis.* 83 : 1070

Kumaresan, S. and ManibhushanRao, 1991. Studies on the biological control of rice sheath blight disease. *Indian J. Plant Pathol.* 9 : 64-70

Laha, G.S. and Verma, J.P. 1998. Role of fluorescent pseudomonads in the suppression of root rot and damping off of cotton. *Indian Phytopath* 51 : 275-278

Laha,G.S.,Venkataraman,S. Reddy,A.P.K 2000. Alternate approaches in the management of sheath blight of rice. Proceedings of the International Conference on Integrated Plant Disease Management for Sustainable Agriculture. Vol I Indian Phytopathological Society, New Delhi. pp.225-226

Lee and Le Tourneau, D.J. 1958. Chlorogenic acid content and Verticillium wilt resistance of potatoes. *Phytopathology* 48 : 268-274

Leong, J. 1986. Siderophores – their biochemistry and possible role in the biological of plant pathogens. *Annu. Rev. Phytopathol.* 24 : 187-209

Lewis,J.A and Papavizas,G.C.1985. Effect of mycelial preparation of *Trichoderma* and *Gliocladium* on population of *Rhizoctonia solani* and the incidence of damping off. *Phytopathology* 75:812-817

Lifshitz, R., Lifshitz, S. and Baker, R. 1985. Decrease in incidence of Rhizoctonia pre emergence damping off by use of integrated chemical and biological control. *Plant Dis.* 69 : 431-434

*Lin, B.T., Wu. S.Z., Xu, V.M. and Mew, T.W. 1992. Screening of antagonistic bacteria for biocontrol of rice sheath blight Chinese. *J. Rice. Sci.* 6 : 77-82

*Mah, S.Y., Cheah, U.B. and Syed, A.R. 1988. Masalah sisa racun kulat pada sayur-sayurandi semenanjung Malaysia MARDI Teknol. *Sayur-Sayuran Ltd* 4 : 39-45

Mahadevan,A.1966. Biochemistry of infection and resistance. *Phytopath. Z.* 57 : 96-96

Marshall. D.S. 1982. Effect of *Trichoderma harzianum* seed treatment and *Rhizoctonia solani* inoculum concentration and damping off on snap bean in acidic soils. *Plant Dis.* 66 : 788-789

Maxwell, D.P. and Bateman, D.F. 1967. Changes in the activities of some oxidases in extracts of Rhizoctonia infected bean hypocotyls in relation to lesion maturation. *Phytopathology* 57 : 132-136

Mayee, C.D. and Datar, V.V. 1986. *Phytopathometry.* Technical Bulletin-1 (*Special Bulletin-3*), Marathwada Agricultural University, p. 146

Mayer, A.M., Harel, E. and Shaul, R.B. 1965. Assay of catechol oxidase-a critical comparison of methods. *Phytochemistry* 5 : 783-789

*Meena, B., Radhajeyalakshmi, R., Vidhyasekaran, P. and Velazhahan, R. 1999. Effect of foliar application of *Pseudomonas fluorescens* on activities of phenylalanine ammonia lyase, chitinase, B, 1, 3 glucanase and accumulation of phenolics in rice. *Acta Phytopathologica et Entomologica Hungarica* 34 : 307-315

Meena, B., Ramamoorthy, V., Muthuswamy, M. 2001. Morphological and pathological variations in isolates of *Rhizoctonia solani* causing sheath blight of rice. *Pl. Dis. Res.* 16 : 166-172

Mehta, R.D., Patil, K.A., Rao, K.K. and Mehta, M.H. 1995. *Trichoderma harzianum* as biological control agent in formulated product. *Indian J. Mycol. Pl. Pathol.* 25 : 129

Mew, T.W. and Rosales, A.M. 1986. Bacterization of rice plants for control of sheath blight caused by *Rhizoctonia solani*. *Phytopathology* 76 : 1260-1264

Mohan, R. 1976. Studies on the sheath rot of rice caused by *Acrocylindrium oryzae*. Sawada. M. Sc. (Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore, p.78

