ETIOLOGY AND MANAGEMENT OF STOLON ROT OF MINT (*Mentha viridis* Linn.)

Thesis submitted in part fulfilment of the requirements for the degree of
MASTER OF SCIENCE (AGRICULTURE) in **PLANT PATHOLOGY**
to the Tamil Nadu Agricultural University, Coimbatore - 641 003.

By

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CERTIFICATE

This is to certify that the thesis entitled " **ETIOLOGY AND MANAGEMENT OF STOLON ROT OF MINT** (***Mentha viridis*** **Linn.)**" submitted in part fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a record of bonafide research work carried out by **Miss. MERIN BABU** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles, prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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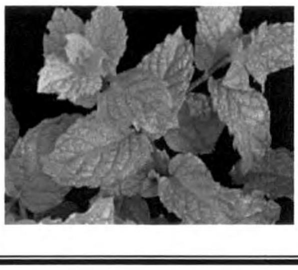

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Merin

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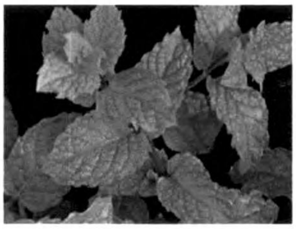

Abstract

ABSTRACT

ETIOLOGY AND MANAGEMENT OF STOLON ROT OF MINT (*Mentha viridis* Linn.)

By

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Severe incidence of stolon rot of mint (*Mentha viridis* Linn.) was noticed in some areas of Coimbatore district during the year 2001. The disease incidence varied from 16.48 to 36.81 per cent. The highest incidence was observed in Chittepalayam area of Coimbatore district. *Rhizoctonia solani* Kühn was found to be the incitant of mint stolon rot. Symptoms consisted of stolon rot and drying of shoots, which led to death of the entire plant in advanced stages. All the *Mentha* species tested *viz.*, *Mentha viridis* Linn., *Mentha citrata* Ehrh., *Mentha arvensis* Linn. var. *piperascens* Malvid. and *Mentha piperita* Linn. were found susceptible to the disease except *Mentha spicata* Huds. Stolon rot incidence reduced the essential oil content of the herb. The pathogen was capable of infecting ten crop species belonging to eight families.

A temperature of 30°C and pH levels 6.0 and 6.5 were found conducive for the *in vitro* growth of the pathogen. Disease incidence increased with increase in inoculum levels. Older plants showed higher degree of resistance to *R. solani* infection than the younger ones. The population of *R. solani* in mint rhizosphere increased with age of the plants. Oilcake extracts significantly inhibited *in vitro* growth of *R. solani.* Under glasshouse condition, application of neem cake @ 10 g/kg of soil significantly reduced the disease incidence. *Trichoderma viride* and *Trichoderma* sp. (isolate-1) were effective in inhibiting the growth of *R. solani* under *in vitro* condition. Stolon treatment with talc based formulation of *T. viride* and *Trichoderma* sp. (isolate - 1) @ 4 g/kg of stolon effectively controlled the disease. Application of oilcakes and antagonists not only reduced the disease incidence but also promoted the germination of stolons and enhanced the plant growth.

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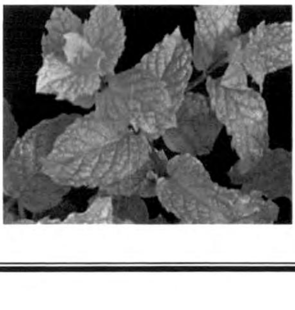


Introduction

CHAPTER I

INTRODUCTION

Mentha viridis Linn., commonly known as mint, is an aromatic perennial herb belonging to the family Lamiaceae. It is a multipurpose crop valued for its spicy flavour, aromatic and medicinal properties. The above ground herb of mint on distillation, yields essential oil containing a number of aromatic isolates like menthol, carvone, linalylacetate and linalool, which are used in pharmaceutical, perfumery and cosmetic industries.

The genus *Mentha* comprises of about 25 species. Only five species *viz.*, *Mentha arvensis* Linn.var. *piperascens* Malvid. (Japanese mint), *Mentha spicata* Huds. (Spearmint), *Mentha citrata* Ehrh. (Bergamot mint or Lemon mint), *Mentha piperita* Linn. (Pepper mint) and *Mentha viridis* Linn. (Pudina) are commercially cultivated in India (Bhattacherjee, 2000).

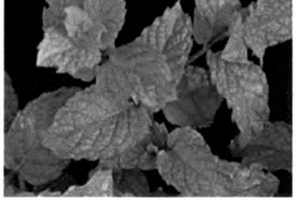
Mints are extensively cultivated in USA, Europe and South East Asian countries. In India, commercial cultivation of mint is confined to Uttar Pradesh, Punjab, Jammu and Kashmir and Tamil Nadu. Japanese mint is cultivated in an area of 50,000 ha in India (Gupta, 2001). During 1997-98 India earned 87-88 crores of foreign exchange by exporting 2525 tonnes of mint oil (Datta *et al.*, 2001). The area under mint is registering an upward trend mainly because of attractive prices both in international and domestic markets.

Crop loss due to diseases has been identified as a major production constraint for mint. Mint has been reported to be affected by several diseases like rust, powdery mildew, leaf blight, leaf spot, stolon rot and stem rot (Gupta, 1995; Jain, 1995; Shukla *et al.*, 2001). Among the various diseases, the stolon rot is very important in view of severe crop losses. The disease appears as blackening and rotting of stolon tissues, which leads to extensive drying up of shoots and leaves which in turn reduce the oil yield. In advanced stages, there will be death of the entire plant. The stolon rot occurred in a severe form in the year 2001 in mint growing areas of Coimbatore district.

Mint is propagated by stolons. Thus the reduction in stolon production by the disease will be a limiting factor for expanding commercial cultivation. Besides, the stolons obtained from the infected crop, if used for planting, may cause poor crop stand which in turn reduce the yield of green herb.

Considering the heavy losses incurred due to this disease, investigations were taken up with the following objectives:

- Survey of mint growing areas of Coimbatore district to assess the intensity of disease.
- To isolate the causal organism of the disease and establish the pathogenicity.
- To study the factors favouring pathogen and disease development.
- To assess the reaction of various species of mint to the stolon rot pathogen.
- To develop a management strategy with emphasis on use of organic amendments and biocontrol agents to combat the stolon rot of mint.



Review of Literature

CHAPTER II
REVIEW OF LITERATURE

Mint is a herb bestowed with immense medicinal values. With the rise in global demand for plant based drugs, mint cultivation has gained momentum during the last decade. Susceptibility of mint plants to pests and diseases is a major problem faced by mint cultivators. Among the various diseases, stolon rot is a major biotic constraint for the production of healthy stock of mint plants.

2.1. Pathogen and symptoms

Stolon rot has been reported to be caused by different pathogens. The association of soil-borne fungi with stolon rot of mint was first reported by Green (1961). *Rhizoctonia* spp. and *Fusarium* spp. were isolated from stolons showing distinct, slightly sunken, reddish brown lesions, which gradually became extensive resulting in stolon decay.

Husain and Janardhanan (1965) reported *Rhizoctonia bataticola (Macrophomina phaseoli)* as the causal agent of stolon rot of Japanese mint (*Mentha arvensis* ssp. *haplocalyx* var. *piperascens*). Pinkish brown lesions initially appeared on the stolons turned to dark brown - black patches which increased in size resulting in soft decay of stolons. The affected plants showed yellowing, stunting and wilting followed by death and desiccation of above ground parts.

Stolon rot of *Mentha arvensis* Linn. caused by *Thielavia basicola* (*Thielaviopsis basicola*) was first reported by Sattar and Husain (1976). The symptoms were similar to those produced by *R. bataticola* infection. Singh (1991) reported stolon rot of *M. arvensis* as a disease complex caused by two fungal pathogens – *R. solani* and *M. phaseoli*. The disease was observed during March - April at Indian Institute of

Horticultural Research Experimental Farm, Bangalore. The symptoms consisted of blackening of stolon tissues in the earlier stages, which led to extensive drying up of shoots and leaves and subsequent death of the entire plant.

2.2. *In vitro* studies on the growth of *R. solani*

2.2.1. Effect of temperature

The effect of temperature on the growth of the *R. solani in vitro* has been studied by a number of scientists. The optimum temperature for hyphal growth and sclerotial production of *R. solani,* under *in vitro* condition was reported as 30°C (Sangeetha, 1988; Lakpale *et al.*, 1997).

A temperature of 28°C was found to be optimum for growth and production of sclerotia by *R. solani* (Singh and Malhotra, 1994; Dubey, 1997). Tiwari (1997) reported 25°C as the most favourable temperature for growth of *R. solani in vitro.*

Xu *et al.* (1997) reported the optimum temperature range for the hyphal growth of *R. solani* as 26-32°C. In this temperature range, the sclerotia germinated and produced hyphae within 10–12 h.

2.2.2. Effect of pH

On potato dextrose agar, *R. solani* causing poinsettia root rot grew over a pH range of 3.9-7.5. The best growth occurred at pH 5.8 (Bateman, 1962). For *R. solani* causing web blight of winged bean, the optimum pH recorded was 6.0 (Singh and Malhotra, 1994).

Dubey (1997) reported that pathogen grew over a wide range of pH *viz.*, 3.0-10.0 and pH 6.5 supported the maximum growth and excellent sclerotial production. The mycelial growth and sclerotial formation were optimum at pH 6.0-7.0 for rice isolate of *R. solani* (Tiwari, 1997).

2.3. Factors favouring disease incidence

The process of infection of plant by pathogen is influenced by environmental and host factors.

2.3.1. Soil moisture

Soil-borne diseases have close relationship with soil moisture. Some diseases are favored by high soil moisture while others are favoured by low moisture level.

R. solani has been known to cause disease in plants over a wide range of soil moisture *viz.*, 20 - 80 per cent (Wright, 1957; Cook and Papendick, 1972; Sangeetha, 1988). Gill *et al.* (2001) conducted a study to know the effect of soil moisture on root rot of wheat caused by *R. solani* and found that the disease severity decreased up to 60 per cent, as the soil became wet.

2.3.2. Soil reaction (Soil pH)

Soil pH influences the development and severity of certain diseases caused by soil-borne pathogens. Manipulation of soil pH has been used successfully for many years in the control of several soil-borne diseases.

Many investigators have found that *R. solani* attacked its hosts over a wide range of soil pH. Roth and Riker (1943) found that the damping-off of red pine seedlings appeared in a broad pH range with a maximum disease incidence at pH 4.4. Bateman (1962) concluded that soil pH do not have any major influence on poinsettia root rot development since the disease occurred over the entire pH range tested (pH 4.0-7.5).

2.3.3. Inoculum level

The incidence of damping-off of tomato caused by *R. solani* increased with increase in inoculum (Hadwan and Khara, 1992). The disease incidence ranged from 19 per cent in pots containing a 1: 24 level of inoculum to 90 per cent at 1: 3 level.

Vanitha (1992) observed that the degree of *R. solani* infection in rice increased progressively with increase in inoculum load. Sivakumar (1994) also made similar observations. The incidence of wire stem of cabbage caused by *R. solani* increased with increase in inoculum density (Keinath, 1995).

2.4. Effect of organic amendments on *in vitro* growth of the pathogen

Cold water extracts of pinnai (*Calophyllum inophyllum*) cake inhibited sclerotial production at 10 per cent concentration (Ezhilan *et al.*, 1994).

Dubey and Patel (2000) reported that mahuva cake extract strongly inhibited the *in vitro* growth of *R. solani* followed by karanj and groundnut cakes.

Neem cake extract (10 per cent) inhibited the mycelial growth of *R. solani* causing sheath blight of rice (Meena *et al.*, 2000).

2.5. Effect of antagonists on *in vitro* growth of the pathogen

T. harzianum strongly inhibited the *in vitro* mycelial growth of *R. solani* causing root rot of french bean (Mathew and Gupta, 1998).

Sudhakar *et al.* (1998) observed that in dual culture technique, *T. viride* (Coimbatore-2 isolate) was superior in inhibiting the *R. solani* growth followed by *T. harzianum* and *Gliocladium virens*. *T. viride* MNT 7 effectively inhibited the growth of *R. solani* inciting collar rot of teak seedlings (Ramesh, 2000).

Sharma and Saxena (2001) tested *T. harzianum, T. viride, G. virens* and four strains of *Pseudomonas fluorescens* against *R. solani*. They found that all the biocontrol fungi tested inhibited the growth of *R. solani* in dual culture.

Wijesundera and Herath (1994) reported that a deep purple pigmented isolate of *Bacillus subtilis* obtained from paddy soil inhibited the growth of *R. solani in vitro*.

2.6. Management of the disease

2.6.1. Effect of organic amendments

Application of decomposable organic matter is one of the cheapest and effective methods of alteration of physical, chemical and biotic environment of soil. Dry or green crop residues, oil cakes, sawdust, compost *etc.* are used as organic amendments.

The application of organic amendments such as oilcakes and sawdust reduced the incidence of black scurf of potato caused by *R. solani* (Singh *et al.*, 1972). Among the oilcakes tested, mustard cake gave the best control.

Lakshmanan and Nair (1984) studied the effect of different oilcakes under dry and flooded conditions on the incidence of sheath blight of rice and found that groundnut cake or neem cake application after the harvest of the crop and *Bassia longifolia* cake application under puddled conditions were the best.

The efficacy of oilcakes and other organic materials in managing *R. solani* diseases has been reported by many authors (Papavizas and Davey, 1960; Singh, 1968; Rajan, 1980; Kannaiyan and Prasad, 1981a; Dath, 1982; Kundu and Nandi, 1985; Rao *et al.*, 1989; Tiyagi and Alam, 1995).

2.6.2. Effect of biocontrol agents

2.6.2.1. *Trichoderma* spp.

Soil application of wheat bran culture of *T. harzianum* significantly controlled *R. solani* causing stem rot of carnation (Elad *et al.*, 1981). The spread of web blight caused by *R. solani* in cowpea foliage was restricted effectively by spraying *T. koningii* spore suspension (Latunde- Dada, 1991).

Sowing *T. viride* treated seed of *Eucalyptus camaldulensis* in coir pith compost successfully controlled the damping-off disease (Kumar and Marimuthu, 1994).

Soil application of the antagonist also reduced the damping-off by 95 per cent and increased the seedling vigour. *T. harzianum* effectively controlled the collar rot of passion fruit caused by *R. solani* (Sawant *et al.*, 1995).

Application of wheat bran inoculum preparation of *T. viride* at the rate of 5 g/kg of soil suppressed the bean seedling damping-off caused by *R. solani* (El-Farnawany and Shama, 1996). Seed or soil treatment of *T. harzianum* and *T. viride* effectively controlled root rot of *Phaseolus vulgaris* caused by *R. solani* (Hazarika and Das, 1998).

