## EXPLORATION OF THE FEASIBILITY OF BIOLOGICAL CONTROL OF POST HARVEST DISEASES OF SOLANACEOUS VEGETABLES

By

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DEPARTMENT OF PLANT PATHOLOGY College of Agriculture Vellayani, Thiruvananthapuram 1995

# **Dedicated to**

my dear friend Ms. Magimma Zacharias, B.Sc. (Ag.)

#### DECLARATION

I hereby declare that this thesis entitled "Exploration of the feasibility of biological control of post harvest diseases of solaraceous vegetables" 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 to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

ral work

Vellayani,

6.7.1995

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#### CERTIFICATE

Certified that this thesis entitled "Exploration of the feasibility of biological control of post harvest diseases of solanaceous vegetables" is a record of research work done independently by Ms.Reeny Mary Zachariah under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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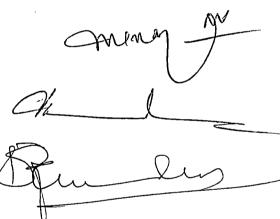
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# INTRODUCTION

#### 1. INTRODUCTION

Post harvest loss. of fresh vegetables and fruits is one of the pressing problems encountered by cultivators and consumers in a tropical country like India. Attention to the concept of post harvest loss reduction as a significant means to increase food availability was drawn by the World Food Conference held in Rome during 1974.

India has emerged as an important fruit and vegetable producer of the world ranking second in vegetable production next to China. It is estimated that by the year 2000 A.D the vegetable requirement in India will be about 83 million tonnes (Jain, 1995).

has been established that post harvest loss reduction is It cheaper than an equivalent increase in food production. Unfortunately the post harvest management of fruits and vegetables is inadequately dealt with. As a result wastage due spoilage estimated at 20-30 percent of the total to microbial production occurs. The situation is evidently compensated by the higher consumer prices.

The widely accepted method for the control of post harvest diseases is the use of fungicides. But this pauses potential

oncogenic risks to the consumers. The hazardous impact of fungicides or other agrochemicals in the ecosystem is also very conspicous.

Biological control of plant diseases is suggested as an alternative to chemical control (Cook, 1977) and is considered as a cost effective and an environmentally friendly technique. Among the biocontrol agents, the mycoprasites have attained a significant position. Boosalis and Mankau as early as 1965 suggested that efforts on the biological control of plant diseases can be achieved only through parasitism and predation.

Eventhough a number of mycorporasites have been recognised (Elad <u>etal.</u>, 1980; Janisiewiz, 1988) their role in combating the post harvest diseases of common vegetables like tomato, brinjal and chilli is very little.

The present study was therefore aimed at overcoming the above difficulty. The major items of work included

- i) Isolation and identification of the fungal pathogens associated with tomato, brinjal and chilli after harvest.
- ii) Correlation studies on fungal incidence with temperature, relative humidity and rainfall for a continuous period of one year.

- iii) Qualitative study of the naturally existing phylloplane mycoflora of tomato, brinjal and chilli (from seedling to adult plant).
- iv) <u>in vitro</u> studies of the common phylloplane fungi with the major pathogens of tomato, brinjal and chilli for evaluating suitable antagonistic fungi.
- v) Mechanism of action of antagonism.
- vi) Effect of selected mycoparasite against the important fruit rot pathogens.

# **REVIEW OF LITERATURE**

#### 2. REVIEW OF LITERATURE

# 2.1 Isolation and identification of pathogens associated with solanaceous vegetables.

Post harvest decay of vegetables is a major problem encountered by cultivators and consumers all over the world. The major items of work carried out in India and abroad on post harvest deterioration of common solanaceous vegetables viz., tomato, brinjal and chilli are reviewed below.

#### 2.1.1. Tomato (Lycopersicon esculentum Mill.)

Critopoulos (1954) in his studies on tomato fruits in California recorded the symptoms due to infection by three species of <u>Phytophthora</u> viz., <u>P. capsici</u>, <u>P. drechsleri</u> and <u>P. parasitica</u>. Of these <u>P. capsici</u> was the vigorous type and effected rapid decay by forming compact tuft of sporangia and mycelia over the surface.

Symptoms due to infection by <u>Fusarium</u> spp. viz., <u>F</u>. <u>nivale</u> and <u>F</u>. <u>moniliforme</u> were studied extensively by Thakur and Yadav (1971). The symptoms started as water soaked lesions and gradually increased in size. The tissues beneath the lesions became depressed and sunken with irregular cracks and wrinklings

while the tissues near the lesions became soft and the surface was covered completely by the cottony growth of the fungus.

Pearson and Hall(1975) recorded fruit rot by <u>Alternaria</u> <u>alternata</u>. The symptoms consisted of brown to black sunken spots with or without a definite border. The neighbouring spots coalesced and became ivory black in colour. The inner tissues turned soft and became dark brown to black with a cylindrical dry core. Under humid conditions dense velvetty olive green spore masses were visible with profuse mouldy growth on the lesions. Similar symptoms were also noticed at the site of the growth cracks. Infected fruits were devoid of any bad odour.

Fruit rots caused by Corynespora cassicola, Aspergillus flavus and F. equiseti were reported by Khanna and Chandra (1976). Corynespora rot was generally observed on ripe fruits. The disease made its appearance in the form of small olive green coloured lesions occasionally surrounded by a chlorotic zone during the months of December and January. The lesions gradually increased in size and included deeper tissues of the fruit. With age their colour changed to olivaceous black. Even well developed lesions showed a regular outline. The infected portion of the fruits became soft and pulpy.

<u>Aspergillus</u> rot was observed on fully ripe fruits. The infection started in the form of water soaked spots at any place on the skin of the fruits. The spots gradually increased in size. The affected skin was ruptured and produced open lesions. Gradually, the deeper tissues of the fruit became affected and developed rotting. Finally major portion of the fruit became rotted and it turned into a soft shapeless mass with pulpy tissue inside.

<u>Fusarium</u> rot appeared as water soaked lesion generally on the stalk end of the fruit. This extended gradually but retained its regular out line. The affected skin ruptured and a lesion involving deeper tissues developed. Older lesions showed white to salmon coloured cottony growth of the fungus consisting of profusely branched hyphae with spores. The affected part of the fruit soon turned into a soft pulp. Sometimes a foul odour with a watery exudate was noticed.

Thapa and Sharma (1976) in their studies on the incidence of fruit rot diseases of tomato under storage conditions in Solan Colletotrichum phomoides recorded fungi like and area parasitica to have caused maximum damage, while Fusarium sp. Ρ. and <u>Rhizoctonia</u> <u>solani</u> caused only negligible losses. Thomas et al. (1981) in their studies from Pennysylvania on green and fruits recorded several pathogens. ripe These included

<u>C. coccodes</u>, <u>A. solani</u>, <u>A. tenuis</u>, <u>F. roseum</u> and <u>Phomopsis</u> sp. on ripe tomatoes and <u>A. tenuis</u> and <u>R. solani</u> on green tomatoes. <u>A. solani</u> and <u>A. tenuis</u> were observed from both green and ripe tomatoes. Studies on the fruit rot infection cycle in packing operations from Florida by Sonoda <u>et al.</u> (1982) recorded <u>Rhizopus stolonifer</u> as the most damaging pathogen.

Moline and Kuti (1984) made comparative studies with <u>Mucor</u> species from Czechoslovakia. They recorded <u>M. mucedo</u> and <u>M. piriformis</u> on mature green and ripe red tomato fruits. The fungus also caused maximum decay of tomatoes within two to three days.

Sharma and Sumbali (1993) conducted an extensive survey at wholesale, retailer and consumer level to assess the various vegetable rot causing fungi in parts of North India. A number of fungi causing various rots in tomato were reported. They included Pythium butleri (cottony leak), Sclerotinia sclerotiorum (wet rot), C. capsici (anthracnose), F. solani (Fusarium rot), Cladosporium sphaerospermum and С. cladosporioides (Cladosporium rot), Penicillium aurantiogriseum (blue mold rot), Geotrichum candidum (sour rot), A. alternata (dark olive green rot), <u>Curvularia lunata (Curvularia</u> rot), <u>Bipolaris spicifera</u>, (Bipolaris rot) and R. stolonifer (soft rot).

### 2.1.2. Brinjal (<u>Solanum melongen</u>a L)

Pawar and Patel (1957) observed a Phomopsis blight and fruit brinjal. They reported disease symptoms ranging from rot of seedling blight to fruit rot. In the fruit, the symptoms manifested as minute sunken dull purple lesions which coalesced to form large rotten areas. Numerous pycnidia of the fungus also visible on these spots. Markov and Ahtpakhora (1958)were recorded anthracnose of brinjal caused by C. melongena. This induced extensive lesions and led to complete rotting of the fruits. Ramakrishnan and Wilson (1968) reported Rhizopus rot with profuse white growth and Diplodia rot with dark growth on the fruit surfaces with extensive rotting of the tissues. Similar results were also obtained by Alice and Pailey (1978) in their studies on post harvest spoilage of vegetables by fungi. Lakshminarayana and Reddy (1979) recorded C. capsici, A. niger, lycopersici, R. solani, Ascochyta Myrothecium roridum, Helminthosporium spiciferum and Cladosporium cladosporioides to have caused considerable damage. Detailed studies on the varietal susceptibility of brinjal fruit to soft rot by P. vexans at Jabalpur showed maximum percentage of rotting in the fruits of long purple variety, followed by Oval green, Round purple and Dwarf green within 20 days of incubation (Chowdhury and Hasiza, 1979).