Mondal, K.K., Singh, R.P., Dureja, P. and Verma, J.P. 2000. Secondary metabolites of cotton rhizobacteria in the suppression of bacterial blight of cotton. *Indian Phytopath.* 53 : 22-27

Mukherjee, P.K. and Ghosh. J.J. 1975. Phenol oxidase activity in relation to resistance of rice to infection by *Helminthosporium oryzae*. *Sci. Cult.* 411 : 433-434

Mukherjee, P.K. and Mukhopadhyay, A.N. 1995. In situ myco-parasitism of *Gliocladium virens* on *Rhizoctonia solani*. *Indian Phytopath.* 48 :101-102

Muthamilan, M. 1994. Management of diseases of chickpea and rice using fluorescent pseudomonads. Ph.D thesis, Tamil Nadu Agricultural University, Coimbatore, p. 182

Nandakumar, R., Babu,S., Viswanathan, R., Raghuchander, T. and Samiyappan, R. 2001. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. *Soil Biol. Biochem.* 33 : 603-612

Oku, H. 1967. Role of parasitic enzymes and toxins in development of characteristic symptoms in plant disease *The Dynamic Role of Molecular constituents in plant parasite interactions*. (eds. Mirocha, C.J. and Uritani. I.). Bruce Publishing Co., Minneapolis, Minn, pp. 237-253

*Padhi,. B. and Chakrabarti, N.K. 1984. Carbohydrate changes in rice plants inoculated with races of *Pyricularia oryzae Cav. Oryzae* 21 : 170-172

Padmakumary, G. 1989. Survival of *Rhizoctonia solani* Kuhn with special reference to antagonistic soil microflora. Ph.D. thesis, Kerala Agricultural University, Thrissur, p. 159

*Palo, M.A.1926. Rhizoctonia diseases of rice:A study of the disease and the influence of certain conditions upon the viability of sclerotial bodies of the causal fungus. *Philippine Agric.* 15 : 361-376

Papavizas. G.C. 1985. *Trichoderma* and *Gliocladium*. Biology and potential for biocontrol. *Annu. Rev. Phytopathol.* 23 : 23-54

Papavizas, G.C., Dunn, M.T., Lewis J.A. and Beagle-Ristaino, J. 1984. Liquid fermentation technology for experimental production of biocontrol fungi. *Phytopathology* 74 : 1171-1175

Parmeter, J.R. Jr and Whitney, H.S. 1970. Taxonomy and nomeclature of the imperfect state *Rhizoctonia solani Biology and Pathology*. (ed. Parmeter, J.R.Jr). University of California Press, Berkeley, p 255

Patil, S.S., Powelson, R.L. and Young, R.A. 1962. The relation of chlorogenic acid and total phenols in potato to infection by *Verticillium albo-atrum*. *Phytopathology* 52 : 364-369

Phillip, J.M. and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55 : 158-161

Podile, A.R., Kumar, B.S.D. and Dube, H.C. 1990. Antibiosis of rhizobacteria against some plant pathogens. *Indian J. Microbiol.* 28 : 108-111

Prasad , R.D. and Rangeshwaran, R. 2000. Effect of soil application of granular formulation of *Trichoderma harzianum* on *Rhizoctonia solani* incited seed rot and damping off of chickpea. *J. Mycol. Pl. Pathol.* 30 : 216-220

Prasad, R.D., Rangeshwaran, R. and Sreeramakumar, P. 1997. Mass production of *Trichoderma harzianum* and itself life in different formulations. I.P.S. Golden Jubilee International Conference Nov. 10-15, 1999, India. *Abstract* : 212

*Qui, X., Pei, Y., Wang, Y.N. and Zhang, F.X. 1990. Isolation of pseudomonads from cotton plants and their effect on seedling diseases. *Acta Phytophylaica Sinica* 17 : 303-306

Rabindran, R. 1994. Biological control of rice sheath blight caused by *Rhizoctonia solani* and blast caused by *Pyricularia oryzae* using *Pseudomonas fluorescens*. Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore, p. 181

Rai, M., Acharya, D., Singh, A. and Varma, A. 2001. Positive growth responses of medicinal plants *Spilanthus calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. *Mycorrhiza*. 11 : 123-128