Das and Dutta (1999) evaluated the efficacy of *T. harzianum* as seed treatment along with four different carriers against stem rot of soybean caused by *R. solani.* The lowest disease index was observed when seeds were treated with *T. harzianum* and methylcellulose with a significant increase in yield over the control.

Kurzawinska and Gajda (2001) reported that *T. viride* had high antagonistic ability against *R. solani* infecting potato. *T. viride* reduced the infection percentage to 46 per cent as against 100 per cent in control.

2.6.2.2. *Gliocladium virens*

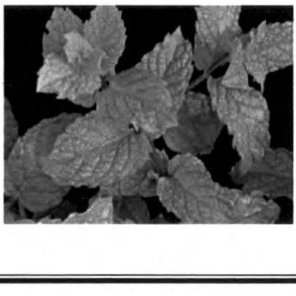
Mishra and Mukhopadhyay (1996) reported significant reduction in stolon and root rot caused by *R. bataticola* by the sucker treatment of *Mentha* with *G. virens* and in field experiments the disease incidence was reduced from 67 to 22.2 per cent.

Application of *G. virens* alone or in combination with Thiram effectively controlled the web blight of groundnut caused by *R. solani* (Dubey, 2000).

2.6.2.3. *Bacillus* spp.

In field trials, the stem canker and black scurf of potato caused by *R. solani* was reduced to 21.1, 49.4, 41.4 and 74.5 per cent by *Bacillus* spp., *Streptomyces* spp., *G. roseum* and *Trichoderma* spp. respectively (Schmiedeknecht, 1993). Noronha *et al.* (1995) reported that seed treatment with *B. subtilis* isolate AD - 183 significantly reduced the disease incidence under field conditions.

Damping-off of tomato caused by *R. solani* was suppressed by the soil application of *B. subtilis* RB 14 (Asaka and Shoda, 1996) or by the combined application of *B. subtilis* RB14 -C and Flutolanil (Kondoh *et al.*, 2000). The co-utilization of *B. subtilis* and Flutolanil decreased the amount of Flutolanil used from 375 μg/plot to 94 μ g/plot, while exerting the same effect in reducing the disease.



Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3.1. Survey on the occurrence of mint stolon rot incidence

Survey was conducted in important mint growing areas *viz.*, Karamadai, Chittepalayam, Thondamuthur, Therkupalayam and Subbanaikanpudur of Coimbatore district to assess the severity of stolon rot. At each place four fields were selected and 10 plots, each of $1m^2$ area were randomly fixed per field and observations were recorded and per cent disease incidence was calculated.

3.2. Quantitative determination of *Rhizoctonia solani* in soils from different places

For the quantitative determination of *R. solani* in naturally infested soils, the method given by Ko and Hora (1971) was followed. Accordingly, one gram of soil was moistened with sterile distilled water, compacted with a spatula and evenly distributed into 10 clumps on a Petri plate containing *Rhizoctonia* selective medium (RSM). Fifteen plates were used for each soil sample. The perimeter of each soil clump was observed under the microscope after 24 h of incubation at room temperature in dark.

3.3. Isolation of pathogen

Mint plants showing typical symptoms of stolon rot collected during survey were used for the isolation of pathogen. The isolations were made from the diseased stolon following the tissue segment method (Rangaswami, 1972). Axenic culture of the pathogen was obtained by single hyphal tip method and maintained in potato dextrose agar (PDA) slants.

R. solani obtained from different places were maintained as separate isolates *viz.*, *R.s.*1 (Karamadai), *R.s.*2 (Chittepalayam), *R.s.*3 (Thondamuthur), *R.s.* 4 (Therkupalayam) and *R.s.*5 (Subbanaikanpudur).

3.4. Testing the virulence of *R. solani* isolates grown on different media

Two types of laboratory grown inoculum of *R. solani viz., R. solani* grown on potato dextrose broth and sand maize medium were used in this study to assess the ability of *R. solani* isolates to incite stolon rot of mint.

3.4.1. Inoculation using potato dextrose broth grown inoculum

Conical flasks (250 ml), each containing 100 ml potato dextrose broth were sterilized at 1.04 kg/cm^2 pressure for 20 minutes. These flasks were inoculated with 6 mm diameter mycelial disc of *R. solani* and incubated at room temperature for ten days. The mycelial mat was harvested, homogenized and applied @100 ml/kg of soil. Sieved garden soil was sterilized in an autoclave at 1.04 kg/cm^2 pressure for three h on two successive days. Inoculation was done in both sterilized and non-sterilized soils. After five days, rooted mint cuttings were planted in artificially infested soil. Per cent disease incidence was recorded 30 days after planting.

3.4.2. Inoculation using sand maize medium grown inoculum

R. solani isolates were multiplied in sand maize medium (Riker and Riker, 1936). Sand and ground maize grains were mixed in 19:1 ratio, moistened with water and autoclaved at 1.04 kg/cm^2 pressure for two h on three successive days. The sterilized medium was inoculated with mycelial disc of *R. solani* and incubated at room temperature for 15 days. Sieved garden soil was sterilized in an autoclave at 1.04 kg/cm^2 pressure for three h on two successive days. The pathogen multiplied on sand maize medium was applied at the rate of 100 g/kg of soil. The pathogen was inoculated into sterilized and non-sterilized soils. Five days after inoculation of pathogen, mint cuttings were planted and per cent disease incidence was recorded 30 days after planting. The causal organism was reisolated from infected plants obtained from the above mentioned methods.

3.5. Reaction of various *Mentha* species to *R. solani*

The reaction of various species of mint to this pathogen was investigated. Planting materials of different *Mentha* spp. *viz.*, *Mentha citrata* Ehrh., *Mentha piperita* Linn., *Mentha arvensis* Linn. var. *piperascens* Malvid. and *Mentha spicata* Huds., were brought from Horticultural Research Station, Ooty and grown in glasshouse for further studies.

3.5.1. Incidence of stolon rot in various *Mentha* spp.

For assessing the stolon rot incidence in various mint species, the stolons of the four species were planted individually in sterilized soil inoculated with pathogen multiplied on sand maize medium (100 g/kg soil). The stolon rot incidence was recorded 30 days after planting and per cent disease incidence was calculated.

3.5.2. Estimation of essential oil content

The essential oil content of infected and healthy plants was determined following the method given by Langenau (1948). Leaves were shade dried and finely powdered. Thirty gram powder was transferred to the distillation flask with few glass beads to avoid frothing. The flask was fitted to an essential oil extraction apparatus. The distillation was made on a thermostatically controlled heater. A temperature of 90°C was maintained initially till boiling and subsequently 70°C was maintained for three hours. The distillate was cooled down to room temperature and allowed to settle till the oil layer became clear. The volume was read and the oil content was arrived using the formula.

$$\text{Volatile oil per cent (v/w)} = \frac{\text{Volume of oil (ml)}}{\text{Weight of sample (g)}} \times 100$$

3.6. Host range

The host range of *R. solani* isolated from mint was studied in a pot culture experiment. Fifteen plant species belonging to nine families were inoculated to study their reaction to the pathogen. For monocots, inoculation was done by placing sclerotia of the pathogen between stem and leaf sheath. The inoculated spots were covered with absorbent cotton and tied with parafilm. It was then moistened with distilled water. In case of dicots, seeds were sown in sterilized soil incorporated with sand maize medium @ 100 g/kg of soil. The inoculated plants were observed for symptom development.

3.7. *In vitro* studies on growth of *R. solani*

3.7.1. Growth of pathogen on various media

Variation in growth of *R. solani* on different media *viz.*, PDA, Corn meal agar, Rose bengal agar, Water agar, Czapek's Dox agar and Richard's agar (Appendix) were studied. For this, a 6 mm diameter mycelial disc taken from seven-day-old culture of the pathogen was placed on the centre of each plate containing the medium. The inoculated plates were incubated at room temperature for 72 h and the diameter of the colony was measured in each case.

3.7.2. Effect of temperature on the growth of the pathogen

3.7.2.1. Solid medium

Petri plates containing PDA were inoculated with 6 mm diameter mycelial disc of *R. solani* at the centre and were incubated at 20, 25, 30, 35 and 40°C for 72 h to study the effect of temperature on growth of the pathogen. Five plates were maintained at each temperature and the mycelial growth was recorded after the incubation period.

3.7.2.2. Liquid medium

Six millimetre diameter mycelial disc of the pathogen was inoculated into 250 ml conical flasks, each containing 100 ml of potato dextrose broth. The inoculated flasks were incubated at different temperatures *viz.*, 20, 25, 30, 35 and 40°C for 10 days. The mycelial mat was separated using previously weighed filter paper, dried at 60°C and the dry weight was determined.

3.7.3. Effect of pH on the growth of the pathogen

The growth of the pathogen at different pH levels in various liquid media was assessed following the method given by Singh and Malhotra (1994). Potato dextrose broth, Czapek's Dox broth and Richard's broth at nine pH levels *viz.*, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were prepared and sterilized. A 6 mm diameter mycelial disc of *R. solani* was inoculated to each 250 ml conical flask containing 100 ml of broth and incubated for 10 days at room temperature. Five replications were maintained for each treatment. The mycelial mat in each flask was separated using previously weighed filter paper, dried at 60^0C and the mycelial dry weight was determined.

3.8. Factors favouring disease development

3.8.1. Effect of inoculum level and age of plants on disease incidence

In order to find out the critical stage of susceptibility to the pathogen and the level of inoculum required to incite stolon rot, a pot culture trial was conducted in glasshouse. Sieved garden soil was taken in pots and autoclaved at 1.04 kg/cm^2 pressure for two hours. The pathogen multiplied on sand maize medium was added @ 25, 50, 75 and 100 g/kg of soil. Mint plants of 10, 20, 30, 40, 50 and 60 days age were planted at each inoculum level simultaneously. Disease incidence was recorded 30 days after planting. Suitable control was maintained without the inoculum.

3.8.2. Effect of soil moisture on stolon rot incidence

Soil moisture was adjusted to six levels, *viz.*, 30, 40, 50, 60, 70 and 80 per cent by gravimetric method (Keen and Raczkowski, 1921). The pots were watered by draining the excess water using a filter paper placed at the bottom of the pot containing the soil. A known weight of soil was dried in an oven at 110^0C for 24 h. The weight of water added was calculated by difference, which was considered as 100 per cent moisture holding capacity. Then the pots were adjusted to 30, 40, 50, 60, 70 and 80 per cent moisture level. The pots were weighed every day and the loss of moisture was recouped daily. The pathogen multiplied in sand maize medium was added @ 100 g/kg of soil and left for five days. Mint cuttings were planted after five days and stolon rot incidence was recorded 30 days after planting.

3.8.3. Effect of soil pH on stolon rot incidence

A pot culture experiment was carried out to find out the influence of soil pH on stolon rot incidence. The soil pH was adjusted to different levels *viz.,* 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 using sodium hydroxide and sulphuric acid (Bateman, 1962). After adjusting the pH, the soil was allowed to wither for seven days and pH was once again checked and readjusted wherever necessary. The pathogen multiplied in sand maize medium was added @ 100g / kg of soil. Mint cuttings were planted five days after inoculation of pathogen. Stolon rot incidence was recorded 30 days after planting and the results were tabulated.

3.8.4. Determination of *R. solani* population in mint rhizosphere at various intervals

For studying the population dynamics of *R. solani* in mint rhizosphere, artificial infestation was carried out by mixing the pathogen multiplied in sand maize medium with soil @ 2,5, 5.0, 7.5 and 10.0 per cent (w/w). Mint cuttings were planted in the infested soil. The population of *R. solani* was monitored from the initial period up to 60 days after planting. Soil samples were collected from mint rhizosphere at 15 days interval and the inoculum load was estimated following the method given by Ko and Hora (1971) as described earlier.

3.9. Disease Management

3.9.1. Management using oilcakes

3.9.1.1. *In vitro* evaluation of inhibitory effect of oilcake extracts on growth of *R. solani*

The procedure given by Dubey and Patel (2000) was adopted for this experiment with slight modification. Seven locally available oilcakes *viz.*, neem cake, coconut cake, sesame cake, pungam cake, ellupai cake, groundnut cake and cotton seed cake were evaluated *in vitro* against *R. solani*. The oilcakes were powdered and soaked overnight in sterile distilled warm water @ 1 g of oilcake per ml of water. It was then ground using pestle and mortar and filtered through fine cloth which formed the standard oilcake extract (100 per cent). Ten ml of this extract was added to 90 ml of PDA medium and autoclaved. A 6 mm diameter mycelial disc of the pathogen was placed on the centre of the Petri plate containing 15 ml PDA medium amended with oilcake extracts. Suitable control was maintained by inoculating pathogen disc in Petri plate containing non-amended PDA medium. The plates were incubated at room temperature and the growth of the pathogen was recorded after 72 h.

3.9.1.2. Effect of oilcakes on stolon rot incidence, germination and growth of mint under glasshouse condition

A pot culture experiment was laid in completely randomized design to test the efficacy of oilcakes in controlling the stolon rot disease of mint. Treatments consisted of neem cake, coconut cake, sesame cake, pungam cake, ellupai cake, groundnut cake and cotton seed cake. These oilcakes, already tested *in vitro* were applied basally @ 10 g/kg of soil and allowed to wither for 10 days. The pathogen multiplied in sand maize medium was applied at the rate of 100 g/kg of soil. Four replications were maintained for each treatment. Mint stolons were planted in inoculated pots and the germination percentage was recorded 15 days after planting. The stolon rot incidence was recorded at 15, 20, 30, 45 and 60 days after planting.

3.9.1.3. Effect of oilcakes on the population of *R. solani* in the soil

Soil samples were taken from the rhizosphere of the treated plants, sixty days after planting. The population of *R. solani* in these soil samples was estimated using the method given by Ko and Hora (1971).

3.10. Management using biocontrol agents

3.10.1. Isolation of native antagonists

Dilution plate technique (Waksman, 1952) was followed for the isolation of antagonistic microbes from rhizosphere of mint plants. One gram of the rhizosphere soil was taken and a series of dilutions (up to 10^{-7}) were made using sterile distilled water. One millilitre of 10^{-3} dilution was transferred to a sterilized Petri plate and 15 ml of *Trichoderma* selective medium (Elad and Chet, 1983) was added. The plates were incubated at room temperature for five days. The colonies of *Trichoderma* spp. were transferred to potato dextrose agar slants. For the isolation of *Bacillus subtilis* 15 ml of nutrient agar was added to sterilized Petri plates containing 1 ml of soil suspension (10^{-6} dilution). The plates were incubated at room temperature($28 \pm 2^{0}C$) for 24-48 h and the *B. subtilis* colonies were transferred to nutrient agar slants.