Brinjal fruit rot by <u>F</u>. <u>moniliforme</u> was recorded as a new pathogen by Datar (1980). The variety Manjiri Gota was found to be susceptible. The lesions were sunken and brown in colour. Profuse pinkish growth was visible on the calyx and stemend region of fruits.

A fruit scab characterised by scabby growth with light cracks due to <u>Cladosporium tennuissimum</u> was also recorded (Mandal and Das Gupta, 1980). Ali and Shukla (1981) reported soft watery rot accompanied by tissue discolourations during November to March from Gwalior due to infection by <u>R. oryzae</u>. Later the infection progressed rapidly and the pathogen completely covered the fruit by 15-20 days.

et al. (1986) reported pathogens like A. nidulans, Kumar Cephalosporium acremonium, F. moniliforme, F. oxysporum and R. stolonifer from market samples in Punjab. Aspergillus' rot started as a brown water soaked lesion with brown centre and light brown margin. Soon they became brown and the margins diffused with the original colour of the fruit. Cephalosporium rot started as small water soaked black spots. Later they increased in size rapidly and resulted in the complete rotting of the fruit within 3-4 days. The rotten area was watersoaked and glossy in appearance with a pulpy texture. The stylar end of the fruit was also rotten where the mycelium of the fungus was

present. In the case of <u>F</u>. <u>oxysporum</u>, the rot gradually extended from the stylar end to stalk end of the fruit and in a few days whole fruit became soft and rotten. Misra and Rath (1986) also reported similar symptoms with <u>F</u>. <u>moniliforme</u> and <u>F</u>. <u>equiseti</u> from samples collected from the markets of Bhubaneswar. Sundaresan <u>et al</u>. (1986) isolated <u>P</u>. <u>vexans</u>, <u>Botryodiplodia</u> <u>theobromae</u> and <u>Rhizopus</u> sp. in addition to <u>Fusarium</u> sp.

Studies conducted at the College of Agriculture, Vellavani by John (1991) revealed the occurrence of fruit rot by <u>P. vexans</u>. The rots appeared as minute, circular, sunken, greyish spots with brownish halo which later enlarged to produce concentric rings with yellow and brown zones. The outer most ring got separated from the healthy fruit surface. Lesions increased in size and formed large rotten areas with abundant pycnidia.

Sharma and Sumbali (1993) during their survey of vegetable markets in North India reported various fungal rots of brinjal. They reported soft rot caused by <u>R</u>. <u>stolonifer</u>, cottony leak by <u>P</u>. <u>diliensii</u>, anthracnose by <u>C</u>. <u>capsici</u>, <u>Fusarium</u> rot by <u>F</u>. <u>oxysporum</u>, sour rot by <u>G</u>. <u>candidum</u>, dark olive green rot by <u>A</u>. <u>alternata</u>, grey mold rot by <u>Botrytis cinerea</u> and <u>Paecilomyces</u> rot by <u>Paecilomyces variotii</u>.

## 2.1.3. Chilli (<u>Capsicum annuum</u> L)

Rao (1965) recorded storage diseases of chilli fruits from Bombay due to infection by <u>Diplodia</u> sp. Ramakrishnan and Wilson (1968) in their studies on the post harvest diseases of chilli reported anthracnose as a common disease of chilli causing damage in the field and storage. Wrinkled lesions appeared on fruits and profuse dark dots represented the acervuli of the fungus. The rotting of the tissues was completed within 3-4 days.

Alice (1969) reported infection due to <u>R</u>. <u>arrhizus</u>, <u>Fusarium</u> sp., <u>Diplodia</u> sp. and <u>A</u>. <u>carbonarius</u> from the College of Agriculture, Vellayani. <u>Rhizopus</u> produced a wet rot with the entire fruit covered by the mycelium, while <u>Fusarium</u> rot changed the colour to black and rotting was over by eight days. In <u>Diplodia</u> fruit rot the purple colour of the fruit was changed to dark brown. Black pycnidia developed on the surface of the fruits in advanced stages. <u>Aspergillus</u> produced dry rot with the black conidial head on the rotten surface.

Sharma <u>et al</u>. (1980) reported fruit rot due to infection by <u>F. solani</u> and <u>F. diversisporum</u>. In the former case, a light yellow colour was noticed at the site of incision. With further advancement of the rotting, tissues became soft and mycelium was visible externally. A foul smell and a watery secretion was also

visible. Finally the tissues became yellow and soft. <u>F. diversisporum</u> produced complete degradation within 5 days of inoculation. The pedicel infection resulted in the secretion of milky juice. Rots caused by <u>Verticillium</u> <u>psalliote</u>, <u>F. moniliforme</u>, <u>Fusarium</u> sp. and <u>A. alternata</u> were reported by Uma (1981).

Adisa (1985) from Nigeria described chilli fruit rots caused by <u>R. oryzae</u>, <u>R. stolonifer</u>, <u>Cladosporium</u> sp., <u>F. oxysporum</u>, <u>F. equiseti</u>, <u>A. fumigatus</u>, <u>A. flavus</u> and <u>P. multicolor</u> and accounted for 40-45 and 25-35 percent of the losses during the wet and dry season respectively. The rots were widely distributed and produced serious damage. Similar studies were also made by Datar and Ghule (1985).

Misra and Rath (1986) reported <u>F. oxysporum</u> and <u>F. solani</u> in chilli from the markets of Bhubaneswar. The incidence of Fusarium in rotten samples ranged from 47-77 percent. Based on extensive survey conducted in parts of North India at wholesale, retailer and consumer level to assess the various vegetable rot fungi, Sharma and Sumbali (1993) recorded <u>C. capsici</u>, <u>F. equiseti</u> ond <u>F. moniliforme</u>, <u>A. flavus</u>, <u>C. cladosporioides</u> and <u>A. alternata</u> from chilli.

#### 2.2 Incidence of fruit rot in relation to environment factors

Growth and sporulation of fungi are initiated by relative humidity and temperature of the atmosphere. Depending upon these factors variations in symptoms also occur. Thus Chand <u>et al</u>. (1968) reported a high relative humidity favoured the disease development by <u>Gloeosporium</u>. The incidence by <u>R</u>. <u>solani</u> Was maximum at 100 percent relative humidity (Ali, 1970). Effect of temperature and relative humidity on the pathogenesis of <u>A</u>. <u>solani</u> and <u>A</u>. <u>tenuis</u> have been studied in detail by Mehta <u>et al</u>. (1975). Disease symptoms were not observed when the fruits were incubated at 8°C or at 45°C for both the pathogens.

Similarly development of fruit rot by the two pathogens was also recorded at 100 percent relative humidity.

Effect of temperature was also prominent. Thus Garg and Gupta (1979) reported the fruit rot of tomato by <u>F. solani</u> to be maximum at temperatures of  $28-30^{\circ}$ C. Excessive low or high temperature retarded the fungal spread and rotting.

Hasija and Batra (1979) observed the incidence of fruit rot by <u>Phoma</u> <u>destructiva</u> at a temperature range of  $15-35^{\circ}$ C with maximum rotting at  $25^{\circ}$ C. A relative humidity of 100 percent favoured the maximum occurrence of <u>Phoma</u> rot. Bartz (1980)

noticed the incidence of tomato rot by <u>G</u>. <u>candidum</u> in severe proportions during shipment in Florida at 25-27°C and 80 percent relative humidity. Temperatures of  $25\pm2°$ C and relative humidity of about 90 percent favoured fruit rot by <u>Nigrospora oryzae</u> and <u>Stemphylium vesicarium</u> (Chary <u>et al</u>. 1980). Severity of tomato fruit rot by <u>C</u>. <u>tenuissimum</u> was maximum at 25°C and 80 percent relative humidity (Narain and Rout, 1981). <u>M</u>. <u>mucedo</u> caused complete rotting of the fruit within 3-4 days at 20°C. Surface <u>the</u> growth and sporulation of the pathogen on the fruit was maximum at high relative humidity.

Highest rotting of brinjal fruits by P. vexans occurred at 25°C and above 75percent relative humidity (Pawar and Patel, 1957). Mehta and Mehta (1989) found no incidence of fruit rot by oxysporum and F. moniliforme at 8°C and 45°C respectively. F. Maximum rotting was observed at 28°C for both the organisms. The maximum disease development was found to be parallel to the rate radial mycelial growth of the pathogens. Sharma and Sumbali of (1993) observed that high relative humidity favoured infection and spread of <u>R. stolonifer</u>, <u>P. diliensii</u>, C. capsici, F. oxysporum, G. candidum and B. cinerea on brinjal fruits.

Adisa (1985) observed that <u>Cladosporium</u> sp. grew best at 25°C on chilli but no spore germination occurred at low relative humidity. Dasqu**ptots** and <u>Mandal</u> (1989) reported that high

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humidity favoured the incidence of rots by <u>C</u>. <u>capsici</u> and <u>Fusarium</u> spp. viz., <u>F. solani</u> and <u>F. equiseti</u>. Sharma and Sumbali (1993) observed that <u>C</u>. <u>capsici</u>, <u>F. equiseti</u>, <u>F. moniliforme</u>, <u>A. flavus</u>, <u>C. cladosporioides</u> and <u>A. alternata</u> caused enourmous post harvest wastage of chilli at high relative humidity.