Raji, P. and Lekha, B.N. 2003. *Pseudomonas fluorescens* for enhancing plant growth and suppressing sheath blight of rice. Proceedings of the 6th International Workshop on Plant Growth Promoting Rhizobacteria. Oct 5-10, pp. 208-211

Rama,S., Singh,H.V. and Singh,Y. 2000. Effect of *Trichoderma* spp. against *Rhizoctonia solani* and selection of substrates for their mass production. Proceedings of the International Conference on Integrated Plant Disease Management for Sustainable Agriculture. Vol II Indian Phytopathological Society, New Delhi pp.393-394

*Rao, M.K., Mohammed, Z. and Matsuyama, N. 1988. Phenol metabolism and plant disease resistance. *Acta Phytopath Ent. Hung.* 23 : 103-114

*Reddy, M.K. and Ravindranath, V. 1988. Polyphenols in relation to rust resistance in ground nut genotypes. *Nat. Acad. Sci.* (India) Letters 11 : 39-41

Rosales, A.M., Thomashow,L.S., Cook, R.J. and Mew, T.W. 1995. Isolation and identification of antifungal metabolites produced by rice associated antagonistic *Pseudomonas* sp. *Phytopathology* 85 : 1028-1032

Sadasivam, S. and Manickam, A. 1992. *Biochemical Methods for Agricultural Sciences*. Wiley Eastern Company, New Delhi, p. 220

Sahay, N.S. and Varma, A. 1999. *Piriformospora indica* : a new biological hardening tool for micro propagated plants. *FEMS Microbiol Let.* 181 : 297-302

Saikia, M.K. and Gandhi, S.K. 2003. Comparative activities of three antagonistic fungi against cauliflower stem rot pathogen, *Rhizoctonia solani*. *J. Mycol. Pl. Pathol.* 33 : 138-140

Saksena, A.K. and Chaubey, R.D. 1972. Banded blight disease of Paddy Rice Pathology Research at U.P.I.A.S., Kanpur. Paper presented in All India Co-ordinated Rice Improvement Project

Sakthivel, N., Sivamani, E., Unnamalai, N. and Gnanamanickam, S.S. 1986. Plant growth promoting rhizobacteria in enhancing plant growth and suppressing plant pathogens. *Curr. Sci.* 55 : 22025

Sarkar, D.K., Sharma, N.R. and Shahjahan, A.K.M. 1992. Antagonistic soil bacteria for biological control of rice sheath blight disease. *Int. Rice Res. Newsl.* 17 : 22-23

Savant, N.K. and Sawant, A.S. 1995. Nutrient composition of rice seedlings as influenced by rice hull ash application to seed bed. *Oryza* 32 : 82-84

Schippers, B., Baker, A.W. and Baker, P.A.H.M. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annu. Rev. Phytopathol.* 25 : 339-358

Schneider, S. and Ullrich, W.R. 1994. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. *Physiol. Mol. Plant Pathol.* 45 : 291-304

Shahjahan, A.K.M., Fabellar, N. and Mew, T.W. 1987. Relationship between growth rate, sclerotia production and virulence of isolates of *Rhizoctonia solani*. *Int. Rice Res. Newsl.* 12 :28-29

Shamina,A. Sarma,Y.R. 2000. B1,3-Glucanase, Peroxidase and Polyphenol oxidase in relation to tolerance of Black Pepper to *Phytophthora capsici*. Proceedings of the International Conference on Integrated Plant Disease Management for Sustainable Agriculture. Vol. II. Indian Phytopathological Society, New Delhi, p.1129-1130

Sindhan. G.S. and Jaglan. B.S. 1988. Role of phenolic compounds and carbohydrates in resistance of ground to tikka leaf spot. *Indian J. Mycol. Pl. Pathol.* 19 : 141-144

Singh, S.K., Satyanarayanan, K. and Reddy, A.P.K. 1990. Studies on morphology. growth habit. hyphal anastomosis and virulence pattern of five isolates of sheath blight pathogen of rice. *Indian Phytopath.* 43 : 368-371