3.10.2. *In vitro* evaluation of antagonistic effect of biocontrol agents

The isolates of antagonists were evaluated *in vitro* against *R. solani* by dual culture technique (Dennis and Webster, 1971) to select the most effective one. *T. viride* (obtained from Department of Plant Pathology, Coimbatore), *Trichoderma* spp. (native isolates 1 and 2) and *B. subtilis* were used in this study. A 6 mm diameter mycelial disc of *R. solani* was taken from the growing edge of the colony and placed at one end of the Petri dish containing PDA medium. A similar disc of the *Trichoderma* spp. was placed at the opposite end approximately 70 mm away from the pathogen. In case of bacterial antagonist, one loopful of *B. subtilis* was streaked on one end of the Petri plate containing PDA and 6 mm diameter mycelial disc of *R. solani* was placed at the other end. Five replications were maintained for each treatment and the plates were incubated at room temperature. Radial growth of the pathogen in dual culture was recorded after 72 h of incubation.

3.10.3. Preparation of talc based formulation of biocontrol agents

Potato dextrose broth was inoculated with mycelial disc of *Trichoderma* and incubated for 10 days. The biomass was homogenized and mixed with talc powder at 1:2 ratio and shade dried. In case of *Bacillus subtilis* instead of potato dextrose broth, nutrient broth was used.

3.10.4. Effect of biocontrol agents on stolon rot incidence, germination and growth of mint under pot culture condition

The biocontrol agents tested *in vitro* were evaluated in pot culture trial under glasshouse condition. Talc based formulations of boicontrol agents were used for the treatment of stolons. The treatments consisted of:

T_1 - *T. viride* @ 4 g/kg of stolon

T_2 - *Trichoderma* sp. (isolate 1) @ 4 g/kg of stolon

T_3 - *Trichoderma* sp. (isolate 2) @ 4 g/kg of stolon

T_4 - *B. subtilis* @ 4 g/kg of stolon

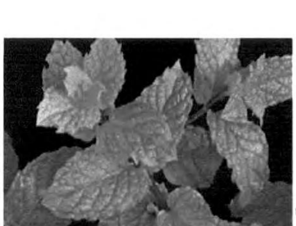
T_5 - *T. viride* + *B. subtilis* each @ 4 g/kg of stolon

T_6 - *Trichoderma* sp. (isolate-1) + *B. subtilis* each @ 4g/kg of stolon

T_7 - *Trichoderma* spss. (isolate-2) + *B. subtilis* each @ 4 g/kg of stolon

T_8 - Control

The pots were filled with soil and inoculated with the pathogen multiplied in sand maize medium @ 100 g/kg of soil. Treated mint stolons were planted in these pots. Four replications were maintained for each treatment. The germination percentage was recorded 15 days after planting. Stolon rot incidence, root and shoot lengths were recorded at 15 days interval up to 60 days.



Experimental Results

CHAPTER IV
EXPERIMENTAL RESULTS

4.1. Symptoms of the disease

Severe incidence of stolon rot of mint (*Mentha viridis* Linn.) was observed in various parts of Coimbatore district during March–April, 2001. Drying of plants in patches was observed in the stolon rot affected fields (Plate 1a). During early stages of infection, symptoms manifested as stunted growth and yellowing of leaves. The disease initiated as slightly sunken black lesions on the stolon, which spread fast to the entire length causing stolon rot (Plate 1b). As disease advanced to the above ground portions drying of shoots occurred. Severe infection led to the death of the entire plant (Plate 1c, d, e).

4.2. Pathogen

Isolations from infected stolons, collected from different places were made on PDA. Most of the isolations yielded the fungus, which was identified as *Rhizoctonia solani* Kühn (Plate 2 and 3). Pathogenicity of the isolated organism was established by proving Koch's postulates.

4.3. Survey on the occurrence of stolon rot incidence

Major mint growing areas of Coimbatore district were surveyed to record the severity of mint stolon rot incidence. It is evident from the data (Table 1) that the disease was prevalent in all the areas surveyed and the incidence ranged from 16.48 to 36.81 per cent. The highest stolon rot incidence (36.81 per cent) was recorded from Chittepalayam area.

4.4. Population of *R. solani* in soils from different places

To assess the inoculum load in naturally infested soils, population of *R. solani* in soil samples collected from various places was determined. The highest population (2.18 propagules per gram of soil) was recorded from soil sample collected from Chittepalayam. The lowest inoculum load (0.87 propagules per gram of soil) was recorded from Thondamuthur soil sample (Table 1).

Plate 1a. Stolon rot affected mint field

Plate 1b. Stolon rot affected mint plants

A. Healthy

B. Infected

PLATE 1a

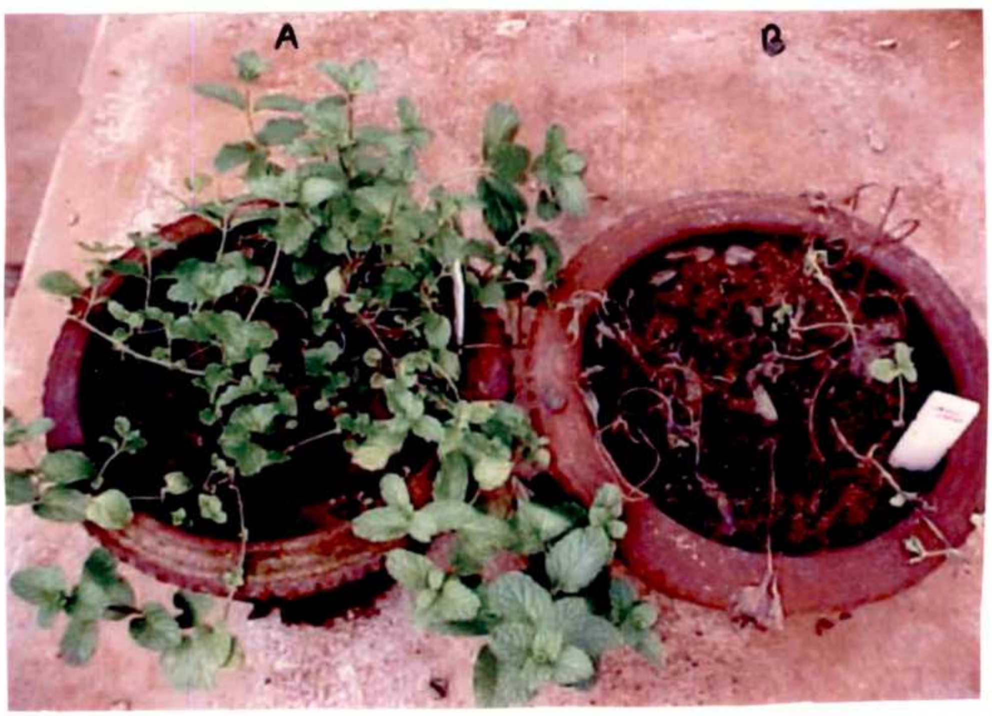


PLATE 1b

Plate 1c. Healthy and infected stolons

1. Healthy
2. Infected

Plate 1d. Drying of shoots

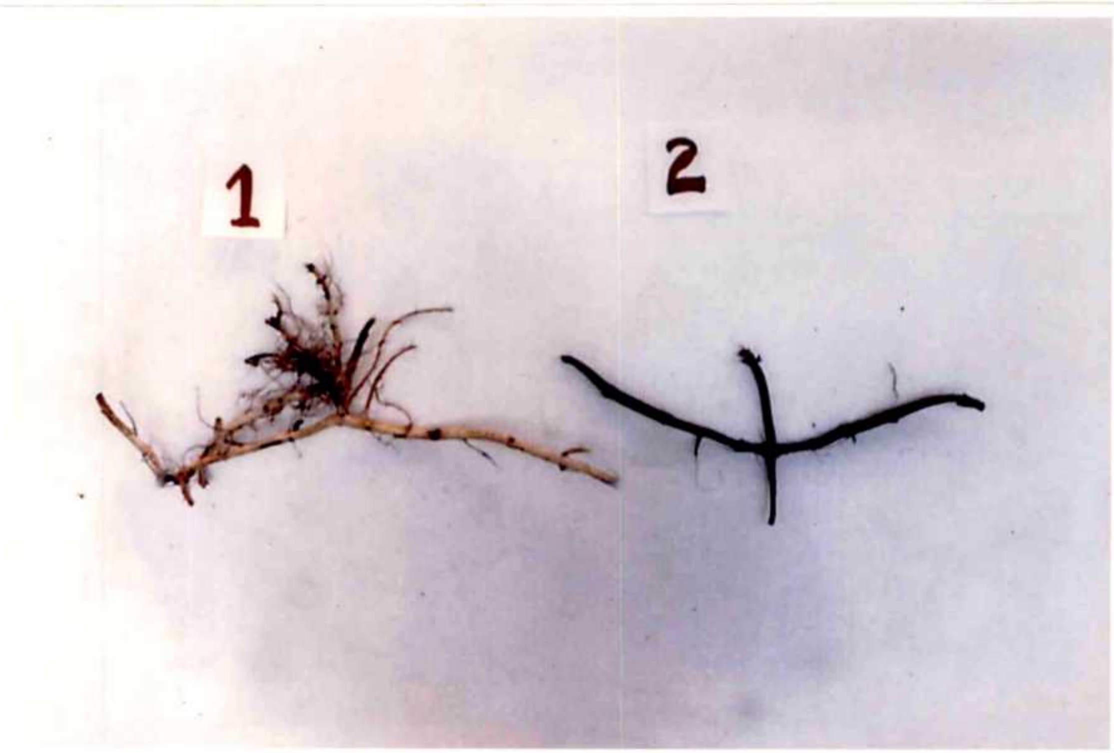


PLATE 1c

PLATE 1d

Plate 1e. Stages of stolon rot development

1. Stolon infection
2. Stolon and shoot infection
3. Drying of the entire plant

PLATE 1e

Plate 2. Growth of *Rhizoctonia solani* Kühn. from infected mint stolon on PDA

Plate 3. Pathogen causing stolon rot - *R. solani*

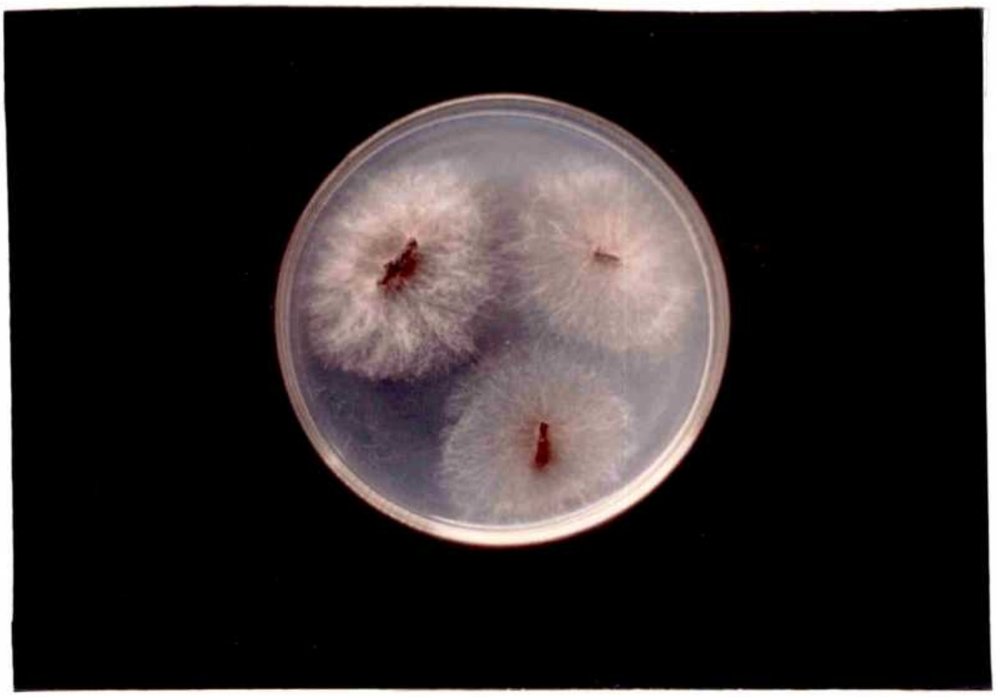


PLATE 2

PLATE 3

Table 1. Occurrence of mint stolon rot incidence in Coimbatore district

Sl. No.	Location	Stolon rot incidence (Per cent)	Population of *R. solani* * (No. of propagules per g of soil)
1	Karamadai	33.60	1.73
2	Chittepalayam	36.81	2.18
3	Thondamuthur	16.48	0.87
4	Therkupalayam	31.75	1.65
5	Subbanaikanpudur	28.96	1.18
	CD (P=0.05)		0.16

* Mean of four replications

4.5. Virulence of *R. solani* isolates grown on different media

The virulence of *R. solani* isolates multiplied on potato dextrose broth and sand maize medium was tested in sterilized and non-sterilized soils. *R.s.*2 (Chittepalayam) isolate multiplied on sand maize medium exhibited the highest degree of virulence (93.75 per cent disease incidence). The sand maize medium inoculum induced higher levels of infection in both sterilized and non-sterilized soils than the potato dextrose broth for all the isolates (Table 2). The most virulent isolate (*R.s.* 2) was used for subsequent studies.

4.6. Reaction of various *Mentha* spp. to *R. solani*

4.6.1. Stolon rot incidence on different *Mentha* spp.

Out of the five *Mentha* species screened, four species *viz., M. viridis, M. piperita, M. arvensis* and *M. citrata* were found susceptible to stolon rot. Per cent disease incidence in susceptible species ranged from 63.10 per cent in *M. citrata* to 91.67 per cent in *M. viridis. M. spicata* was found free from stolon rot disease (Table 3). In *M. arvensis*, the fungus produced sclerotia on the above ground portions of affected plants (Plate 4a, b, c).

4.6.2. Effect of *R. solani* infection on essential oil content of mint

The results of the studies (Table 4) indicated that *R. solani* infection reduced essential oil content in all the susceptible *Mentha* spp. and the extent of reduction varied with the species. There was a significant reduction in the essential oil content of *M. citrata* (0.750 per cent) and *M. piperita* (0.752 per cent) as a result of *R. solani* infection.

4.7. Host range of the pathogen

Out of the 15 plant species inoculated, 10 expressed symptoms and the symptoms manifested varied between species. Reactions of various hosts to mint isolate of *R. solani* are summarized in Table 5. It is clear from the data that the mint isolate caused damping-off and lesions in collar region and leaf blight in plants belonging to different families (Plate 5a– f).