2.3 Phylloplane mycoflora of tomato, brinjal and chilli

The leaf surface or the phylloplane constitutes a distinct and is inhabited by a varied assemblage microhabitat of saprophytic and parasitic organisms (Preece and Dickinson, 1971). Sinha (1971) in his studies on the phylloplane microflora of chilli, brinjal and tomato through out its growth period observed several species of fungi. This included M. hiemalis, Spicariase S. F. moniliforme, A. niger, A. flavus, C. cladosporioides, A. tenuissima and A. solani throughout the growing season on tomato while R. nigricans and C. herbarum were confined to moderately cold and cold weather. Kashyap and Levkina (1977) isolated <u>Penicillium</u> <u>brevicompactum</u>, Trichoderma viride. <u>Cladosporium</u> sphaerospermum and <u>Acremonium</u> spp. from the leaves of tomato while Nair (1977) found A. alternata, C. lunata and R. bataticola in the phyllosphere of tomato plants. Khara and Singh (1981) in two crop seasons obtained Achaetomium sp.,

<u>A. globosum, Aureobasidium pullulans, Phomopsis</u> sp., <u>Sepedonium</u> <u>ochraceum, Sphaeronema allahabadense, A. alternata, Aspergillus</u> sp., <u>Fusarium</u> sp., <u>Curvularia</u> sp. and <u>Epicoccum purpurascens</u> from different tomato varieties.

Similar studies on brinjal yielded <u>M</u>. <u>hiemalis</u>, <u>R</u>. <u>nigricans</u>, <u>Fusidium</u> sp., <u>Sporotrichum</u> sp., <u>C</u>. <u>cladosporioides</u>, <u>A. solani</u> and <u>C. siddiquii</u> throughout the growing seasons and <u>C. herbarum</u> and <u>Alternaria</u> sp. during cold weather. <u>Syncephalastrum</u>, sp., <u>F. moniliforme</u>, <u>A. niger</u>, <u>A. flavus</u>, <u>A. sydowii</u>, <u>Aspergillus</u> sp., <u>Penicillium</u> sp., and <u>A. tenuissima</u> were common during cold and moderately warm weather. During cold weather <u>A. sulphureus</u> was confined to young leaves while <u>Fusarium</u> and <u>A. fumigatus</u> were exclusively associated with mature leaves only.

Capsicum leaves also yielded fungi like M. hiemalis, Cunninghamella sp., F. moniliforme, Fusidium sp., A. niger, P. janthinellum, A. solani and C. siddiqui throughout the growing season and C. herbarum and Alternaria sp. during moderately cold cold weather. <u>R. nigricans, T. koningii, A. flavus</u>, and Aspergillus sp., <u>C. cladosporioides</u>, <u>Heterosporium</u> sp., tenuissima and Papulaspora sp. were common during cold A. and moderately warm weather. Fungi like P. candidum and Strachybotrys 50, were found on young leaves during cold weather

whereas moderately warm weather promoted the population of <u>Syncephalastrum</u> sp., <u>A. niveus</u> and <u>Fusarium</u> sp. Those fungi exclusively associated with mature leaves included <u>Cephalosporium</u> sp., during cold and warm weather and <u>A. fumigatus</u> and <u>C. verruculosa</u> during the cold weather.

#### 2.4 Studies on mycoparasitism, selection of suitab antagonists and mechanism of action of antagonism

Naturally occuring microbial antagonists and their interaction have been studied extensively by various workers. Thus Weindling (1932) recorded T. liqnorum as a parasite on soil fungi like A. niger, Penicillium sp. and F. lateritium. Boosalis (1954) observed parasitism of <u>R</u>. solani by <u>Penicillium</u> sp. through hyphal penetration while England (1969) reported the parasitism of <u>C</u>. <u>cucurbitarum</u> by <u>Piptocephalis</u> <u>virginiana</u> through production of abnormal branching manifested by swollen hyphae, witches broom or coiling of hyphae. Turner and Tribe (1976) observed the parasitic action of Coniothyrium minitans on <u>Sclerotinia</u> <u>sclerotiorum</u> by invasion of sclerotia <u>in vitro</u>. (1977) has reported <u>Syncephalis</u> <u>californica</u> as Hunter an aggressive parasite capable of attacking R. oryzae under a wide range of soil environments. Tronsmo and Raa (1977) reported the the apple rot pathogen suppression of Β. cinerea by T. pseudokoningii through growth inhibition. Huang (1978) found

<u>Gliocladium catenulatum</u> as a hyperparasite of <u>S</u>. <u>sclerotiorum</u> and <u>Fusarium</u> spp. and caused the affected cells to collapse and disintegrate.

Singh <u>et al</u>. (1978) noted <u>Acremonium</u> <u>sordidulum</u> to be mycoparastic on <u>C</u>. <u>dematium</u>. f. <u>truncata</u> in India. EI-shafie and Webster (1979) reported <u>Curvularia</u> sp. parasitised <u>R</u>. <u>stotonifer</u>, <u>R</u>. <u>sexualis</u> and <u>R</u>. <u>arrhizus</u> by penetrating the rhizoidal hyphec, sporangiophores and sporangia. Chlamydospore formation by the parasite inside the host was also hoted. They also explained the antagonistic action of <u>Drechslera</u> <u>specifera</u> and <u>A</u>. <u>alternata</u> against <u>R</u>. <u>arrhizus</u> through penetration of vegetative hyphae, sporangiophores and sporangia.

Arora and Dwivedi (1980) and also observed the mycoparasitic activity of <u>Fusarium</u> spp. viz., <u>F. oxysporum</u>, <u>F. semitectum</u> and <u>F. udum</u> towards <u>R. solani</u> through hyphal penetration and coiling. Elad <u>et al.</u> (1980) observed <u>T. harzianum</u> as a biocontrol agent effective against <u>S. rolfsii</u> and <u>R. solani</u>. The mechanism of action was by hyphal interaction and also through the production of cell wall lytic enzymes resulting in the lysis of the mycelia. Hyphal parasitism of <u>F. oxysporum</u> f. sp. <u>lycopersici</u> on <u>R. nigricans</u> was characterised by coiling, penetration and ramification inside the host. Rupture of the host hyphae and

chlamydospore formation by the parasite was also noted. The host hyphae bulged out and deposited a 'wall like' barrier and developed further (Pathak <u>et al</u>., 1981).

Dwivedi and Mishra (1982) studied the hyperparasitism of <u>C</u>. <u>cladosporioides</u> to the hyphae of <u>R</u>. <u>orvzae</u>. They observed coiling, penetration and remification of the hyphae inside the host along with the granulation of cytoplasm. Brame and Flood (1983) reported the inhibition of <u>A</u>. <u>solani</u> through the production of antibiotics by the antagonist <u>Aureobasidium</u> pullulans.

Padmakumari (1989) in her studies found A. niger, Chaetomium globosum, F. semitectum, F. solani, G. virens, Neurospora crassa, P. citrinum, P. oxalicum, P. wortmanii, R. oryzae, R. stolonifer, т. harzianum and T. viride to be antagonistic to R. solani in vitro conditions. Further studies with in pot culture experiments showed the effectiveness of T. viride and T. harzianum as antagonistic organisms in reducing the intensity of sheath blight of rice by restricting the survival of R. solani.

# MATERIALS AND METHODS

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#### 3. MATERIALS AND METHODS

#### 3.1 Isolation and identification of pathogens associated with solanaceous vegetables - Tomato, (Lycopersicon esculentum Mill.), Brinjal (Solanum melongena L) and Chilli (Capsicum annuum L).

Samples of tomato, brinjal and chilli with disease symptoms were collected from the local markets of Thiruvananthapuram and Instructional farm, College of Agriculture, Vellavani. The materials were collected in polythene bags, brought to the laboratory and isolations were made within 24 hrs. of collection. Care was taken to avoid rotted materials.

#### 3.1.1 Isolation and identification of the organisms

Diseased portion of each sample was cut into small pieces and surface sterilised by dipping in 0.1 per cent aqueous mercuric chloride for two minutes followed by washing in three changes of sterile distilled water. The pieces were kept asceptically 2cm. apart on sterilized and melted potato dextrose agar medium (Appendix - I) and incubated at room temperature (28±1°C) for 7 days. The fungal growth was transferred to potato agar slants after purification dextrose by single spore Subsequent subculturing was isolation. done at monthly Morphological and cultural characteristics intervals. of the fungi isolated were studied and identified by slide culture technique of Riddel (1950).

Plain agar was melted and poured into petridishes to a thickness of 2mm and after solidification, blocks of 6mm square were cut out using a sterile needle. One such square was placed in the centre of a sterile microscopic slide and all the four sides of the agar block was inoculated with small culture bits of the fungus. A cover slip was placed on the top of the agar block and the slide was kept in a moist chamber and incubated at room temperature for seven days. After this the cover slip was lifted off gently, a drop of 15 percent alcohol was placed in the centre and before drying, the coverslip was mounted using lactophenol cotton blue. The culture was examined and identification made.

The pathogenecity of the isolates was proved following Koch's postulates. Fresh samples of tomato, brinjal and chilli were collected from the local markets of Thiruvananthapuram, the vegetables were surface sterilised as detailed earlier and inoculations with different pathogens were carried out using the technique of Grainger and Horne (1924). The method consisted of making a hole of 8mm diameter on the fruit surface by a sterile cork borer. A fungal bit from an actively growing culture was kept in the hole and plugged with the cut piece and incubated at room temperature for 7-10 days. The isolates that proved their pathogenecity were taken up for further studies.

Mature and intact fruits of uniform size and age were selected and inoculated with the selected pathogens as given below.