Sivakumar. G. and Narayanaswamy, N.T. 1998. Biological control of sheath blight of rice with *Pseudomonas fluorescens*. *Oryza* 35 : 57-60

Sivakumar. G. and Sharma, D.C. 2003. Induced biochemical changes due to seed bacterization by *Pseudomonas fluorescens* in maize plants. *Indian Phytopath.*56: 134-137

Skidmore. A.M. and Dickinson, C.H. 1976. Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Trans.Br. Mycol. Soc.* 66 : 57-64

Smitha. K.P. 2000. Management of foliar blight of amaranthus (*Amaranthus tricolor*) caused by *Rhizoctonia solani* Kuhn using microbial antagonists. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p. 73

Snedecor, G.W. and Cochran, W.G. 1967.*Statistical Methods* edn 6: Oxford and IBH publishing Co, New Delhi, p. 69

Sridhar, R.. 1970. Physiology of the rice plant as influenced by *Pyricularia oryzae* and Nitrogen fertilization. Ph.D thesis. Annamalai University, Annamalai Nagar, Tamil Nadu, p. 172

Srivastava. S.K. 1987. Peroxidase and polyphenol oxidase in *Brassica juncea* infected with *Macrophomina phaseolina* (Tassi.) Goid and their implications in disease resistance. *J. Phytopathol.* 120 : 249-254

Sundaram. P. 1980. Studies on certain biochemical and physiological changes in rice plants as influenced by potassium fertilization and *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris* (Frank) Donk) inoculation. M. Sc. (Ag.) thesis. Annamalai University. Annamalai Nagar, Tamil Nadu, p. 115

Suslow, T.V. 1982. Role of root colonizing bacteria in plant growth *Phytopathogenic prokaryotes* Vol. 1. (eds. Mount, M.S. and Lacy, G.H.). Academic Press, London, p. 187-223

Suslow, T.V. and Schroth, M.N. 1982. Rhizobacteria of sugar beet: Effects of seed application and root colonization on yield. *Phytopathology* 72 : 199-206

Talbot. P.M.B. 1970. Taxonomy and nomenclature of the perfect stage *Rhizoctonia solani. Biology and Pathology.* (ed. Parmeter,J.R.). University of California Press, Berkeley, p.255

*Tohamy. M.R.A., El-Kader. D.A.A., Abou-Zaid, M.F. and Hanna,A.I. 1993. Effect of *Trichoderma* spp. and filtrates on some chemical components of onion plants infected with *Sclerotium cepivorum* and *Fusarium oxysporum* f. sp *cepae*. *Egyptian J. agric. Res.* 71 : 389-400

*Umaerus, V. 1959. The relationship between peroxidase activity in potato leaves and resistance to *Phytophthora infestans. Am. Potato. J.* 36 : 124-131

Varma. A., Singh, A., Sudha, N.S., Sharma, J., Roy,A.,Kumari, M., Rana, D., Thakran, S., Deka, D., Bharti, K., Hurek, T., Blechert, O., Rexer, K.H., Kost, G., Hahn, A., Maier, W., Walter, M., Strack, D. and Kranner, I. 2001. *Piriformospora indica* : an axenically culturable mycorrhizal- like endosymbiotic fungus. *Mycota IX Fungal Associations* (ed. Hock, B) Springer- Verlag,Berlin Heidelberg,New York, pp. 123-150

Varma, A., Verma, S., Sahay, N.S., Butehorn, B and Franken, P. 1999. *Piriformospora indica*, a Cultivable Plant- Growth Promoting Root Endophyte. *Appl. Environ. Microbiol.* 65 : 2741-2744

Varshney, S. and Chaube, H.S. 1999. Biocontrol potential of some selected isolates of fluorescent pseudomonads naturally occurring in rhizosphere of tomato. *Indian J. Plant Pathol.* 17 : 59-61

*Velazhahan, R., Samiyappan, R. and Vidhyasekaran, P. 1999. Relationship between antagonistic activities of *Pseudomonas fluorescens* isolates against *Rhizoctonia solani* and their production of lytic enzymes. *Zeitschrift fur Pflanzen krankheiten und Pflanzenschutz.* 106 : 244-250