Table 2 . Virulence of *R. solani* isolates grown in different media

Sl. No.	*R.solani* isolates	Stolon rot incidence* (Per cent)			
		Sterilized soil		Non-sterilized soil	
		Sand maize medium	Potato dextrose broth	Sand maize medium	Potato dextrose broth
1	*R.s.* 1	87.50 (71.93)	77.08 (61.64)	83.33 (66.26)	68.17 (56.13)
2	*R.s.* 2	93.75 (77.42)	81.25 (64.95)	89.58 (73.76)	77.08 (61.48)
3	*R.s.* 3	39.59 (38.97)	33.33 (35.18)	31.25 (33.87)	29.17 (32.63)
4	*R.s.* 4	85.42 (67.73)	68.75 (56.41)	72.92 (58.68)	60.42 (51.03)
5	*R.s.* 5	70.84 (57.37)	54.17 (47.43)	58.34 (49.87)	47.92 (43.80)
6	Control	0.00	0.00	0.00	0.00
	CD (P=0.05)	9.48	9.97	9.33	5.05

* Mean of five replications

Figures in parantheses are arcsine transformed values.

Table 3. Stolon rot incidence on various *Mentha* spp.

Sl.No.	Species	Stolon rot incidence * (Per cent)
1	*Mentha viridis* Linn.	91.67 (74.56)
2	*Mentha piperita* Linn.	73.81 (59.43)
3	*Mentha arvensis* Linn.	86.90 (70.59)
4	*Mentha citrata* Ehrh.	63.10 (52.84)
5	*Mentha spicata* Huds.	0.00
	CD (P = 0.05)	7.85

* Mean of seven replications

Figures in parantheses are arcsine transformed values

Reaction of Japanese mint (*Mentha arvensis* Linn. var. *piperascens* Malvid.) to *R. solani*

Plate 4a. Healthy and infected mint plants

PLATE 4a

Reaction of Japanese mint (*Mentha arvensis* Linn. var. *piperascens* Malvid.) to *R. solani*

Plate 4b. Stem infection

Plate 4c. Sclerotia produced on stem

PLATE 4b

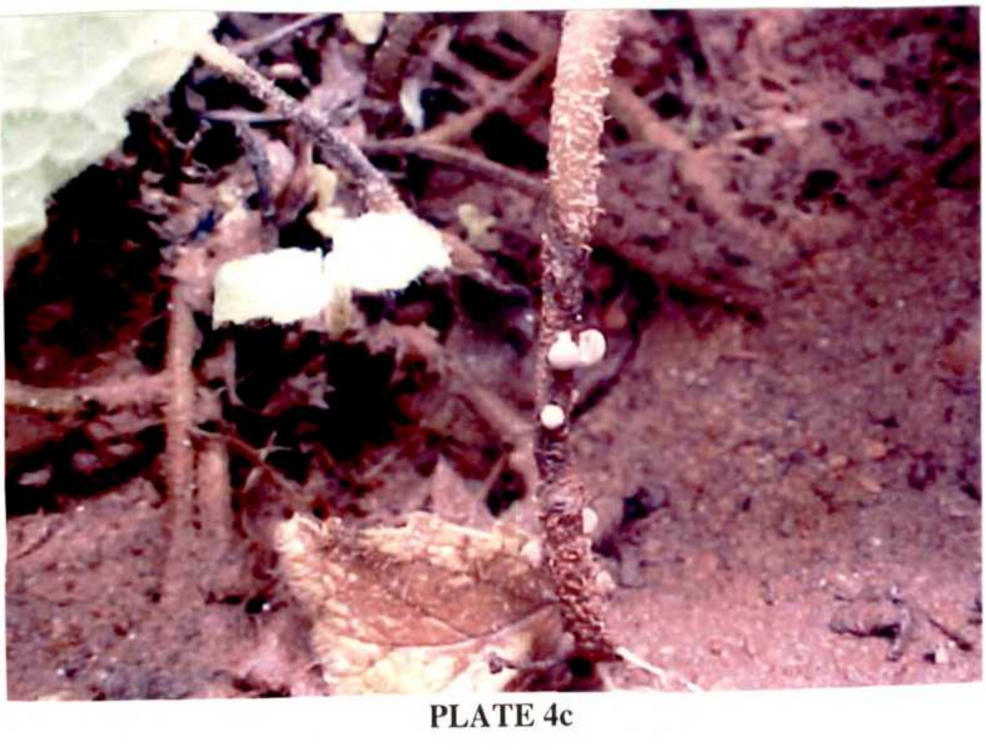


PLATE 4c

Table 4. Effect of *R. solani* infection on essential oil content

Sl. No	Name of the species	Essential oil content* (Per cent)	
		Healthy	Infected
1	*Mentha viridis* Linn.	1.083	0.668
2	*Mentha citrata* Ehrh.	1.753	0.750
3	*Mentha arvensis* Linn.	1.835	0.918
4	*Mentha piperita* Linn.	1.165	0.752
5	*Mentha spicata* Huds.	1.533	1.528
	CD=(P=0.05)	0.024	0.340

* Mean of four replications

Table 5. Host range of mint isolate of *R. solani*

Sl. No.	Family	Scientific name	Symptoms
1.	Amaranthaceae	*Amaranthus viridis* L.	Leaf blight
2.	Asteraceae	*Helianthus annuus* L.	No symptom
3.	Cucurbitaceae	*Cucurbita moschata*(Duch.) Poir	Water soaked lesions on collar region and stem
4.	Fabaceae	*Arachis hypogaea* L.	No symptom
5.	Fabaceae	*Vigna unguiculata* L.	Water soaked lesions which later turned to blight on leaves
6.	Fabaceae	*Vigna radiata* L.	No symptom
7.	Fabaceae	*Vigna mungo* L.	No symptom
8.	Poaceae	*Oryza sativa* L.	Symptoms of sheath blight
9.	Malvaceae	*Abelmoschus esculentus* L.	Rotting on collar region seedlings toppled down
10.	Pedaliaceae	*Sesamum indicum* L.	Root rot
11.	Solanaceae	*Capsicum annuum* L.	Damping-off
12.	Solanaceae	*Lycopersicon esculentum* Mill.	Damping-off
13.	Solanaceae	*Solanum melongena* L.	Damping-off
14.	Zingiberaceae	*Curcuma domestica* Valet.	No symptom
15.	Zingiberaceae	*Zingiber officinale* Roscoe.	Symptoms of banded leaf blight

Host range of *R. solani*

Plate 5a *Amaranthus viridis* L.

Plate 5b. *Cucurbita moschata* (Duch.) Poir.

PLATE 5a

PLATE 5b

Plate 5c. ***Oryza sativa*** **L.**

Plate 5d. ***Abelmoschus esculentus*** **L.**

PLATE 5c

PLATE 5d

Plate 5e. ***Solanum melongena*** **L.**

Plate 5f. ***Zingiber officinale*** **Roscoe.**

PLATE 5e

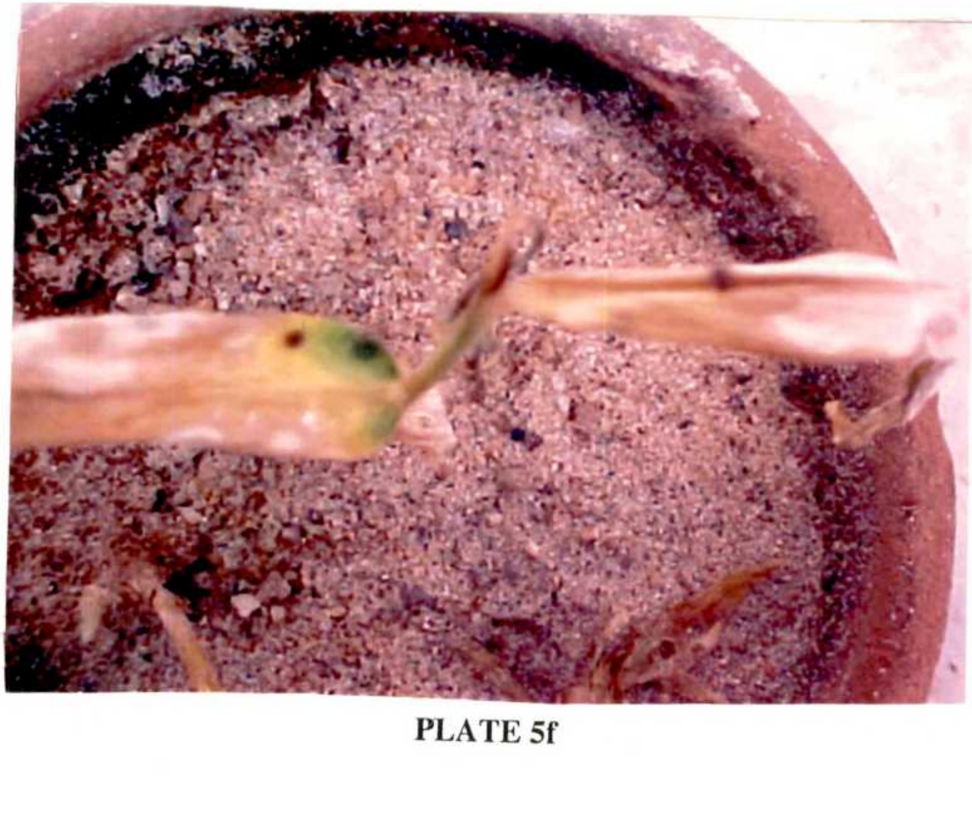


PLATE 5f

4.8. *In vitro* studies on growth of *R. solani*

4.8.1. Growth of *R. solani* on various media

The results of the present study indicated that PDA (88.80 mm), Corn meal agar (88.60 mm) and Water agar (88.60 mm) supported the maximum growth of *R. solani* isolate from mint (Table 6). Richard's agar, Czapek's Dox agar and Rose bengal agar have recorded lesser growth compared to other media.

4.8.2. Effect of temperature on growth of *R. solani in vitro*

Studies on the influence of temperature on the growth of *R. solani* under *in vitro* condition showed that the pathogen could grow over a temperature range of 20-35°C and attained maximum growth at 30°C. At this temperature, mycelial growth of 88.00 mm and mycelial dry weight of 812.00 mg were recorded from PDA and potato dextrose broth grown cultures of *R. solani* respectively. Complete inhibition of mycelial growth occurred at 40°C (Table 7; Plate 6).

4.8.3. Effect of pH on the growth of *R. solani in vitro*

Data on the influence of pH levels (Table 8) on the growth of *R. solani* revealed the ability of the pathogen to grow on a wide pH range of 4.0 to 8.0. Potato dextrose broth at pH 6.0 was found to be optimal for the growth of the pathogen (845.20 mg). Richard's broth and Czapek's Dox broth were found to be not conducive for the growth of *R. solani* at all the pH levels tested.

4.9. Factors favouring the disease incidence

4.9.1. Effect of plant age and inoculum level on stolon rot incidence

Data presented in Table 9 indicated that inoculum level of pathogen and age of the plant had a marked effect on the stolon rot incidence. The disease incidence showed a positive correlation with inoculum level and negative correlation with age of the plant. Maximum stolon rot incidence (89.58 per cent) was recorded from 10-day-old crop planted in soil at 10 per cent inoculum level.

Table 6. Growth of *R. solani* on various media

Sl. No	Media	Growth of *R. solani* * (mm)
1	Water agar	88.60
2	Richard's agar	55.00
3	Corn meal agar	88.60
4	Potato dextrose agar	88.80
5	Rose bengal agar	29.80
6	Czapek's Dox agar	34.40
	CD (P=0.05)	0.81

* Mean of five replications

Table 7. Effect of temperature on the growth of *R. solani in vitro*

Sl. No	Temperature (°C)	Mycelial growth* (mm)	Mycelial dry weight * (mg)
		Potato dextrose agar	Potato dextrose broth
1	20	54.00	497.00
2	25	71.20	709.00
3	30	88.00	812.00
4	35	60.60	677.00
5	40	0.00	0.00
	CD (P=0.05)	1.56	9.58

* Mean of five replications

Plate 6. Effect of temperature on growth of *R. solani in vitro*

1. 20°C
2. 25 °C
3. 30 °C
4. 35 °C
5. 40 °C

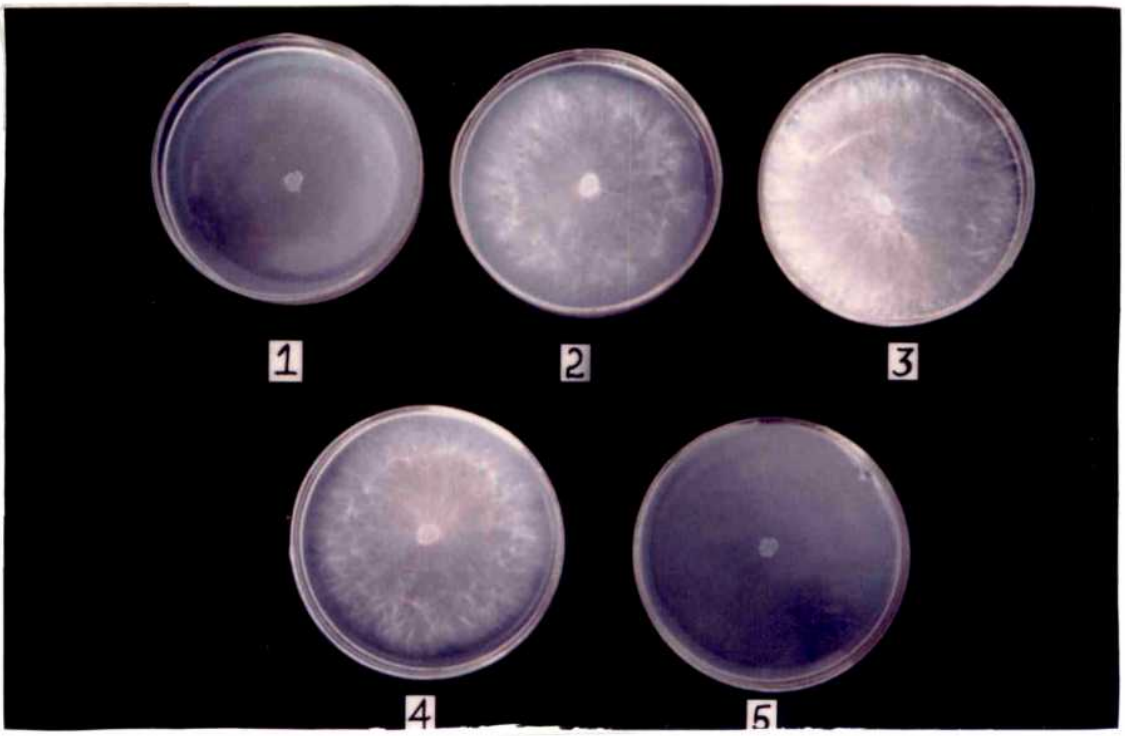


PLATE 6

Table 8. Growth of *R. solani* at various pH levels

Sl. No	pH	Mycelial dry weight* (mg)		
		Potato dextrose broth	Richard's broth	Czapek's Dox broth
1	4.0	475.00	265.40	192.00
2	4.5	505.40	296.60	220.40
3	5.0	689.40	341.80	242.20
4	5.5	787.60	404.00	287.80
5	6.0	845.20	473.00	315.80
6	6.5	805.60	443.00	365.00
7	7.0	735.20	396.00	390.40
8	7.5	719.20	355.20	281.00
9	8.0	567.40	311.60	109.20
	CD (P=0.05)	11.61	18.96	11.05

* Mean of five replications

Table 9. Influence of inoculum level and age of the plant on stolon rot incidence

Sl. No	Age of the crop (days)	Stolon rot incidence* (Per cent)					Mean
		Inoculum level (%)					
		0.0	2.5	5.0	7.5	10.0	
1	10	0.00	31.25 (33.95)	54.17 (47.40)	83.33 (66.26)	89.58 (73.76)	51.67
2	20	0.00	22.92 (28.52)	39.59 (38.97)	45.84 (42.60)	79.17 (63.11)	37.50
3	30	0.00	18.75 (25.57)	29.17 (32.63)	37.50 (37.73)	58.33 (49.83)	28.75
4	40	0.00	12.50 (20.44)	16.67 (23.74)	35.42 (36.42)	39.58 (38.93)	20.84
5	50	0.00	4.17 (8.39)	14.59 (22.27)	18.75 (25.22)	20.84 (27.05)	11.67
6	60	0.00	2.08 (4.19)	6.25 (12.58)	8.33 (14.41)	10.42 (18.61)	5.42
	Mean	0.00	15.28	26.74	38.20	49.65	

CD (P=0.05)

Age	:	3.23
Inoculum level	:	2.95
Age x inoculum level	:	7.23

* Mean of four replications

Figures in parantheses are arcsine transformed values.