#### Tomato

<u>Aspergillus flavus</u> <u>Fusarium solani</u> <u>Fusarium oxysporum</u> Rhizoctonia solani

Mucor hiemalis

Choanephora cucurbitarum

Colletotrichum gloeosporioides

Alternaria solani

Chilli

Mucor hiemalis

Penicillium italicum

Fusarium oxysporum

<u>Fusarium solani</u>

Phoma sp.

Colletotrichum capsici

Phytophthora capsici

<u>Cladosporium</u> sp.

Brinjal Alternaria solani Fusarium solani Botrytis cinerea Rhizopus nigricans Curvularia lunata Botryodiplodia theobromae Mucor sp.

Penicillium sp.

The nature and extent of damage at different periods (4, 8, 12 & 16 days) were recorded. Three replications were maintained for each treatment. The pathogens which recorded maximum damage were used for further studies.

# 3.2.Occurrence of fungal pathogens and their correlation with weather parameters

The pathogens associated with the spoilage of tomato, brinjal and chilli during storage were monitored for a continuous period of one year during 1993 at monthly intervals. The occurrence of pathogens were correlated with weather parameters like temperature, relative humidity and rainfall collected from the meteorological observatory, Department of Agronomy, College of Agriculture, Vellayani.

#### 3.3.Isolation and identification of phylloplane mycoflora

Fourteen days old seedlings of tomato, brinjal and chilli obtained from the Instructional farm, College of Agriculture, Vellayani were raised in earthern pots of 35 cm. diameter filled with garden soil. Manures and fertilizers were given as per the package of practice recommendations of KAU (1993). Two plants were maintained in each pot and six replications were kept for each crop. Leaf samples were collected at 15 days interval for a continuous period of 4 months.

The phylloplane population in the different samples was determined as per the soild dilution and plate technique outlined Timonin (1940) with slight modifications are given below. bv Uniform leaves were tagged and samples collected. Leaf bits of One sq. cm. were cut, pooled together, and the weight was adjusted to This was then added to 99 ml. of sterile distilled water lqm. and flasks were shaken by a mechanical shaker for 20 minutes. of the suspension was pipetted from each flask andml. One transferred to 99 ml. of sterile water in 250 ml conical flasks. This dilution of 10<sup>4</sup> was used for estimating the population of One ml. of this dilution was transfered in to sterile fungi. petridish using sterile pipette. About 15 ml of the medium dextrose agar with rose bengal streptomycin and peptone (Appendix-II), melted and cooled to 45°C was dispensed into the and rotated to ensure uniform spread of the petridishes Three replications were maintained. suspension in the medium. The plates were incubated at room temperature and isolations made five days after plating.

## 3.4. Studies on mycoparasitism

studies of phylloplane mycoflora with the In vitro 3.4.1. common pathogens of tomato, brinjal and chilli for the selection of suitable mycoparasite

The method outlined by Skidmore and Dickinson (1976) was for studying the interactions of pathogens with followed phylloplane fungi. Potato dextrose agar blocks of 3mm. diameter containing seven day old growth of mycelia of both pathogen and the phylloplane fungi were placed 3.5cm. apart on PDA in a petridish and incubated at room temperature for 12 days.

The pathogens were selected based on the frequency of occurrence and extent of damage made by them.

The selected pathogens and the common phylloplane fungi are given below.

Phyllonlane fungi

Crop	Pathogens	Phylloplane fungi
Tomato	l. <u>Fusarium solani</u>	1. <u>Botryodiplodia</u> <u>theobromae</u>
	2. <u>Rhizoctonia</u> <u>solani</u>	2. <u>Pestalotic</u> palmarum
		3. <u>Phoma</u> sp.
		4. Trichoderma viride
		5. <u>Aspergillus niger</u>
		6. <u>A</u> . <u>flavus</u>

7. A. terreus

Brinjal	l. <u>Alternaria</u> <u>solani</u>	1. <u>B. theobromae</u>
	2. <u>Fusarium</u> <u>solani</u>	2. <u>P</u> . <u>palmarum</u>
		3. Phoma sp.
	- /	4. <u>T</u> . <u>viride</u>
		5. <u>A</u> . <u>niger</u>
		6. <u>A</u> . <u>flavus</u>
		7. <u>A</u> . <u>terreus</u>
Chilli	l. <u>Fusarium</u> . <u>solani</u>	1. <u>B</u> . <u>theobromae</u>
٩	2. <u>Colletotrichum</u> <u>capsici</u>	2. <u>P. palmarum</u>
		3. <u>Phoma</u> sp.
		4. <u>T. viride</u>
		5. <u>A. niger</u>
		6. <u>A</u> . <u>flavus</u>
		7. A. terreus

Three replications were maintained for each treatment. The paired cultures were examined after 12 days and the nature of reactions were noted. Interaction types were assigned according to the method adopted by Purkayastha and Bhattacharya (1982) as follows.

A. Homogenous intermingling between organisms

B. Overgrowth ~ Pathogen overgrown by mycoparasite (test fungus)

C. Cessation of growth at line of contact

D. Clear zone of inhibition

E. Overgrowth - mycoparasite overgrown by the pathogen

3.4.2. Mechanism of action of antagonism

The dual culture technique of Dennis and Webster (1971) was used for studying the mechanism of antagonism. In sterile petridishes melted PDA was poured and allowed to solidify. Sterilised cellophane discs of 90mm. diameter were placed over this so as to lie flat on the medium, using a pair of sterile forceps. An agar disc of 5mm diameter containing the mycelium of the fruit rot pathogen cut from the margin of an actively growing culture of the fungal pathogen was placed 2cm. apart along with a 5mm. agar disc of the test fungus. The plates were incubated at 28±1°c for seven days. Direct observations were carried out after incubation period under a light microscope at the zone of hyphal contact. Microscopic observation for hyphal interaction was also made by cutting out one cm<sup>2</sup> portions of cellophane containing intermingling hyphal growth and mounted on glycerine. The different mechanisms of mycoparasitism exhibited by the efficient antagonists of fruit rot pathogens were also studied.

3.4.3. Selection of suitable mycoparasites

The fungi that showed inhibition of the fruit rot pathogen in <u>in vitro</u> studies were selected as effective mycoparasites. From the different mycoparasites so obtained only <u>Trichoderma</u> <u>viride</u> was utilised further for <u>in vivo</u> studies as shown below.

Vegetables	used	Pathogens	Mycoparasite selected
Tomato	1.	<u>Fusarium</u> solani	<u>Trichoderma</u> viride
	2.	<u>Rhizoctonia solani</u>	
Brinjal	1.	<u>Fusarium solani</u>	<u>Trichoderma</u> <u>viride</u>
	2.	<u>Alternaria solani</u>	
Chilli	1.	<u>Fusarium</u> <u>solani</u>	<u>Trichoderma</u> <u>viride</u>
	2.	<u>Colletotrichum capsici</u>	

Fresh fruits of tomato, brinjal and chilli were selected, surface sterilised, washed with two changes of sterile water and sprayed with the conidial suspension of <u>T</u>. <u>viride</u> from seven days old cultures and air dried. They were then inoculated with their respective pathogens using pin prick method viz., 5 pricks were made near the stemend portion of the fruit using a sterile pin and culture discs of 5mm. diameter were placed over the pinpricks. The fruits were then incubated at room temperature. Fresh fruits surface sterilised, sprayed with sterile water and

inoculated with the respective pathogens served as control. Three replications were maintained for each treatment. Observations on keeping quality and extent of damage were recorded at 4 days interval for a period of 16 days.

# RESULTS

#### 4. RESULTS

# 4.1 Isolation and identification of pathogens associated with solanaceous vegetables

Several fungi were found to be associated with the spoilage of solanaceous vegetables viz., tomato, brinjal and chilli under storage conditions. They are listed below.

4.1.1. Tomato

- 1. Aspergillus flavus Link ex Fr.
- 2. Fusarium solani (Martius) Sacc.
- 3. <u>F. oxvsporum</u> Schlect
- 4. <u>Rhizoctonia solani</u> Kühn
- 5. <u>Mucor hiemalis</u> Wehmer
- 6. Choanephora cucurbitarum (Berk & Ray) Thaxt.
- 7 <u>Colletotrichum gloeosporioides</u> Penz.
- 8. Alternaria solani Sorauer
- 9. Phoma sp.
- 10. <u>Geotrichum candidum</u> Link. ex Pers
- 11. Rhizopus stolonifer (Ehrenb, ex Link) Lind
- 12. Cladosporium sp.

Fungi like <u>F. solani</u>, <u>F. Oxysporum</u>, <u>C. glososporioides</u>, <u>A. solani</u>, and <u>A. flavus</u> were present throughout the study and can be considered as the common pathogens associated with tomato while <u>R. Solani</u>, <u>M. hiemalis</u> and <u>C. cucurbitarum</u> were seasonal in appearance.

4.1.1.1. Nature of damage

The nature of damage obtained by selected fungi is described below.

#### 4.1.1.1.1. Aspergillus rot c.o : Aspergillus flavus

The rot started initially as water soaked lesions. It gradually increased in size, ruptured the skin and the juice leaked out with a fermentative odour. The tissues became soft and mycelia with greenish sporulation was observed on the outer surface. (Plate 1.1)

## 4.1.1.1.2 Fusarium rot c.o : Fusarium solani, F. oxysporum

Infection started as water soaked lesions with initially later depressed centre. Irregular cracking of raised but the fruits was common. Dirty white mycelia was seen along the cracks. The disease progressed rapidly, disintegrated the inner tissues which lost their turgidity and resulted in the exudation of yellow juice with an unpleasant odour. (Plate 1.2 and Plate 1.3).