Venkatasubbiah, P., Safeeulla, K.M. and Somasekhar, R.K. 1984. Efficacy of *Trichoderma harzianum* as a biocontrol agent for *Rhizoctonia solani*, the incitant of collar rot of coffee seedlings. *Proc. Ind. Natn. Sci. Acad.* Part B 50 : 525-529

Verma, S., Varma, A.,Rexer,K.H., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Butehorn, B. and Franken, P.1998. *Piriformospora indica*,gen.et sp.nov.,a new root colonizing fungus. *Mycologia* 90 :896-903

Vidhyasekaran, P. 1974. Changes in phenolic contents in ragi leaves due to susceptible and resistant helminthosporiose disease reactions. *Indian J. Exp. Biol.* 12 : 592-596

Vidhyasekaran, P. 1988. *Physiology of disease resistance in plants.* Vol. I. CRC press, Florida (USA)

Vidhyasekaran, P. and Muthamilan, M. 1999. Evaluation of powder formulation of *Pseudomonas fluorescens* Pf 1 for control of rice sheath blight. *Biocon. Sci. Technol.* 9 : 67-74

Vidhyasekaran, P. and Muthamilan, M.1995. Development of formulation of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis.* 79 : 782-786

Vijayakumar, C.S.K. and Rao, A.S. 1980. Physiological changes in Alternaria infected wheat leaves. *Phytopath. Z.* 98 : 76-83

Vijayan, M. 1986. Strain variation in *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris* (Frank) Donk) causing sheath blight of rice. Ph.D thesis, Kerala Agricultural University, Thrissur, p. 130

Vincent, J.M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 159 : 850

Viswakumar, P. 1989. Biological control of *Rhizoctonia solani* Kuhn on rice using mycoparasites. M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p.72

*Walker, J.C., Link, K.P. 1935. Toxicity of phenolic compounds to certain onion bulb parasites. *Bot. Gaz.* 96 : 468-484

Wasfy, E.H., Sheir, H.M., El-meteny, A.Y. and Darweesh, M.M. 1984. Changes in peroxidase isoenzyme pattern of bean hypocotyl due to infection with *Rhizoctonia solani*. *Trans. Br. Mycol. Soc.* 82 : 154-156

Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology* 22 : 837-847

Weller, D.M. 1988. Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.* 26 : 379-407

Whipps, J.M. 1992. Status of biological disease control in horticulture. *Biocon. Sci. Technol* 2 : 3-24

*Williams, G and Williams, P. 1993. Baking soda vs powdery mildew. Not a new idea ! *Hort. Ideas* p. 62

172205

*Williams.G and Williams. P. 1985. Baking soda for powdery mildew control. *Hort. Ideas* : 101-102

Windham.M.T and Baker.R.1986. A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopathology*. 76:518-521

*Wolk. M. and Sarkar. S. 1993. Antagonism *in vitro* of fluorescent pseudomonads against *Rhizoctonia solani* and *Pythium aphanidermatum*. *Zentralblatt for Mikrobiologie* 148: 237-248

Wu, L.C. 1972. *Biochemical Regulation in Diseased Plants on Injury* (eds. T. Hirai. Z. Hidaka and I. Uritani), p. 321-334

Wu, L.C. 1973. Changes in some enzymes of mungbean seeds germinated on mycelial macerates of *Rhizoctonia solani*. *Physiol. Pl. Path.* 3 : 19-27

Wu, W.S. 1980. Biological and chemical seed treatments of soybeans (*Trichoderma pseudokoningii* and *Trichoderma harzianum*) *Mem. Coll. Agric. Natn.* Taiwan Univ. 20 : 1-16

Yamamoto, H., Hoken, H. and Tani, T. 1978. Peroxidase and polyphenol oxidase in relation to crown rust resistance of oat leaves. *Phytopath. Z.* 91 : 193-202

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

Zacharia, G. 1990. Integrated management of sheath blight disease of rice. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p. 104

Ziy. O. and Zitter, T.A. 1992. Effects of bicarbonates and film forming polymers on cucurbit foliar diseases. *Plant Dis.* 76: 513-517