4.9.2. Effect of soil moisture on stolon rot incidence

Studies on the relationship between soil moisture and stolon rot development revealed that the soil moisture had profound influence on stolon rot incidence. Moderate to high soil moisture levels (60-80 per cent MHC) favoured disease incidence. Disease incidence increased with increase in soil moisture level and the highest stolon rot incidence (89.59 per cent) was recorded at 80 per cent MHC (Table 10). At low soil moisture level (30 per cent MHC), the stolon rot incidence was less (33.33 per cent).

4.9.3. Effect of soil pH on stolon rot incidence

Soil pH of 6.0 to 7.5 favoured the stolon rot development to a maximum extent. Maximum disease incidence occurred at pH 6.5 (87.49 per cent). Highly acidic and highly alkaline soils were found to be not favourable for stolon rot development (Table 11).

4.9.4. Population of *R. solani* in mint rhizosphere

The rhizosphere population of *R. solani* showed positive correlation with age of the plant and inoculum level. A perusal of the data (Table 12) showed that the pathogen population increased with time. The highest population of 6.68 propagules per gram of soil was recorded 60 days after inoculation from soil at 10 per cent inoculum level.

4.10. Management of the disease

4.10.1. Management using oilcakes

4.10.1.1. *In vitro* inhibition of *R. solani* by water extracts of oilcakes

The inhibitory effect of water extracts of different oilcakes was tested against *R. solani in vitro* and the results are summarized in Table 13. Significant reduction in radial growth of *R. solani* was obtained by all the oil cake extracts with the highest inhibition being provided by neem cake (26.00 mm). Extracts of pungam cake (29.75 mm) and ellupai cake (37.75 mm) were also found effective (Plate 7).

Table 10. Effect of soil moisture on mint stolon rot incidence under glasshouse condition

Sl. No	Soil moisture (MHC Per cent)	Stolon rot incidence* (Per cent)
1	30	33.33 (35.18)
2	40	43.75 (41.37)
3	50	62.50 (52.38)
4	60	72.92 (58.84)
5	70	77.08 (61.64)
6	80	89.59 (71.39)
	CD (P=0.05)	7.46

* Mean of four replications

Figures in parantheses are arcsine transformed values

Table 11. Effect of soil pH on mint stolon rot incidence under glasshouse condition

Sl. No	Soil pH	Stolon rot incidence* (Per cent)
1	4.0	54.17 (47.43)
2	4.5	58.33 (49.99)
3	5.0	62.50 (52.42)
4	5.5	70.84 (57.37)
5	6.0	83.33 (68.98)
6	6.5	87.49 (71.93)
7	7.0	77.02 (61.48)
8	7.5	75.00 (61.66)
9	8.0	66.67 (54.93)
	CD (P=0.05)	11.61

* Mean of four replications

Figures in parantheses are arcsine transformed values

Table 12. Population of *R. solani* in mint rhizosphere

Sl. No.	Inoculum level (%) / Days after inoculation	Population of *R. solani** (No. of propagules per gram of soil)					
		0.0	**2.5**	**5.0**	**7.5**	**10.0**	**Mean**
1	0	0.00	1.98	2.60	3.17	4.18	2.37
2	15	0.00	2.52	3.03	3.68	4.68	2.78
3	30	0.00	3.40	4.08	4.53	5.56	3.51
4	45	0.00	4.35	4.85	5.47	6.25	4.18
5	60	0.00	4.87	5.25	5.89	6.68	4.54
	Mean	0.00	3.42	3.96	4.55	5.47	

CD (P=0.05)

Days	:	0 .08
Inoculum	:	0.08
Days x inoculum	:	0.19

* Mean of four replications

Table 13. ***In vitro*** **evaluation of water extracts of oilcakes against** ***R. solani***

Sl. No.	Treatments	Growth of *R. solani** (mm)
1	Neem cake	26.00
2	Pungam cake	29.75
3	Ellupai cake	37.75
4	Cotton seed cake	45.25
5	Coconut cake	51.75
6	Groundnut cake	62.00
7	Sesame cake	77.75
8	Control	88.25
	CD (P = 0.05)	1.59

* Mean of four replications

Plate 7. Effect of water extracts of oilcakes on *in vitro* growth of *R. solani*

1. Neem cake
2. Pungam cake
3. Ellupai cake
4. Coton seed cake
5. Coconut cake
6. Groundnut cake
7. Sesame cake
8. Control

PLATE 7

4.10.1.2. Effect of oilcakes on germination of mint stolons under glasshouse condition

Soil application of oilcakes significantly promoted the germination of mint stolons. Highest germination percentage was recorded from neem cake applied pots (93.75 per cent) followed by pungam cake (85.42 per cent) and ellupai cake (79.17 per cent). The control pots showed poor germination of stolons (45.84 per cent) (Table 14).

4.10.1.3. Effect of oilcakes on the stolon rot incidence and growth of mint

Amendment of soil with oilcakes was quite effective in reducing the stolon rot incidence as compared to untreated control (Table 15). Among the oilcakes tested, neem cake was found to be the most effective (37.88 per cent) in controlling the disease and was on par with pungam cake (41.36 per cent) and ellupai cake (44.44 per cent). Application of oilcakes also resulted in enhanced plant growth (Plate 8). Shoot and root lengths were maximum with neem cake at all the observation intervals.

4.10.1.4. Effect of oilcakes on the soil population of *R. solani*

Application of oilcakes significantly reduced the inoculum load of *R. solani* in soil. Neem cake highly suppressed the *R. solani* population (1.25 propagules per g of soil). The highest population of 6.3 propagules per gram of soil was recorded from the control (Table 16).

4.10.2. Management using bio control agents

4.10.2.1. *In vitro* inhibition of *R. solani* by biocontrol agents

All the isolates used in the current study restricted the radial growth of *R. solani* on dual culture plates (Table 17; Plate 9; Plate 10). *Trichoderma viride* exhibited the highest antagonistic activity (31.52 mm) and overgrew the pathogen after seven days.

Table 14. Effect of oilcakes on the germination of mint stolons under glasshouse condition

Sl. No.	Treatments	Stolon germination* (Per cent)
1	Neem cake	93.75 (77.42)
2	Pungam cake	85.42 (67.73)
3	Ellupai cake	79.17 (62.95)
4	Cotton seed cake	70.84 (57.37)
5	Coconut cake	60.42 (51.03)
6	Groundnut cake	58.33 (49.83)
7	Sesame cake	68.75 (56.05)
8	Control	45.84 (42.60)
	CD (P = 0.05)	6.13

* Mean of four replications

Figures in parentheses are arcsine transformed values

Table 15. Effect of oilcakes on mint stolon rot incidence and growth under glasshouse conditions

Treatments	Stolon rot incidence* (%)				Shoot length* (cm)				Root length* (cm)			
	15 DAP	30 DAP	45 DAP	60 DAP	15 DAP	30 DAP	45 DAP	60 DAP	15 DAP	30 DAP	45 DAP	60 DAP
Neem cake	28.79 (32.43)	31.06 (33.83)	35.79 (36.64)	37.88 (37.96)	8.95	16.48	23.43	31.58	7.23	9.80	13.10	14.48
Pungam cake	31.59 (34.18)	34.32 (35.78)	39.09 (38.69)	41.36 (40.02)	8.20	15.08	20.15	27.48	6.75	8.68	10.03	12.20
Ellupai cake	34.17 (35.74)	39.44 (38.88)	42.22 (40.48)	44.44 (41.77)	7.45	13.35	17.85	26.03	6.10	8.00	9.28	10.28
Cotton seed cake	40.63 (39.44)	46.87 (43.19)	49.66 (44.79)	52.78 (46.60)	6.73	12.05	16.88	23.20	5.80	7.05	8.60	9.08
Coconut cake	42.36 (40.58)	48.61 (44.20)	51.39 (45.80)	54.52 (47.61)	5.93	10.20	15.98	21.53	5.15	6.30	7.45	8.46
Ground-nut cake	46.43 (42.95)	54.17 (47.43)	57.29 (49.24)	60.86 (51.30)	5.15	9.18	13.50	20.20	4.80	6.00	7.15	7.93
Sesame cake	50.00 (45.00)	56.70 (48.86)	60.86 (51.30)	64.44 (53.44)	5.03	8.50	12.17	17.93	4.08	5.13	6.60	6.83
Control	72.50 (58.71)	81.67 (64.67)	86.67 (71.31)	90.83 (77.33)	4.15	6.18	10.18	13.90	2.43	3.30	4.35	6.23
CD (P=0.05)	6.36	5.15	8.70	8.69	0.56	0.60	0.97	1.51	0.13	0.54	0.77	0.91

*Mean of four replications

Figures in parantheses are arcsine transformed values

DAP- Days after planting

Plate 8. Effect of organic amendments on stolon rot incidence of mint

1. Neem cake
2. Pungam cake
3. Ellupai cake
4. Coton seed cake
5. Coconut cake
6. Groundnut cake
7. Sesame cake
8. Control

PLATE 8

Table 16. Effect of oilcakes on soil population of *R. solani*

Sl.No.	Treatments	Population of *R. solani** (No. of propagules per gram of soil)
1	Neem cake	1.25
2	Pungam cake	1.67
3	Ellupai cake	1.83
4	Cotton seed cake	2.92
5	Coconut cake	3.17
6	Groundnut cake	3.87
7	Sesame cake	4.28
8	Control	6.30
	CD (P = 0.05)	0.12

* Mean of four replications

Table 17. Inhibition of mycelial growth of *R. solani* by antagonists *in vitro*

Sl. No.	Treatments	Growth of *R. solani* * (mm)
1	*Trichoderma viride*	31.52
2	*Trichoderma* sp. (isolate-1)	36.34
3	*Trichoderma* sp. (isolate-2)	61.82
4	*Bacillus subtilis*	47.54
5	Control	81.68

CD (P = 0.05) 1.70

* Mean of five replications

Plate 9. Effect of fungal antagonists on *in vitro* growth of *R. solani*

1. ***T.viride***
2. ***Trichoderma* sp. (isolate-1)**
3. ***Trichoderma* sp. (isolate-2)**
4. **Control**