Plate	1.	Nature	of	damage	by	1.	Aspergill	us flavus
						2.	Fusarium	solani
1 distribution						3.	Fusarium	oxysporum



Plate 2. Nature of damage by 1. Rhizoctonia solani 2. Mucor hiemalis

### 4.1.1.1.3 Rhizoctonia rot c.o :R.solani

Lesions were roughly circular with a dark brown border and pale centre. As the infected area enlarged, it became slightly sunken and remained firm. Splitting of the skin occurred in different directions. The whole fruit surface was covered with dense flocculent mycelia within seven days. Profuse growth of sclerotia was also observed. The inner tissues became soft and watery. (Plate 2.1)

#### 4.1.1.1.4. Mucor rot c.o :M. hiemalis

On the fruit surface within two to three days of infection, water soaked areas with fluffy yellowish white mycelia of the fungus was noticed. In advanced stages the fruit was completely shrunken and emitted a fermentative smell. (Plate 2.2.)

#### 4.1.1.1.5. Choanephora rot c.o : Choanephora cucurbitarum

Starshaped cracks occurred at early stages of infection. Centre of the infection became depressed and soft. The skin got loosened from the pulp with a white scanty superficial growth which later became dense and dark. As the lesions advanced, secondary invasion by saprophytic fungi occurred. The rotten fruits emitted an offensive odour. (Plate 3.1)

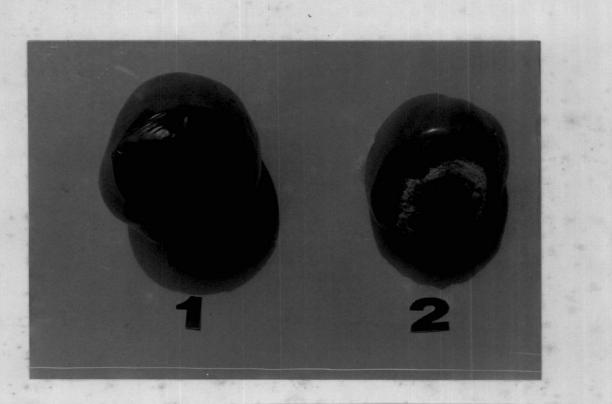


Plate 3. Nature of damage by 1. <u>Choanephora cucurbitarum</u> 2. <u>Colletotrichum gloeosporioides</u>

## 4.1.1.1.6. Colletotrichum rot c.o.: C. gloeosporioides

Lesions were circular at first later became water soaked dark and sunken. White coloured spore masses in concentric circles were often visible. Slight cracking and watery ooze was observed. (Plate 3.2.)

## 4.1.1.1.7. Alternaria rot c.o : A. solani

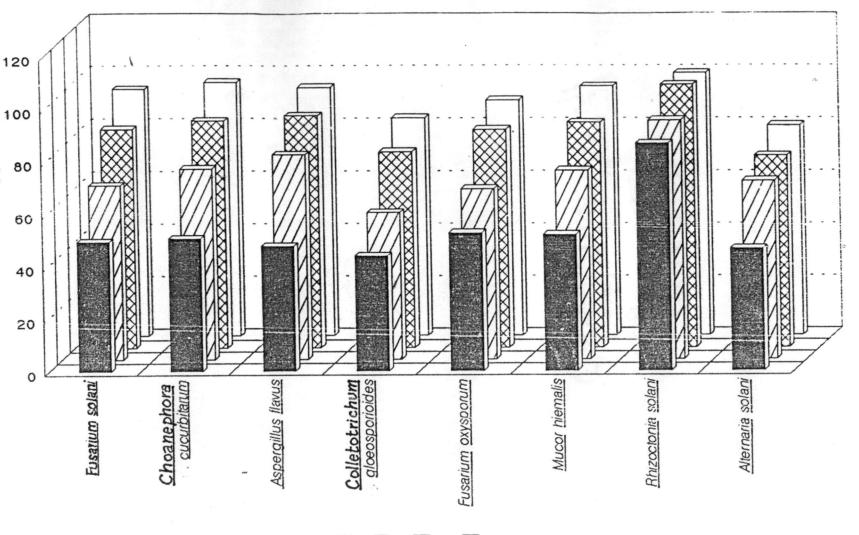
Decayed tissue was firm and dry. The colour became brown to black and extended deep into the fruit. Internal cavity was lined with dark grey growth. Under humid conditions. production of olive green mycelium with abundant spores was visible. Fruits were devoid of any bad odour.

4.1.1.2. Extent of damage

The extent of damage caused by the major pathogens at different periods viz., 4, 8, 12 and 16 days after inoculation are given in table 1, Fig.1 and Appendix-III. <u>R. solani</u> caused maximum damage. The damage was 91 percent by the 8th day and it became 100 percent by the 12th day and the fruit was completely covered by fungal growth. <u>A. flavus</u>, <u>C. cucurbitarum</u>, <u>M. hiemalis</u>, <u>F. solani</u> and <u>F. oxysporum</u> caused more than 80 percent and 90 percent damage by twelth and sixteenth day respectively and were on par.

	Days a	(%)		
Particulars	4	8	12	16
Fusarium solani	49.33	66.33	83.33	94.00
Choanephora cucurbitarum	50.67	72.67	86.33	96.67
Aspergillus flavus	47.67	78.00	88.33	94.67
<u>Colletotrichum</u> gloeosporioides	44.00	56.00	74.67	83.00
Fusarium oxysporum	52.67	65.00	83.00	89.67
Mucor hiemalis	52.00	72.00	85.67	95.00
Rhizoctonia solani	86.67	91.00	100.00	100.00
Alternaria solani	46.67	68.00	73.00	80.00
C.D. for comparing treatment means (0.05)	11.6390	15.269	10.398	9.019

Table 1.Extent of damage by major pathogens to tomato during storage at different periods



24 28 212 16 days after inoculation

Fig. 1. Extent of damage by major pathogens to tomato during storage at different periods

4.1.2. Brinjal

The fungi associated with the spoilage of brinjal are given below.

- 1. Alternaria solani Sorauer
- 2. Fusarium solani (Martius) Sacc.
- 3. Botrytis cinerea Pers.
- 4. Rhizopus nigricans Ehrenb
- 5. Curvularia lunata (Wakker) Boediin
- 6. Botryodiplodia theobromae Pat
- 7. Aspergillus niger Van Tieghem
- 8. Phomopsis vexans (Sacc & Sydow)
- 9. Colletotrichum gloeosporioides Penz
- 10. Phytophthora palmivora Butler
- 11. Trichothecium roseum Link
- 12. Pythium aphanidermatum (Eds.) Fitz
- 13. Rhizoctonia solani Kuhn

14. Mucor sp.

15. Penicillium sp.

<u>Aspergillus niger</u> and <u>T. roseum</u> were found to be present occasionally while fungi like <u>A.solani</u>, <u>F.solani</u>, <u>C.gloeosporioides</u>, <u>B.cinerea</u>, <u>Penicillium</u> sp., <u>R.nigricans</u>, <u>C.lunata</u> and <u>B.theobromae</u> caused considerable damage throughout the year where P. vexans, P. palmivora, P. aphanidermatum and R. solani were seasonal in appearance and were confined to the rainy periods.

4.1.2.1. Nature of damage

# 4.1.2.1.1. Alternaria rot C.O: Alternaria solani

The disease is characterised by small, circular spots with definite margin. Some of the spots were sunken with or without skin breaks. Often these coalesced together to form large patches. The flesh became spongy and turned grey to dark tan. Deep brown to black, scanty superfiscial mycelial mass was visible. (Plate 4.2)

## 4.1.2.1.2 Fusarium rot C.O: Fusarium solani

Symptom started as water soaked areas which advanced and in the later stages turned light brown. The infected areas got decayed, over which the dense white mycelial growth of the fungus was observed. (Plate 4.1)

## 4.1.2.1.3. Botrytis rot C.O : Botrytis cinerea

Infection started as circular brown area with a pale margin. Centre turned greenish due to spore formation. During later stages, the tissues turned brown. A liquid oozed out with a foul smell. (Plate 5.2)



Plate 5	Nature	of	damage		Penicillium sp.		
and the				2.	Botrytis	cinerea 🖌	
			영제 전쟁이	 2.	Rhizopus	nigricans	

### 4.1.2.1.4 Rhizopus rot C.O: Rhizopus nigricans

Water soaked areas were concentrated around injuries and soon this was covered by the mycelial growth. Abundant sporulation was noticed. The mycelial mass bearing brown to black sporangia appeared only along the margin. Brownish liquid with a characteristic odour oozed out. (Plate 5.3)

#### 4.1.2.1.5 Diplodia rot C.O: Botryodiplodia theobromae

Symptom started as a brownish colouration later became brownish black. Skin became rough due to pycnidial encrustations. Fruits became wrinkled and shrivelled. No juicy exudation or foul smell was noticed. (Plate 4.3.)