---

*Original not seen

APPENDICES

APPENDIX - I

Composition of different media

A) Potato dextrose agar

Potato	:	200.00 g
Dextrose	:	20.00 g
Agar	:	20.00 g
Distilled water	:	1 litre

B) King's B medium

Peptone	:	20.00 g
Dipotassium		
hydrogen phosphate	:	1.50 g
Magnesium sulphate	:	1.50 g
Glycerol	:	10 ml
Distilled water	:	1 litre

Adjust pH to 7.2

APPENDIX – II

Preparation of stain

A) Trypan blue stain

Trypan blue	-	0.05 g
Lactophenol	-	100 ml

B) Lactophenol

Lactic acid	-	20 ml
Phenol	-	20 ml
Glycerol	-	40 ml
Water	-	20 ml

APPENDIX - III

Buffers for enzyme analysis

A) 0.1 M sodium phosphate (pH 6.4)

Stock solutions

A – 1.56 g of sodium dihydrogen phosphate in 100 ml

B – 1.42 g disodium orghohydrogen phosphate in 100 ml

68.5 ml A is mixed with 31.5 ml B

B) 0.1 M sodium borate (pH 8.8)

A – 0.2 M sodium of boric acid (12.4 g in 1000 ml)

B – 0.05 m solution of borax (19.05 g in 1000 ml)

50 ml of A is mixed with 30 ml of B, diluted to a total of 200 ml

ECOFRIENDLY MANAGEMENT OF RHIZOCTONIA LEAF BLIGHT OF AMARANTHUS

PRIYADARSINI P.

Abstract of the
thesis submitted in partial fulfilment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2003

Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

ABSTRACT

The present investigation was undertaken at College of Agriculture, Vellayani during the year 2002-2003 to evolve an ecofriendly practice for the management of foliar blight of amaranthus caused by *Rhizoctonia solani*. Biocontrol agents like *Trichoderma harzianum*, *Pseudomonas fluorescens*, a newly discovered root endophyte, *Piriformospora indica* and indigenous materials like turmeric powder-baking soda and rice husk ash were used in the study.

From among the ten isolates of *R. solani*, the isolate R_3 (Vellayani) which produced maximum disease symptoms was selected for the study. Among the four fungal antagonists tested against *R. solani*, *T. harzianum* emerged as the most potential one. The fungus, *P. indica* when subjected to dual culture with *R. solani* did not cause any inhibition on its growth. Among the four isolates of *P. fluorescens* tested against *R. solani*, the isolate B_3 showed maximum inhibition followed by the isolate KK_{16}. The culture filtrate of the isolate of *P. fluorescens viz.*, B_3 alone exerted inhibitory effect on the growth of *R. solani*. The different combinations of turmeric powder-baking soda and rice husk ash did not inhibit the growth of *R. solani*.

Pot culture experiments were conducted to assess the efficacy of biocontrol agents and indigenous materials in managing the disease and enhancing the growth of amaranthus. Among the three concentrations of rice husk ash used, the highest concentration of 1000 g produced maximum disease suppression and enhancement of growth. Rice husk ash exerted significant effects on all the growth parameters. The fungal antagonist *T. harzianum* and bacterial isolates B_3 and KK_{16} were found to be highly effective in reducing the disease and enhancing growth. 10 : 1 combination of turmeric powder-baking soda was found to enhance the

growth significantly and there was a reduction in the disease incidence also. The highest concentration of *P. indica* (2.00 per cent) recorded the maximum root colonization and exerted significant effects on root length and root dry weight.

Changes in the level of total phenols, amino acids carbohydrates and enzymes like PAL, PO and PPO due to the above treatments were estimated. Soil application followed by foliar spray of fungal and bacterial antagonists significantly enhanced the activity of the defense related enzymes. The level of total phenol was found to be higher for plants treated with *T. harzianum*. There was no significant increase in amino acid and carbohydrate content due to these treatments. The results of this study indicate that the efficient strains of *T. harzianum* and *P. fluorescens* (soil or foliar application) can be used in tandem with rice husk ash to evolve a suitable ecofriendly disease management strategy to combat foliar blight of amaranthus.