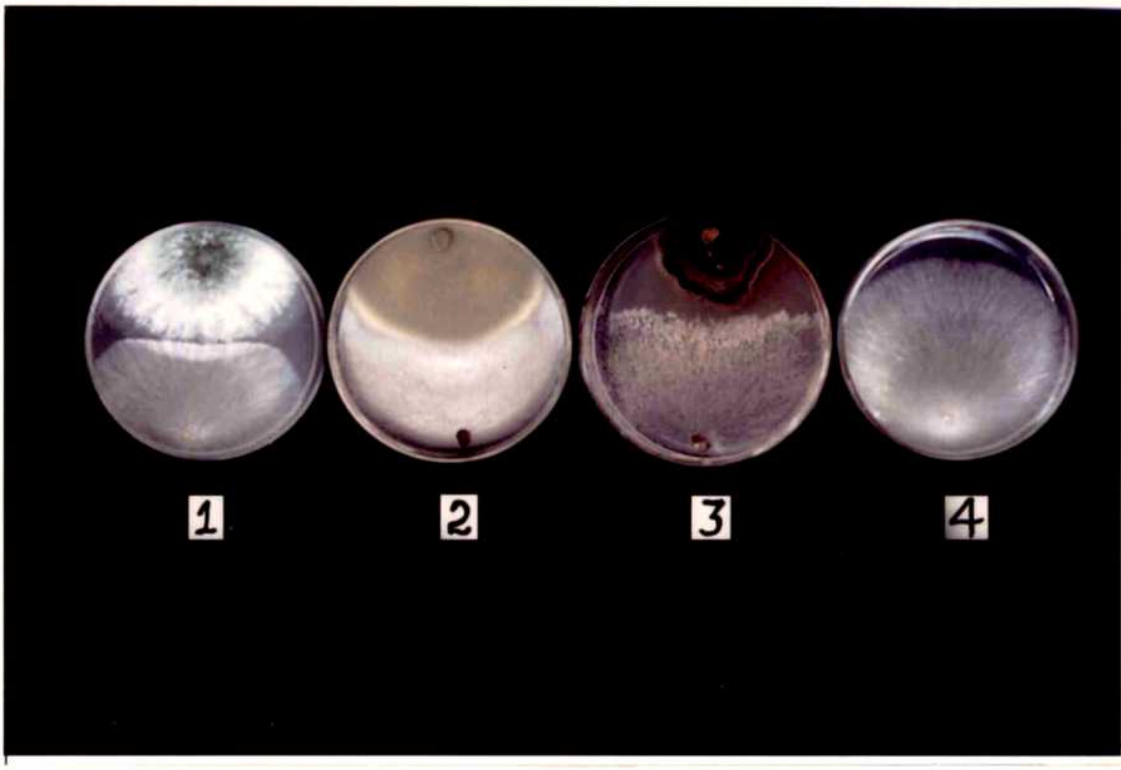


PLATE 9

Plate 10. Effect of bacterial antagonists on *in vitro* growth of *R. solani*

1. *B. subtilis*

2.Control

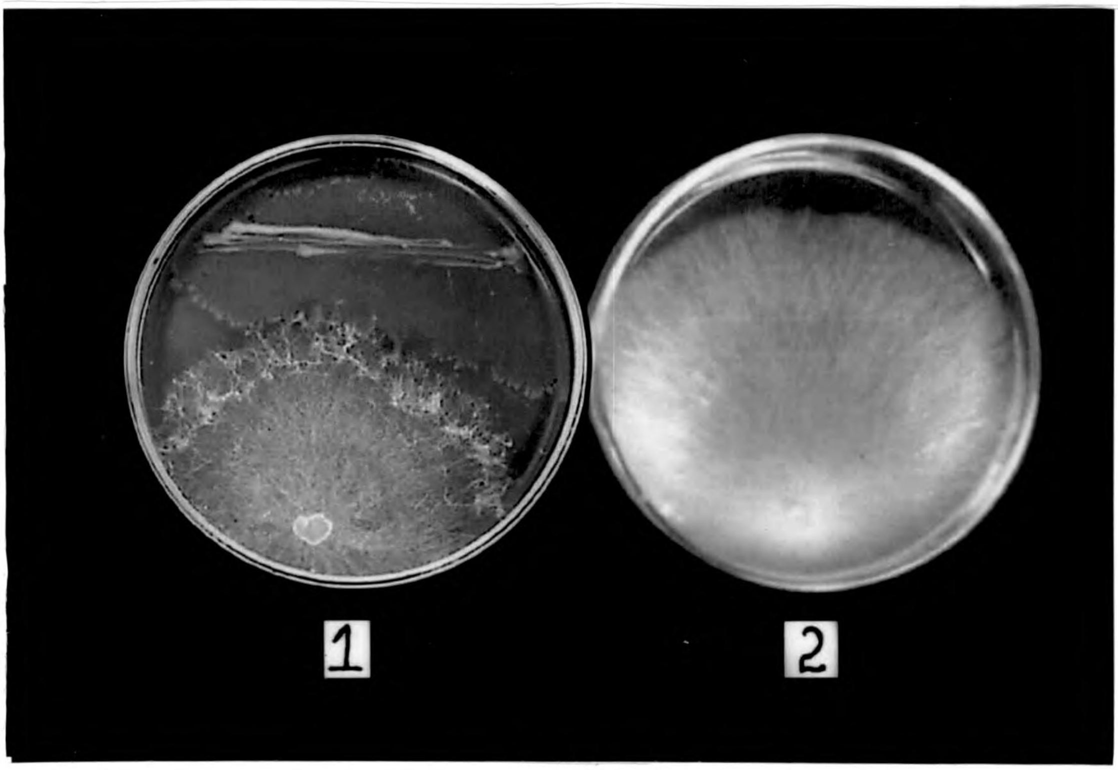


PLATE 10

4.10.2.2. Effect of biocontrol agents on the germination of mint stolons under glasshouse condition

Treatment of mint stolons with biocontrol agents significantly increased the establishment. *T. viride* treated stolons showed the highest germination (95.84 per cent) followed by its combination with *B. subtilis* (91.67 per cent). The lowest germination was recorded from control (47.92 per cent) (Table 18). The results also indicated no significant difference when *Trichoderma* sp. was combined with *B. subtilis.*

4.10.2.3. Effect of biocontrol agents on stolon rot incidence and growth of mint under glasshouse condition

The efficacy of biocontrol agents to contain mint stolon rot incidence was studied under glasshouse condition. There was a marked decrease in the disease incidence in all the treatments when compared to control. *T. viride* treated plants showed the lowest stolon rot incidence (36.90 per cent) followed by its combination with *B. subtilis* (37.69 per cent) at 60 days after planting. All the treated plants exhibited enhanced growth (Plate 11) and *T. viride* treatment resulted in maximum shoot and root lengths at all the observation intervals (Table19).

4.10.2.4. Effect of biocontrol agents on soil population of *R. solani*

Application of biocontrol agents significantly reduced the inoculum load of *R. solani* in soil. *T. viride* isolate alone and in combination with *B. subtilis* effectively reduced the *R. solani* inoculum to 1.03 and 1.12 propagules per gram of soil respectively (Table 20). The highest inoculum load was recorded from control (6.45 propagules per gram of soil).

Table 18. Effect of biocontrol agents on the germination of mint stolons

Sl. No.	Treatments	Germination of mint stolon * (Per cent)
1	*T. viride*	95.84 (81.61)
2	*Trichoderma* sp. (isolate-1)	85.42 (68.09)
3	*Trichoderma* sp. (isolate-2)	72.92 (58.08)
4	*B. subtilis*	79.17 (62.95)
5	*T. viride* + *B. subtilis*	91.67 (78.74)
6	*Trichoderma* sp. (isolate-1) + *B. subtilis*	87.50 (69.56)
7	*Trichoderma* sp. (isolate-2) + *B. subtilis*	75.08 (59.18)
8	Control	47.92 (43.80)
	CD (P = 0.05)	9.18

* Mean of four replications

Figures in parentheses are arcsine transformed values

Plate 11. Effect of biocontrol agents on stolon rot incidence of mint

1. ***T.viride***
2. ***Trichoderma* sp. (isolate-1)**
3. ***Trichoderma* sp. (isolate-2)**
4. ***B. subtilis***
5. ***T.viride* + *B. subtilis***
6. ***Trichoderma* sp. (isolate-1) + *B. subtilis***
7. ***Trichoderma* sp. (isolate-2) + *B. subtilis***
8. **Control**

PLATE 11

Table 19. Effect of stolon treatment with biocontrol agents on stolon rot incidence and growth of mint under glasshouse condition

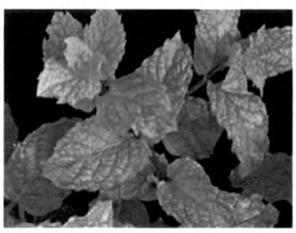
Treatments	Stolon rot incidence* (%)				Shoot length* (cm)				Root length* (cm)			
	15 DAP	30 DAP	45 DAP	60 DAP	15 DAP	30 DAP	45 DAP	60 DAP	15 DAP	30 DAP	45 DAP	60 DAP
T. viride	23.86 (29.16)	28.22 (32.06)	34.85 (36.17)	36.90 (37.41)	7.98	15.73	21.58	27.45	6.13	8.60	10.48	12.68
Trichoderma sp. (isolate-1)	36.51 (37.17)	38.50 (38.32)	43.37 (41.17)	45.64 (42.50)	6.65	10.60	14.90	18.65	5.35	7.30	8.75	9.95
Trichoderma sp. (isolate-2)	42.71 (40.80)	47.27 (42.99)	51.39 (45.80)	54.17 (47.39)	5.33	8.55	12.60	16.25	3.85	4.50	6.88	8.60
B. subtilis	39.44 (38.88)	42.22 (40.52)	44.44 (41.77)	49.72 (49.45)	6.15	9.35	13.28	16.78	4.40	5.48	7.28	8.85
T. viride + *B. subtilis*	24.62 (29.63)	31.06 (33.83)	35.60 (36.63)	37.69 (37.86)	7.33	14.45	18.90	26.77	5.88	8.30	9.05	10.98
Trichoderma sp. (isolate-1) + *B. subtitis*	37.73 (37.80)	40.45 (39.48)	42.95 (40.93)	47.95 (43.81)	6.08	9.98	13.73	18.13	4.90	6.38	7.93	9.95
Trichoderma sp. (isolate-2)+ *B. subtilis*	46.88 (43.19)	49.38 (44.63)	53.13 (46.81)	58.75 (50.06)	5.18	7.30	11.65	15.30	3.08	3.83	5.85	7.63
Control	77.50 (61.88)	87.50 (71.93)	90.83 (77.33)	91.67 (77.93)	4.23	6.70	10.33	13.78	2.70	3.28	4.33	6.70
CD (P=0.05)	5.16	8.03	8.69	8.45	0.96	1.40	1.65	1.47	0.42	0.80	0.75	0.61

*Mean of four replications
Figures in parantheses are arcsine transformed values.
DAP- Days after planting

Table 20. Effect of biocontrol agents on soil population of *R. solani*

Sl.No.	Treatments	Population of *R. solani** (No. of propagules per g of soil)
1	*T. viride*	1.03
2	*Trichoderma* sp. (isolate-1)	1.97
3	*Trichoderma* sp. (isolate-2)	3.10
4	*B. subtilis*	2.68
5	*T. viride* + *B. subtilis*	1.12
6	*Trichoderma* sp. (isolate-1) + *B. subtilis*	2.35
7	*Trichoderma* sp. (isolate-2) + *B. subtilis*	3.58
8	Control	6.45
	CD (P = 0.05)	1.14

* Mean of four replications



Discussion

CHAPTER V

DISCUSSION

Mint (*Mentha viridis* Linn.) is an aromatic herb used in pharmaceutical, food and confectionery industries. A number of diseases attack this crop and cause a heavy toll. Recently, stolon rot occurred in a devastating form in various mint growing areas of Coimbatore district. Hence, the present investigation was undertaken to ascertain the etiology and to work out suitable management practices for the disease.

A survey was conducted in the mint growing pockets of Coimbatore district. The disease occurred in severe form during March-April, 2001. Survey report indicated that the disease incidence was very high in Chittepalayam area where the crop was two-year-old. The increased incidence may be attributed to the population build up of the pathogen in soil, when the crop was grown continuously for a long period. Further, the pathogen produces sclerotia, which can survive in soil for prolonged periods.

5.1. Pathogen

The pathogen responsible for the stolon rot of mint was isolated and identified as *R. solani.* Various fungi have been reported to be associated with the stolon rot of *Mentha* spp. Green (1961) noticed the association of *Rhizoctonia* spp. and *Fusarium* spp. with stolon rot of *M. piperita, M. cardiaca* and *M. spicata.* In Japanese mint (*M. arvensis*) stolon rot has been reported to be caused by *R. bataticola* (Husain and Janardhanan, 1965), *Thielavia basicola* (Sattar and Husain, 1976) and *R. solani* and *M. phaseoli* (Singh, 1991). A perusal of the literature revealed that *R. solani* has not been reported earlier on *M. viridis* from India or abroad. Hence this constitutes a new disease record.

5.2. Virulence of *R. solani* isolates grown on different media

In the present study, *R. solani* isolates exhibited varying degrees of virulence. Variation in the virulence of *R. solani* isolates has been reported by several workers (Shahjahan *et al.*, 1987; Singh *et al.*, 1990).

The sand maize medium grown *R. solani* isolates produced higher disease incidence than potato dextrose broth grown cultures. The difference in pathogenicity may be attributed to the difference in nutrient sources in the two media, which in turn affect the mycelial growth. Earlier workers (Hooda and Grover, 1984; Mehta and Gupta, 1992) had also indicated that the pathogenicity of *R. solani* was greatly influenced by the growth medium.

5.3. Reaction of various *Mentha* spp. to *R. solani*

Variation for resistance to stolon rot was found among the *Mentha* species tested. *M. spicata* was found free from the disease. Stolon rot incidence significantly reduced the essential oil content also.

5.4. Host range of *R. solani*

R. solani is a widespread and destructive plant pathogen. The mint isolate of *R. solani* infected ten plant species belonging to the families Amaranthaceae, Cucurbitaceae, Fabaceae, Poaceae, Malvaceae, Pedaliaceae, Solanaceae and Zingiberaceae.

The ability of *R. solani* to infect a wide range of plant species has been documented by many authors. *R. solani* f.sp. *sasakii*, incitant of maize banded leaf and sheath blight, was found to infect a number of crop plants belonging to families Graminae, Papilionaceae and Solanaceae (Barua and Lal, 1981). The soybean isolate of *R. solani* was found to infect a number of crop and weed species (Bhattacharya, 1996; Black *et al.*, 1996).

The rice isolate of *R. solani* has wide host range and infected plants belonging to 32 families (Saxena, 1997). Sharma and Tripathi (2001) reported that urdbean isolate of *R. solani* was able to infect crop species belonging to Leguminosae, Graminae, Solanaceae, Brassicaceae, Cucurbitaceae and Malvaceae.

5.5. *In vitro* studies on growth of *R. solani*

5.5.1. Growth on various media

In the present study, PDA, corn meal agar and water agar were found to be the best for the growth of *R. solani.* Behera *et al.* (1984) and Meena *et al.* (2001) also observed the suitability of potato dextrose agar medium for promoting the mycelial growth of *R. solani.*

5.5.2. Effect of temperature on *in vitro* growth of *R. solani*

Pathogens differ in their preference for higher or lower temperatures. The optimum temperature for the growth of mint isolate of *R. solani* in PDA and potato dextrose broth was found to be 30°C (Fig. 1). This is in agreement with the observations made by Sangeetha (1988) and Lakpale *et al.* (1997). However, the optimum temperature for the rice isolate of *R. solani* was recorded as 25°C by Tiwari (1997).