### 4.1.2.1.6 Curvularia rot C.O: Curvularia lunata

Infection started as regular circular or irregular concentric lesion with a faded outer zone initially. This later turned grey. The inner tissues became soft. The whole fruit turned brownish black with a velvetty cover due to heavy sporulation. (Plate 6.1)

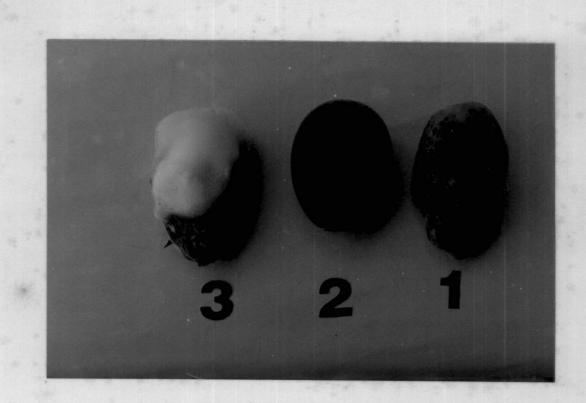


Plate 6. Nature of damage by 1. <u>Curvularia</u> <u>lunata</u> 2. <u>Colletotrichum</u> <u>gloeosporioides</u> 3. <u>Mucor</u> sp. 4.1.2.1.7 Colletotrichum rot C.O: Colletotrichum gloeosporioides

The disease is characterised by a small, circular, water soaked lesion which later turned dark and became sunken. Salmon coloured spore masses in concentric circles were observed. (Plate 6.2)

4.1.2.1.8 Mucor rot C.O: Mucor sp.

Water soaked areas appeared around the injuries and soon this was covered with white fluffy mycelial mat. Brown liquid oozed out in later stages and the fruit became soft and flattened. (Plate 6.3)

4.1.2.1.9. Pencillium rot C.O: Penicillium sp.

Infection started as water soaked lesion which later turned brown. Bluish green spore mass was observed. Fruits were wrinkled in appearance without odour. (Plate 5.1)

4.1.2.2. Extent of damage

The extent of damage caused by different pathogens are given in Table 2, Fig.2 and Appendix IV. <u>B. theobromae</u> caused maximum infection after 4 days followed by <u>B. cinerea</u> and <u>A. solani</u> and were on par. The damage caused by <u>B. theobromae</u> was maximum at 12 and 16 days. The fruit was almost covered by fungal growth

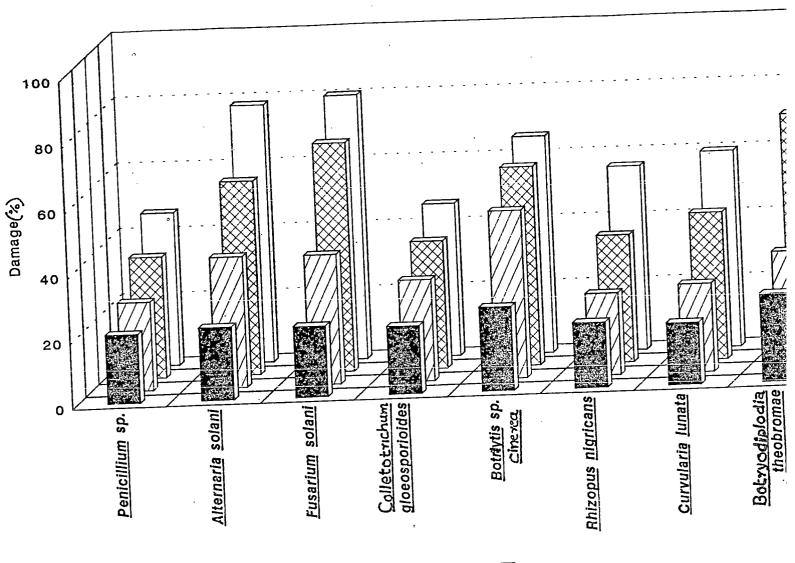
(92 percent) by the 16th day. This was followed by <u>F</u>. <u>solani</u> (81 percent) and <u>A</u>. <u>solani</u> (79 percent).

Table 2. Extent of damage by major pathogens to brinjal

	Days after inoculation (%)					
Particulars	4	8	12	16		
<u>Penicillium</u> s <b>p</b>	21.00	27.00	37.00	46.67		
<u>Alternaria solani</u>	22.33	40.00	59.33	78.67		
<u>Fusarium solani</u>	21.67	39.67	70.00	80.67		
<u>Colletotrichum</u> gloeosporioides	20.73	31.00	39.00	46.67		
<u>Botrytis</u> <u>cinerea</u>	25.67	51.33	60.87	66.33		
Rhizopus nigricans	20.00	25.00	39.00	56.33		
<u>Curvularia lunata</u>	19.00	27.00	45.00	60.00		
<u>Botryodiplodia</u> theobromae	27.00	36.00	74.33	91.67		
C.D. (0.05) for comparing treatment means	7.189	11.192	14.379	17.150		

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▲ 4 🛛 8 🖾 12 🔲 16 days after inoculation

Fig. 2. Extent of damage by major pathogens to brinjal

4.1.3. Chilli

The fungi associated with the spoilage of chilli are given below.

- 1. Mucor hiemalis Wehmer
- 2. <u>Penicillium italicum</u> Wehmer
- 3. Fusarium oxysporum Schlect
- 4. F. solani (Martius) Sacc
- 5. Curvularia lunata (Wakker) Boedijn
- 6. Phoma sp.
- 7. Colletotrichum capsici (Syd.) Butler and Bisby
- 8. Phytophthora capsici sensu lato
- 9. Cladosporium sp.

The fungi like <u>F. solani</u>, <u>C. capsici</u> and <u>F. oxysporum</u> were found throughout the year, while <u>P. capsici</u> and <u>M. hiemalis</u> were noticed only during rainy seasons. <u>Cladosporium</u> sp., <u>Phoma</u> sp. and <u>C. lunata</u> were occasionally recorded.

4.1.3.1. Nature of damage

The nature of damage by fungi were as described below.

## 4.1.3.1.1. Mucor rot C.O: Mucor hiemalis

The affected portions became water soaked and depressed. Fluffy white mycelia was seen and the infected region became soft, watery and the juice leaked out (Plate 7.1).

# 4.1.3.1.2. Penicillium rot C.O: Penicillium italicum

Light brown discolouration advancing in an oval manner was seen initially. Fruits became soft and leaky with bluish green spore mass at the region of the fruit cap. Internal brown discolouration was also observed. (Plate 7.2)

# 4.1.3.1.3 and 4 Soft rot C.O: Fusarium oxysporum and F.solani

Infection started mostly from the stylar end as small, water soaked, brownish area with scanty mycelial growth on the surface. Rotten fruit became soft and a watery exudate oozed out. Under high humid conditions, the fluffy mycelial cottony growth fully covered the fruit. (Plate 7.3 and 9.3)

## 4.1.3.1.5. Curvularia rot C.O: Curvularia lunata

Light brown discolouration later turning to black was observed. Infected area was covered with large number of spores (Plate 8.1).



Plate 7. Nature of damage by 1. Mucor hiemalis 2. Penicillium italicum 3. Fusarium oxysporum



Plate 8. Nature of damage by 1. <u>Curvularia lunata</u> 2. <u>Phoma</u> sp.

3. Colletotrichum capsici

4.1.3.1.6. Phoma rot C.O: Phoma sp.

Slight brown discolouration of the fruit was seen initially. Water soaked spots with light brown border later becoming ashy grey, sunken, leathery but firm with pycnidia was observed. Dirty white mycelial growth was seen over the lesion. Internal discolouration was also noticed. (Plate 8.2)

# 4.1.3.1.7. Anthracnose C.O: Colletotrichum capsici

Symptoms appeared as small water soaked circular and sunken spots. They enlarged gradually and centre of the spot became black in colour with acervuli appearing as black pustules in concentric rings. Under humid conditions, creamy pink spore masses could be noticed along with the sparse grey black mycelium (Plate 8.3)

# 4.1.3.1.8. Phytophethora rot C.O: Phytophthora capsici

Greyish brown discolouration very slowly advancing was observed. The internal tissues became soft and was seen adhering to the loose skin. (Plate 9.1)

# 4.1.3.1.9. Cladosporium rot C.O: Cladosporium sp.

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Olive green to blue green large circular spots were seen. Later spots turned ash coloured, dry and paperv. Fluffy mycelial growth was seen on the surface of the spots (Plate 9.2).



Plate 9. Nature of damage by 1. Phytophthora capsici 2. Cladosporium sp. 3. Fusarium solani

# 4.1.3.2. Extent of damage

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The extent of damage due to various pathogens at different periods viz., 4, 8, 12 and 16 days after inoculation are given in Table 3, Fig. 3 and Appendix V.

the 4th day after inoculation, maximum damage was found On to be induced by C. capsici followed by P. italicum. fungi The like <u>F. solani</u>, <u>Phoma</u> sp., <u>M. hiemalis</u> and <u>F. oxysporum</u> were similar in their effects and were on par. The least damage was causedby P. capsici and Cladosporium sp. while on 12th and 16thday maximum damage was recorded by P. italicum and Fusarium spp. S. solani and F. oxysporum. C. capsici and M. hiemalis viz., The least were also similar in their effects and were on par. damage was noticed by P. capsici and Cladosporium sp. on the 16<sup>th</sup> italicum was occasionally noticed eventhough it also <u>P</u>. day caused considerable damage.

	Days after inoculation (%)					
Particulars	4	8	12	16		
Penicillium italicum	30.67	73.67	100.00	100.00		
<u>Fusarium solani</u>	26.33	73.00	100.00	· 100.00		
Fusarium oxysporum	21.67	°65.33	93.00	100.00		
<u>Colletotrichum</u> <u>capsici</u>	31.00	45.33	73.00	94.67		
<u>Mucor hiemalis</u>	22.67	36.33	79.00	91.67		
<u>Cladosporium</u> sp.	15.33	18.67	25.33	42.67		
<u>Phytophthora</u> capsici	14.00	17.66	20.33	30.00		
<u>Phoma</u> sp.	23.00	31.33	43.33	53.67		
C.D. (0.05) for comparing treatment means	8.57	9.29	14.46	15.17		

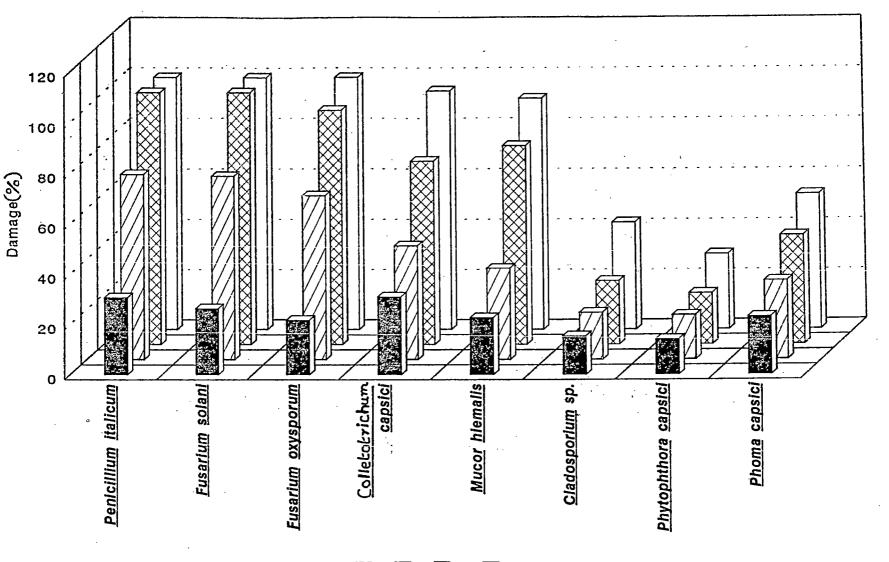
Table 3. Extent of damage by major pathogens to chilli

# 4.2 Occurrence of fungal pathogens and their correlation with weather parameters.

#### 4.2.1. Tomato

The data on the type of fungi associated with the spoilage of tomato during storage dimegiven in Table 4 and in Appendix. VI.

January, February and March were recorded as the dry and hot periods of the year with the maximum temperature ranging from  $30-33^{\circ}C$  and mean relative humidity from 70-80 percent with



4 28 212 16 days after inoculation

Fig. 3. Extent of damage by major pathogens to chilli

occasional rains. Fungi causing storage rots were minimum during this period and included <u>F. solani</u>, <u>A. flavus</u>, C. gloeosporioides, <u>G. candidum</u> and <u>A. solani</u>.

The temperature continued to be high during April eventhough a total rainfall of 21.49 mm was received durng this period. The first week of May continued to be in the grip of high temperature with no rainfall and there after received continuos rains up to the end of August. The mean relative humidity also ranged from 77-91 percent during this period. But June, July and August continuously recorded a mean relative humidity of above 90percent 7 am with continuos rains on all weeks.

The fungal spoilage was also maximum during this period. cucurbitarum, <u>M</u>. hiemalis, by C. Mucorales represented nigricans and R. stolonifer dominated. Rhizoctonia solani R. fungi included recorded considerable damage. Other also Phoma <u>oxysporum, G. candidum</u>, sp. and solani, F. Ά. C. gloeosporioides.

During September, October, November and December the maximum temperature ranged from  $28-31^{\circ}$ C and mean relative humidity from 78-89 percent. Rainfall was also recorded during this period. The fungal flora included <u>A. solani</u>, <u>F. solani</u> and <u>M. hiemalis</u>. In addition to these, <u>Phoma</u> sp. and <u>Cladosporium</u> sp. were also frequently observed.

Month	Storage Pathogens
January	<u>Fusarium solani, Aspergillus flavus, Geotrichum</u> <u>candidum, Colletotrichum</u> gloeosporioides
February	<u>Fusarium solani,Aspergillus flavus,Colletotrichum</u> gloeosporioides,Geotrichum, candidum, Alternaria solani
March	<u>Aspergillus flavus, Alternaria solani, Collestotrichum gloeosporioides, Fumsarium solani, Geotrichum candidum</u>
April	<u>Alternaria solani, Aspergillus flavus, Fusarium oxysporum, Geotrichum candidum</u>
Мау	<u>Alternaria solani, Choanephora cucurbitarum,</u> <u>Fusarium oxysporum, Rhizopus nigricans, Geotrichum</u> <u>candidûm</u>
June	<u>Choanephora cucurbitarum, Fusarium oxysporum,</u> Geotrichum candidum, Phoma sp., <u>Rhizoctonia solani</u>
July	<u>Choanephora</u> <u>cucurbitarum,</u> <u>Colletotrichum</u> gloeosporioides, <u>Fusarium</u> solani, <u>Geotrichum</u> candidum, Rhizoctonia solani, Mucor hiemalis
August	<u>Alternaria solani, Choanephora cucurbitarum, Mucor</u> <u>hiemalis, Phoma</u> sp., <u>Rhizopus stolonifer</u> , <u>Geotrichum candidum</u>
September	<u>Alternaria solani, Colletotrichum gloeosporioides, Fusarium solani, Geotrichum candidum, Mucor hiemalis, Phoma sp.</u>
October	<u>Rhizoctonia solani, Cladosporium</u> sp., <u>Fusarium</u> <u>solani, Mucor hiemalis, Phoma</u> sp., <u>Rhizopus</u> Migricans, Alternaria solani, Aspergillus niger
November	<u>Alternaria solani, Colletotrichum gloeosporioides,</u> Fusarium solani, Phoma sp.
December	<u>Aspergillus flavus, Cladosporium</u> sp., <u>Fusarium</u> <u>solani, Phoma</u> sp., <u>Alternaria solani</u>

Table 4. Incidence of fruit rot pathogens of tomato during 1993 from January to December.

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#### 4.2.2. Brinjal

The data on the type of fungi associated with the spoilage of brinjal during storage digiven in Table 5and Appendix-VI. The common pathogens associated all throughout the year were recorded as <u>A. solani</u> and <u>F. solani</u> followed by <u>P. vexans</u>. Maximum fungal population was noticed during the months of May, June, July and August. This coincided with the low temeperature and high relative humidity and rainfall of the above period. The fungi included <u>M. hiemalis, P. palmivora, P aphanidermatum</u> and <u>R. solani</u> in addition to the earlier mentioned fungi.

The other fungi recorded during September, October, November and December included <u>B</u>. <u>theobromae</u>, <u>C</u>. <u>lunata</u>, <u>R</u>. <u>nigricans</u>, <u>B</u>. <u>cinerea</u> and <u>T</u>. <u>roseum</u>. <u>Phytophthora</u> <u>palmivora</u> and <u>P</u>. <u>aphanidermatum</u> were also noticed during this period. This was favoured by the high relative humidity and rainfall recorded during the above period.

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Month	Storage Pathogens
January	<u>Alternaria</u> <u>solani</u> , <u>Colletotrichum</u> <u>gloeosporiodes</u> , <u>Fusarium solani</u> , <u>Mucor</u> sp., <u>Rhizopus nigricans</u>
February	<u>Alternaria solani,Colletotrichum gloeosporioides, Fusarium solani, Rhizopus nigricans</u>
March	<u>Alternaria solani, Aspergillus niger,</u> <u>Botryodiplodia theobromae, Curvularia lunata</u> , <u>Fusarium solani, Mucor</u> sp., <u>Rhizopus nigricans</u>
April	<u>Alternaria solani, Aspergillus niger,</u> <u>Botryodiplodia theobromae, Curvularia lunata,</u> Fusarium solani, Pencillium sp., Phomopsis vexans
May	<u>Alternaria solani, Aspergillus niger, Fusarium</u> <u>solani, Pencillium</u> sp., <u>Phomopsis</u> <u>vexans</u> , Rhizoctonia solani
June	<u>Alternaria solani, Botrytis cinerea, Fusarium solani, Phomopsis vexans, Phytophthora palmivora, Trichothecium roseum, Rhizoctonia solani, Pythium aphanidermatum</u>
July	<u>Alternaria solani, Botrytis cinera, Fusarium solani, Phomopsis vexans, Phytophthora palmivora, Rhizoctonia solani</u>
August	<u>Alternaria solani, Aspergillus niger,</u> <u>Botryodiplodia theobromae, Curvularia, lunata,</u> <u>Fusarium solani, Phytophthora, palmivora,</u> <u>Penicillium sp., Rhizopus nigricans, Phomopsis</u> <u>vexans</u>
September	Alternaria <u>solani</u> , <u>Aspergillus</u> niger, <u>Botryodiplodia theobromae</u> , <u>Curvularia</u> , <u>lunata</u> , <u>Penicillium</u> sp., <u>Phytophthora palmivora</u> , <u>Pythium</u> <u>aphanidermatum</u> , <u>Mucor</u> sp., <u>Rhizopus</u> nigricans, <u>Phomopsis vexans</u>

Table 5 :Incidence of fruit rot pathogens of brinjal during 1993 from January to December.

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Month	Storage Pathogens
October	<u>Botrytis cinerea, Fusarium solani, Phytophthora</u> <u>palmivora, Pythium aphanidermatum, Mucor</u> sp., <u>Rhizopus nigricans, Trichothecium roseum, Phomopsis</u> <u>vexans</u>
November	Botryt <u>is cinerea, Colletotrichum gloeos</u> por <u>ioides,</u> Phomopsis vexans, Rhizopus nigricans
December	<u>Botrytis cinerea, Colletotrichum gloeosporioides, Phomopsis vexans, Rhizopus nigricans, Trichothecium roseum</u>

4.2.3. Chilli

The incidence of fruit rot pathogens of chilli were given in Table 6 and Appendix-IV.