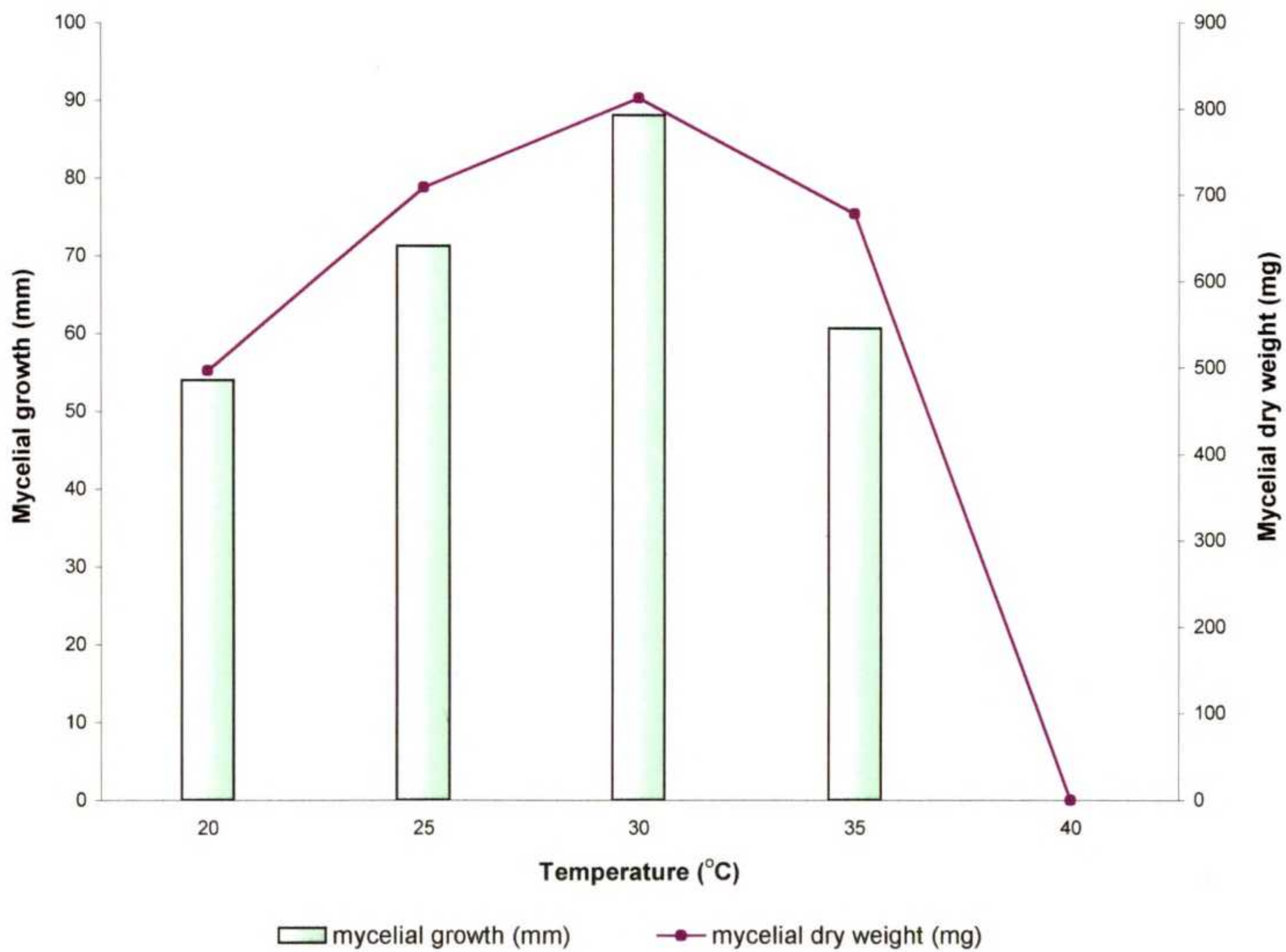
5.5.3. Effect of pH on the growth of *R. solani*

The current study had revealed that *R. solani* causing mint stolon rot could grow over a pH range of 4.0 to 8.0 *in vitro*. The optimum growth occurred at pH 6.0 in potato dextrose broth. The ability of *R. solani* to grow over a wide pH range has been observed by many scientists (Bateman, 1962; Dubey 1997).

5.6. Factors favouring disease incidence

Disease results from the interaction of a pathogen with its host, but the intensity and extent of this interaction is markedly affected by several factors like soil moisture, soil reaction, inoculum potential, age of the host plant, *etc.*

Fig. 1 Effect of temperature on the growth of *R. solani in vitro*



5.6.1. Age of the plant

Susceptibility of crops to diseases varies with the age of the plant. Generally plants are more susceptible to a pathogen at a particular stage of growth. When the crop has crossed that stage, either proneness to infection gets reduced or the crop escapes from disease.

In the present investigation, younger mint plants were found to be highly susceptible to stolon rot than the older ones. The disease incidence decreased with increase in age of the plants, which corroborates with the observations made by Shephard and Wood (1963). They observed that cauliflower plants initially susceptible became resistant as they grew older and the resistance was associated with the appearance of a layer of cells with thickened walls surrounding the vascular tissue which acted as a mechanical barrier to prevent infection.

Saxena (1997) also found that age of plants had significant relationship with the development of banded leaf and sheath blight of maize. He noticed that *R. solani* infection before flowering and silking in maize resulted in complete infection causing no grain formation. Stages beyond sixty days did not favour the cob infection as the grains became hardened during maturity.

In contrast, the rice sheath blight incidence was found to increase with the age of the plants (Sharma and Teng, 1990; Sivakumar, 1994; Vanitha and Narayanaswamy, 1996).

Several biochemical factors may also be involved in the age-based resistance. However, further studies are needed to elucidate the mechanisms involved.

5.6.2. Effect of inoculum level

The stolon rot incidence showed positive correlation with the amount of inoculum and the disease intensity increased with increase in inoculum. Such a phenomenon has been reported by Hadwan and Khara (1992) and Sivakumar (1994). The density of viable sclerotia of *R. solani* AG 2-2 was correlated positively with damping-off and root rot of sugar beet (Hyakumachi and Ui, 1982).

In another study, Venkatasubbaiah and Safeeulla (1983) observed a linear relationship between inoculum density and collar rot (*R. solani*) incidence in coffee. Bruggen *et al.* (1986) noticed that the proportion of infected bean plants increased with inoculum density of *R. solani.* Rice sheath blight incidence increased with the increase in inoculum load (Vanitha, 1992; Damicone *et al.,* 1993; Sivakumar, 1994).

5.6.3. Effect of soil moisture

The geographic distribution of many plant diseases is determined by soil moisture. It is undoubtedly one of the most important factors influencing the development of many soil-borne diseases. Soil moisture either directly affects the activity of pathogens and / or it affects disease incidence through the effect on the host.

In the present study also, soil moisture showed marked influence on stolon rot development. The disease occurred in severe form at higher soil moisture levels (60-80 per cent MHC). However, the pathogen was capable of causing infection even at a low moisture level of 30 per cent MHC.

High soil moisture favoured the development of wire stem or damping – off of cabbage caused by *R. solani* (Wellman, 1932). The damping-off of red pine seedlings was higher at moisture levels between 60-70 per cent (Roth and Riker, 1943). Gonzalez and Owen (1963) reported the optimum moisture content for the development of soil rot of tomato as 60 per cent.

The brown patch disease of wheat caused by *R. solani* was favoured by wet soil (Cook and Papendick, 1972). Sangeetha (1988) reported that at 50 per cent MHC, the pre emergence and post emergence damping-off of blackgram were 38.3 per cent and 23.3 per cent respectively. She observed that at 80 per cent moisture holding capacity, there was no pre emergence damping-off but the post emergence damping off was 36.5 per cent.

5.6.4. Effect of soil pH on stolon rot incidence

The present work has revealed that *R. solani* can cause stolon rot incidence over a wide range of soil pH (4.0 – 8.0). Similar observations have also been made by Roth and Riker (1943) and Bateman (1962). In the present study maximum stolon rot incidence occurred at pH 6.5. The in *vitro* studies have also indicated that the growth of *R. solani* was favoured by a wide pH range of 4.0 to 8.0 with optimum of 6.0. Both the growth *in vitro* as well as the disease incidence in soil were influenced at slightly acidic pH and the pathogen had wide adaptability to various soil conditions (Fig. 2).

5.6.5. Population of *R. solani* in mint rhizosphere

The *R. solani* population increased with time. Since the mint crop is cultivated continuously as a perennial crop, the build up of population of pathogen *R. solani* is expected. The utilisation of nutrient from the plant root exudates may have influenced the population build up. However, further studies are needed to ascertain the mechanism of population build up.

5.7. Management of disease

5.7.1. Management using oilcakes

Oilcakes effectively controlled *R. solani* in both *in vitro* and pot culture studies (Fig. 3). *In vitro* inhibition of *R. solani* by oilcakes has been reported by several authors (Rao *et al.*, 1989; Ezhilan *et al.*, 1994; Dubey and Patel, 2000; Meena *et al.*, 2000).

Fig. 2 Effect of pH on the *in vitro* growth of *R. solani* and stolon rot incidence

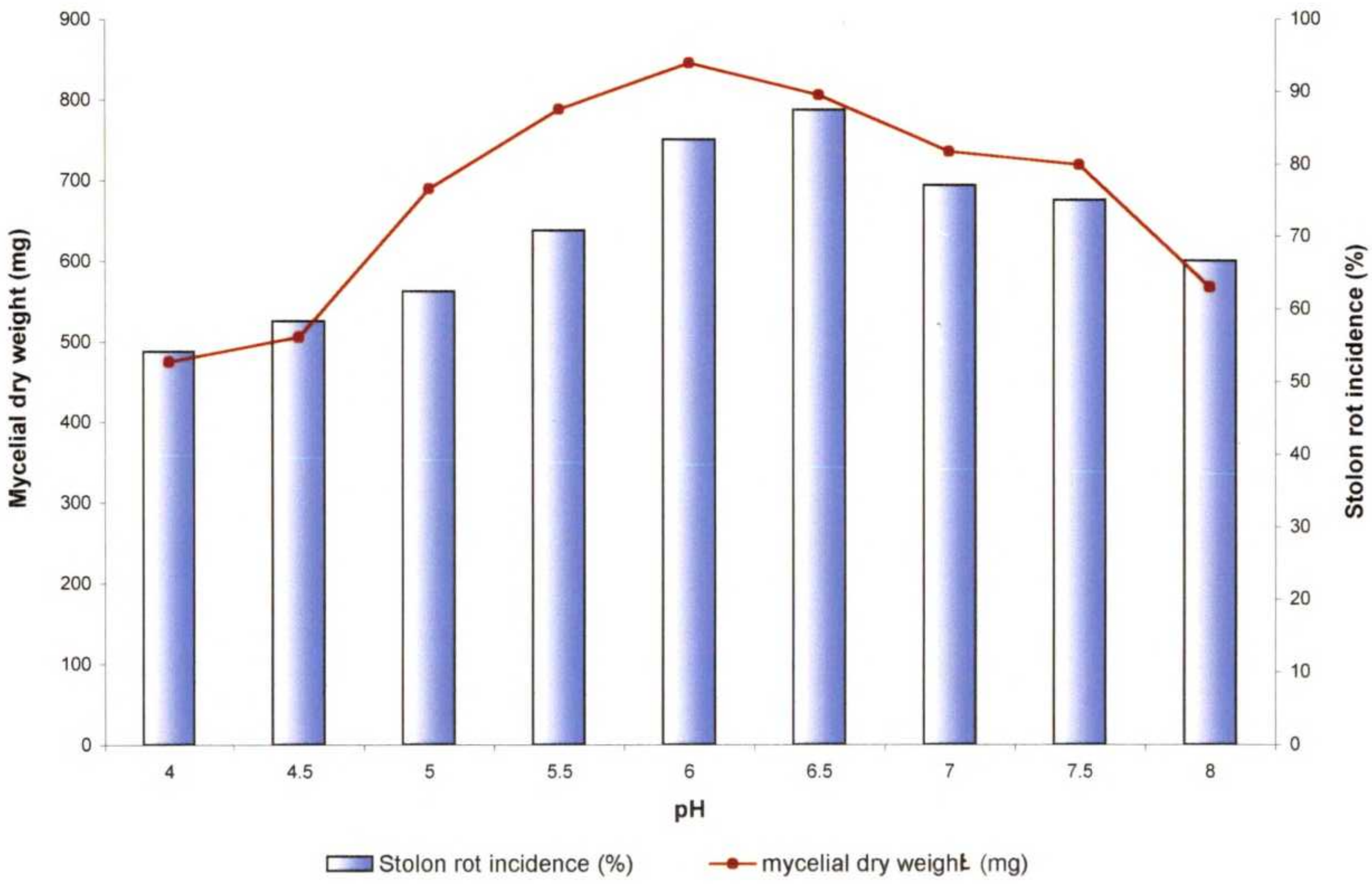
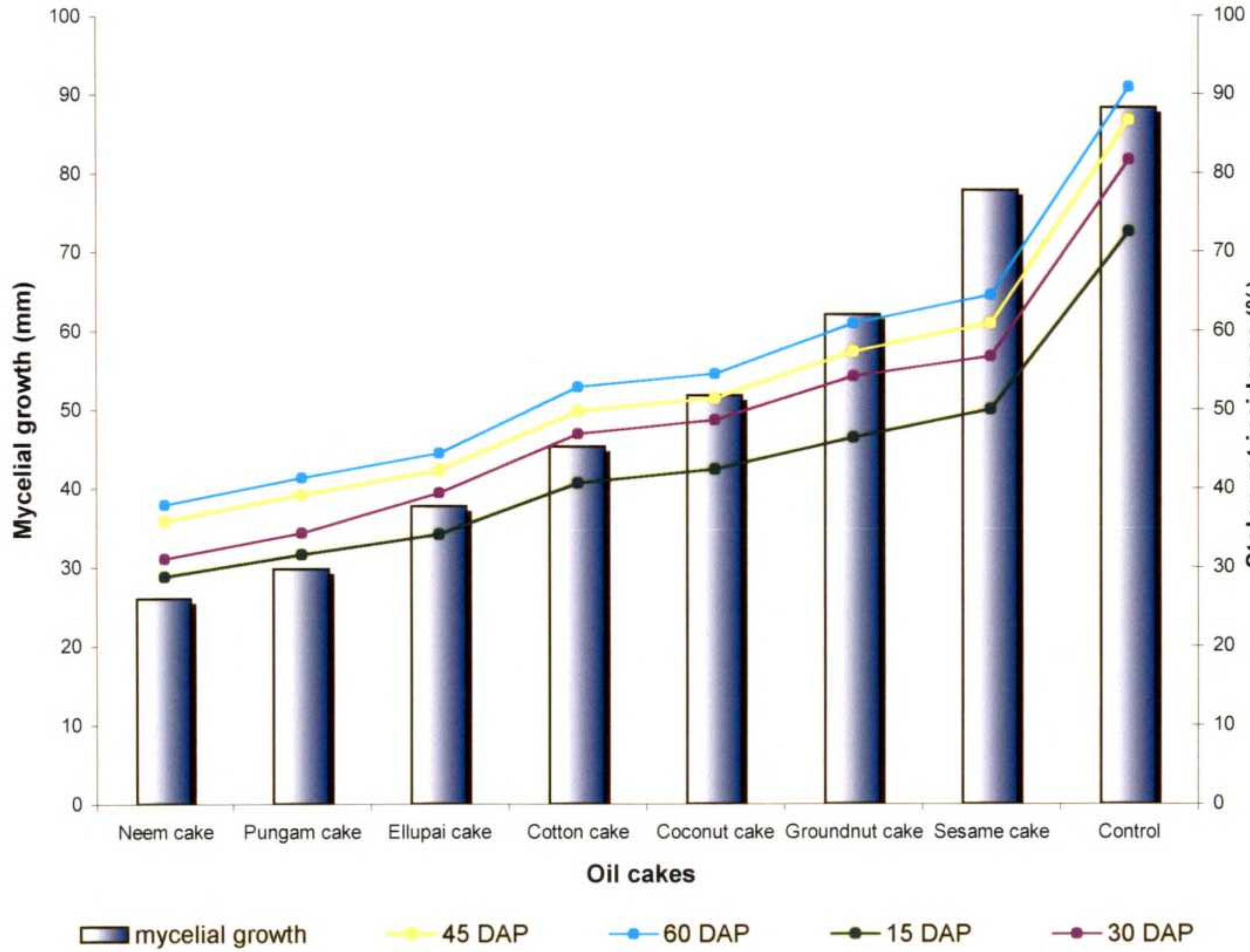


Fig. 3 Effect of oil cakes on *in vitro* growth of *R . solani* and stolon rot incidence



Singh (1968) obtained good control of black scurf of potatoes by amending the soil with mustard cake followed by groundnut, margosa and castor cakes. The soil application of oilcakes significantly reduced the saprophytic survival of *R. solani* sclerotia in soil.

Tiyagi and Alam (1995) reported that oilcakes of neem, castor and mustard effectively reduced the disease incidence caused by *R. solani*, *M. phaseolina* and *Fusarium oxysporum* f.sp. *ciceri.*

Application of groundnut cake in combination with green leaf manure, *Calotropis* significantly reduced the viability of sclerotia of *R. solani* in rice soils (Prasad *et al.*, 1998).

Spraying neem cake extract (10 per cent) reduced the sheath blight incidence (Meena *et al.*, 2000).

Oilcakes significantly reduced the soil population of *R. solani.* The suppression of *R. solani* could be due to the release of toxic products during decomposition of organic materials (Zentmayer, 1963; Gilpatrick, 1969) and the enhancement of natural microbial population having potential antagonism to the pathogen (Rao *et al.*, 1989).

Lumsden *et al.* (1983) reported that increased antagonistic microorganisms in the soil could create an adverse environment to the pathogen through direct parasitism and predation, volatile and nonvolatile fungitoxic factors and lytic enzymes produced by them.

Application of oilcakes not only reduced the stolon rot incidence but also enhanced the growth of the mint plants. Similar observations were made on rice seedlings by Kannaiyan and Prasad (1981b) and Rajan and Menon (1975). The enhanced growth might be due to the change in soil conditions as well as increased nutrient status of the amended soil brought about by the decomposition of organic matter.

5.7.2. Management using biocontrol agents

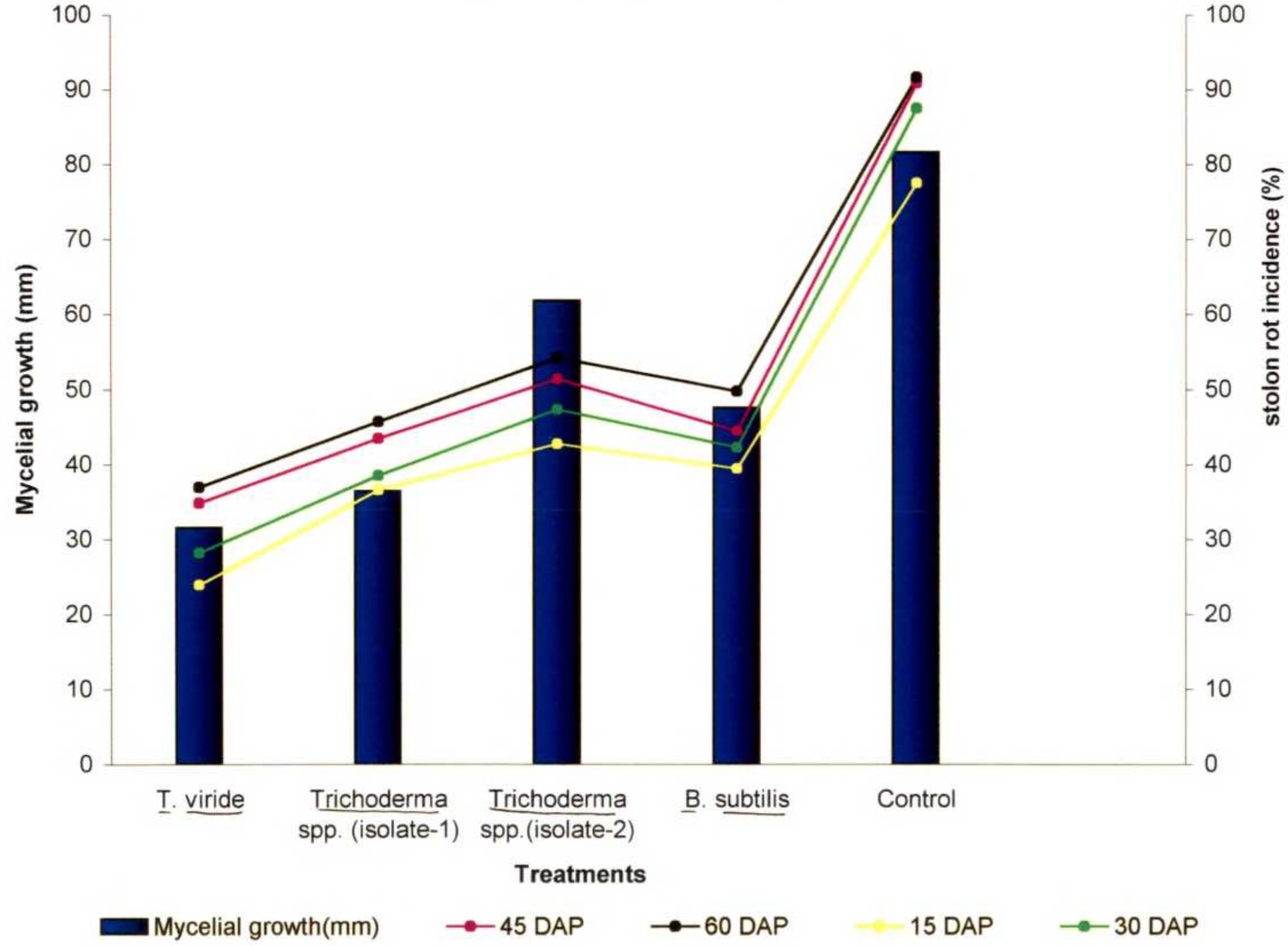
In the present study, the biocontrol agents, T. *viride* and *Trichoderma* sp. (isolate – 1) were found to be highly inhibitory to the growth of *R. solani.* and also reduced the stolon rot incidence in mint (Fig. 4). The treatment with biocontrol agent also significantly increased the plant growth. A spate of literature suggests the beneficial effects of *Trichoderma* spp. in managing plant diseases.

The antagonistic activity of *Trichoderma* spp. to *R. solani* as observed in the present study corroborated with the findings of Hadar *et al.* (1979), Kim and Roh (1987), Roberti *et al.* (1993) and Bhuyan *et al* (1994). The growth inhibitory activity of antagonists could be due to the production of volatile antibiotics (Dennis and Webster, 1971) and hyperparasitism (Wu *et al.*, 1986).

Mint plants are prone to *R. solani* infection in their younger stages. Hence, treatment of stolons with biocontrol agents provides protection in the early stages of establishment. Harman *et al.* (1980) reported that seed treatment with slurry for *T. viride* protected seedlings of pea and radish from infection by *R. solani and Pythium* spp. Seed treatment with *T. viride and B. subtilis* either alone or in combination with adhesive like methylcellulose (2%) effectively reduced the sheath blight and increased grain yield of rice (Das *et al.*, 1998).

Trichoderma spp. have been known to control *R. solani* on a variety of crops (Chet and Baker, 1981). Stem rot of carnation caused by *R. solani* was effectively controlled by the application of wheat bran culture of *T. harzianum* (Elad *et al.*, 1981). They observed a linear correlation between rate of *T. harzianum* preparation applied to soil and degree of disease control.

Fig. 4 Effect of biocontrol agents on *in vitro* growth of *R. solani* and stolon rot incidence

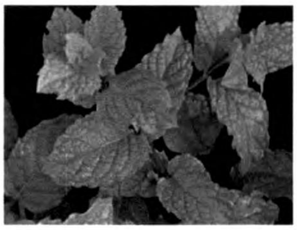


T. harzianum, T. hamatum, T. viride and G. virens effectively reduced the pre and post emergence root rot of French bean in pot culture studies (Mathew and Gupta, 1998). Application of farmyard manure culture of *T. harzianum* significantly reduced the soybean stem rot caused by *R. solani.* The germination, plant height, root and shoot dry weight and grain yield were significantly increased in treated plants (Dutta and Das, 1999).

Trichoderma spp. were known to increase the dry weight of many plant species up to 300 per cent (Chang *et al.*, 1986). The increased growth may be due to control of pathogens and / or due to production of growth stimulatory substances (Windham *et al.*, 1986). This might offer an explanation for the increased germination and enhanced growth of *Trichoderma* treated mint plants.

The present study also indicated reduction in the population of *R. solani* in soils treated with *T. viride* and *B. subtilis.* Lewis and Papavizas (1985) have reported such a phenomenon. They found that mycelial preparations of *Trichoderma* spp. reduced the survival of *R. solani* in soil.

Mint (*Mentha* spp.) is a valued crop used in pharmaceutical and confectionery industries besides being used directly in the food. Since this is a vegetatively propagated crop, it becomes necessary to select disease free stolons. Since the leaves are used in food preparation, use of chemicals for the management of stolon rot disease is ruled out. The present investigation suggests that the use of biocontrol agents for the management of the disease has good potential in view of the awareness on environmental pollution due to chemicals. Studies may be required to standardise the method of stolon treatment to evolve an effective management practice.



Summary

CHAPTER VI

SUMMARY

1. In the survey conducted in different areas of Coimbatore district, mint stolon rot incidence ranged from 16.48 to 36.81 per cent.
2. The incitant of stolon rot of mint (*Mentha viridis* Linn.) was identified as *Rhizoctonia solani* Kühn.
3. Sand maize medium grown inoculum of *R. solani* was found to be the best for inducing stolon rot disease in both sterilized and unsterilized soils.
4. *Mentha viridis* Linn., *Mentha citrata* Ehrh., *Mentha arvensis* Linn. and *Mentha piperita* Linn. were found susceptible to stolon rot while *Mentha spicata* Huds .was found free of the disease. Stolon rot incidence significantly reduced the essential oil content of mint plants and the extent of reduction varied with species.
5. The mint isolate of *R.solani* was capable of infecting ten crop species belonging to eight families.
6. Potato dextrose agar, Corn meal agar and Water agar supported the maximum mycelial growth of *R. solani in vitro*.
7. Under *in vitro* condition, the pathogen grew well at a temperature of 30°C and pH levels 6.0 and 6.5.
8. There was a positive correlation between the disease incidence and inoculum level.
9. Younger mint plants were found more susceptible to stolon rot than the older ones. The disease incidence was higher in 10-day-old plants when compared to 60-day-old plants.
10. High soil moisture levels (60-80 percent MHC) and soil pH 6.0 – 7.5 favoured stolon rot incidence.
11. Population of *R. solani* in mint rhizosphere increased with increase in age of the plant.

12. Extracts of neem cake, pungam cake, ellupai cake, cotton seed cake, coconut cake and groundnut cake inhibited the growth of *R. solani* under *in vitro* condition. Among the various treatments, neem cake extract gave the highest inhibition to *R. solani* growth.
13. Under glasshouse condition, application of neem cake @ 10g / kg of soil recorded maximum stolon germination, reduction in disease incidence and increased root and shoot lengths of mint plants.
14. The antagonists *T. viride,* native isolates of *Trichoderma* sp. and *B. subtilis* inhibited the growth of *R.solani* under *in vitro* conditions. *T.viride* and *Trichoderma* sp. (isolate-1) strongly inhibited the growth of *R. solani in vitro.*
15. Stolon treatment with talc based formulation of *T. viride* (4 g/kg of stolon) effectively controlled the stolon rot incidence, enhanced germination of stolons and promoted the plant growth.

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*Originals not seen

Appendix

APPENDIX

Preparation of the media

The following media were prepared and autoclaved at 1.4 kg cm^{-2} pressure for 20 minutes.

1. Non synthetic medium

Potato dextrose agar (Riker and Riker, 1936)

Potato	:	200.0 g
Dextrose	:	20.0 g
Agar	:	15.0 g
Distilled water	:	1000 ml
pH	:	6.0 to 6.5

2. Synthetic media

A. Solid media

i. Richard's medium (Fahmy, 1923)

Potassium nitrate	:	10.00 g
Potassium dihydrogen orthophosphate	:	5.0 g
Magnesium sulphate	:	2.5 g
Ferric chloride	:	00.02 g
Sucrose	:	50.0 g
Agar	:	15.0 g
Distilled water	:	1000 ml
pH	:	6.6 to 7.2

ii. Czapek's dox medium (Dox, 1910)

Sodium nitrate	:	3.0 g
Dipotassium hydrogen phosphate	:	1.0 g
Magnesium sulphate	:	0.5 g
Potassium chloride	:	0.5 g

Ferrous sulphate	:	0.01 g
Sucrose	:	30 g
Agar	:	15.0 g
Distilled water	:	1000 ml
pH	:	6.8 to 7.2

iii. *Trichoderma* special medium (Elad and Chet, 1983)

Magnesium sulphate	:	0.20 g
Dipotassium hydrogen phosphate	:	0.90 g
Ammonium nitrate	:	1.0 g
Potassium chloride	:	0.15 g
Agar	:	15.0 g
Chlorothalonil	:	0.25 g
Distilled water	:	1000 ml

iv. Nutrient agar (Difco manual, 1953)

Beef extract	:	3.0 g
Peptone	:	5.0 g
Sodium chloride	:	8.0 g
Agar	:	15.0 g
Distilled	:	1000 ml
pH	:	6.8-7.2

v. *Rhizoctonia* selective medium (Ko and Hora, 1971)

Dipotassium hydrogen phosphate	:	1.0 g
Magnesium sulphate. 7 H2O	:	0.5 g
Sodium nitrite	:	0.5 g
Potasium chloride	:	0.50 g
Ferrous sulphate . 7 H2O	:	10.0 mg
Gallic acid	:	0.4 g

Chloramphenicol	:	50.0 mg
Dexon	:	90.0 mg
Streptomycin	:	50.0 mg
Agar	:	20.0g
Distilled water	:	1000 ml

B. Liquid media

i. Potato dextrose broth (Riker and Riker, 1936)

Potato	:	250.0 g
Dextrose	:	20.0 g
Distilled water	:	1000 ml
pH	:	6.0 to 6.5

Richard's broth (Fahmy, 1923)

Potassium nitrate	:	10.0 g
Potassium dihydrogen phosphate	:	5.0 g
Magnesium sulphate	:	2.5 g
Ferric chloride	:	0.02 g
Sucrose	:	50.00 g
Distilled water	:	1000 ml
pH	:	6.8 to 7.2

Czapek's Dox broth (Dox, 1910)

Sodium nitrate	:	3.0 g
Dipotassium hydrogen phosphate	:	1.0 g
Magnesium sulphate	:	0.5 g
Potassium chloride	:	0.5 g
Ferrous sulphate	:	0.01 g
Sucrose	:	30.0 g
Distilled water	:	1000 ml
pH	:	6.8 to 7.2