<u>Fusarium</u> spp. were found to be present through out the year followed by <u>C</u>, <u>capsici</u> and <u>M</u>. <u>hiemalis</u>. <u>P</u>. <u>capsici</u> was recorded during the months of May, June, July, August, September and October. The growth and sporulation were favoured by the low temperature, high relative humidity and the high rainfall recorded during this period.

Table 6 :Incidence of fruit rot pathogens of chilli during 1993 from January to December

Month	Storage Pathogens
January	<u>Fusarium solani, Penicillium italicum</u>
February	<u>Cladosporium</u> sp., <u>Colletotrichum</u> <u>capsici</u> , Fusariumsolani
March	<u>Cladosporium</u> sp., <u>Colletotrichum capsici</u> , <u>Fusarium</u> <u>oxysporum, Phoma</u> sp.
April	<u>Cladosporium</u> sp., <u>Colletotrichum capsici</u> , <u>Fusarium</u> <u>solani</u>
Мау	<u>Cladosporium</u> sp., <u>Fusarium oxysporum</u> , <u>Penicillium</u> <u>italicum</u> , <u>Phoma</u> sp., <u>Phytophthora</u> <u>capsici</u> , <u>Colletotrichum capsici</u>
June	<u>Colletotrichum capsici, Fusarium solani, Fusarium oxvsporum, Phytophthora capsici, Mucor hiemalis</u>
July	<u>Colletotrichum capsici, Fusarium solani,</u> Phytophthora capsici, <u>Mucor hiemalis</u>
August	<u>Fusarium oxysporum, Penicillium italicum, Phythophthora capsici, Mucor hiemalis, Colletotrichum capsici</u>
September	<u>Fusarium solani, Phoma</u> sp., <u>Penicillium italicum,</u> <u>Phytophthora capsici, Mucor hiemalis</u> , <u>Colletotrichum capsici</u>
October	<u>Colletotrichum capsici, Fusarium oxysporum, Phytophthora capsici, Mucor hiemalis, Curvularia lunata</u>
November	<u>Colletotrichum capsici, Curvularia lunata, Fusarium solani, Penicillium italicum</u>
December	<u>Colletotrichum capsici, Curvularia lunata, Fusarium solani, Penicillium italicum</u>

4.3. Isolation and identification of phylloplane mycoflora

4.3.1. Tomato

The following fungi were isolated from the leaf surface of tomato plants.

- 1. <u>Alternaria solani</u> Sorauer
- 2. <u>Aspergillus flavus</u> Link ex Fr.
- 3. Aniger Van Tieghem
- 4. A. terreus Thom
- 5. Acremonium sp.
- 6. Botryodiplodia theobromae Pat
- 7. <u>Choanephora</u> <u>cucurbitarum</u> (Berk & Ray) Thaxt.
- 8. Colletotrichum gloeosporioides Penz
- 9. <u>Clasterosporium flagellatum</u> Schw
- 10. <u>Corynespora</u> <u>cassiicola</u> (Berk. & Curt.) Wel.
- 11. Curvularia lunata (Wakker) Boedijn
- 12. Fusarium solani (Martius) Sacc
- 13. <u>Helminthosporium oryzae</u> Breda de Hann
- 14. Penicillium notatum Westling
- 15. P. wortmanii Kloecker
- 16. Pestalotia palmarum Cooke
- 17. Phoma sp.
- 18. Rhizoctonia solani Kühn



- 19. Rhizopus nigricans Ehrenb
- 20. <u>R</u>. oryzae Went and Geerlings
- 21. <u>Trichoderma</u> viride Pers. ex Fr.

4.3.2. Brinjal

The following fungi were isolated from the leaf surface of brinjal plants.

- 1. <u>Alternaria</u> <u>solani</u> Sorauer
- 2. Aspergillus aculeatus Iizuka
- 3. A. alliaceous Thom and Church
  - 4. <u>A. flavus</u> Link ex Fr.
  - 5. A. niger Van Tieghem
  - 6. A. ochraceous Wilhelm
  - 7. A. panamensis Raper and Thom
  - 8. A. repens (Corda) De Bary & Woron
  - 9. A. restrictus Smith
  - 10. A. terreus Thom
  - 11. A. ustus (Bain) Thom & Church
  - 12. Aureobasidium sp.
  - 13. Botryodiplodia theobromae Pat
  - 14. <u>Curvularia eragrostidis</u> (P.Henn.) Meyer
  - 15. <u>Curvularia</u> sp.
  - 16. Cylindrocladium scoparium Morg.

- 17. Fusarium oxysporum Schlect
- 18. F. solani (Martius) Sacc
- 19. <u>Mucor hiemalis</u> Wehmer
- 20. <u>Penicillium notatum</u> Westling
- 21. Pestalotia palmarum Cooke
- 22. Phoma sp.
- 23. Phomopsis vexams (Sacc & Sydow)
- 24. <u>Rhizopus nigricans</u> Ehrenb
- 25. <u>R. oryzae</u> Went & Geerlings
- 26. <u>Trichoderma viride</u> Pers. ex Fr.

4.3.3. Chilli

The following fungi were isolated from the leaf surface of chilli.

- 1. Alternaria solani Sorauer
- 2. A. candidus Link.
- 3. A. flavus Link ex Fr.
- 4. <u>A. niger</u> Van Tieghem
- 5. <u>A. tamarrii</u> Kita
- 6. <u>A. terreus</u> Thom
- 7. <u>Botryodiplodia</u> theobromae Pat
- 8. <u>Colletotrichum capsici</u> (Syd.) Butler and Bisby
- 9. <u>C. gloeosporioides</u> Penz

- 10. <u>Corynespora</u> <u>cassiicola</u> (Berk & Curt.) Wel.
- 11. Curvul<u>aria lunata</u> (Wakker) Boedijn
- 12. <u>Cladosporium</u> sp.
- 13. <u>Fusarium tricinctum</u> (Corda) Sacc.
- 14. Penicillium notatum Westling
- 15. <u>Pestalotia palmarum</u> Cooke
- 16. Phoma sp.
- 17. Rhizopus nigricans Ehrenb
- 18. R.oryzae Went & Geerlings
- 19. Trichoderma viride Pers. ex Fr.
- 4.4. Studies on mycoparasitism
- 4.4.1. <u>In vitro</u> studies for the selection of suitable mycoparasite

4.4.1.1. Tomato

The type of reactions observed between fruit rot pathogens and phylloplane fungi are given in Table 7.

It was observed that <u>Fusarium solani</u> and <u>Rhizoctonia</u> <u>solani</u> were overgrown by <u>Botryodiplodia</u> theobromae, <u>Trichoderma</u> <u>viride</u> (Plate 10 and 11), <u>Aspergillus niger, A. flavus</u> and <u>A. terreus</u> while <u>Pestalotia</u> <u>palmarum</u> showed cessation of growth at the line of contact. <u>Phoma</u> sp. showed cessation of growth at the line of contact with <u>F. solani</u>. When tried with <u>R. solani</u> over growth by Phoma sp. was observed.

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The mode of action of  $\underline{T}$ . <u>viride</u> alone was studied in detail. It was observed that inhibition of  $\underline{F}$ . <u>solani</u> by  $\underline{T}$ . <u>viride</u> was effected through coiling and penetration of hyphae while with <u>R</u>. <u>solani</u> the inhibition was through coiling and disintegration of hyphae.

Table 7 :Type of reaction of fruit rot pathogens of tomato with the phylloplane fungi.

	Pathogens tried			
Phylloplane fungi (test fungus)	<u>Fusarium solani</u>	<u>Rhizoctonia solani</u>		
Botryodiplodia theobromae	В	В		
<u>Pestalotia</u> palmarum	C	с		
Phoma sp.	с	E		
Trichoderma viride	В	B		
Aspergillus niger	B	В		
<u>Aspergillus flavus</u>	В	В		
<u>Aspergillus</u> terreus	В	В		

B - Overgrowth-pathogen overgrown by the test fungus.

C - Cessation of growth at line of contact.

E - Overgrowth-test fungus overgrown by the pathogen.



Plate 10. Overgrowth. Fusarium solani overgrown by Trichoderma viride



Plate 11. Overgrowth. Rhizoctonia solani overgrown by Trichoderma viride 4.4.1.2. Brinjal

The type of reactions between the fruit rot pathogens and the phylloplane fungi are given in Table 8.

The pathogens viz., <u>Fusarium solani</u> and <u>Alternaria solani</u> were found to be overgrown by the phylloplane fungi viz., <u>Botryodiplodia theobromae</u>, <u>Trichoderma viride</u> (Plate 12 and 13), <u>Aspergillus niger and A. flavus</u>. Homogenous free intermingling was noticed with <u>Phoma</u> sp. and <u>Alternaria solani</u>. Cessation of growth at line of contact was shown by <u>Pestalotia palmarum</u> with both pathogens while a clear zone of inhibition was noticed with <u>A. terreus and F. solani</u>.

The mode of action was studied for  $\underline{T}$ . <u>viride</u> alone. Penetration and coiling of hyphae was noticed with <u>F</u>. <u>solani</u> while penetration and distintegration was common with <u>A</u>. <u>solani</u>.



Plate 12. Overgrowth. <u>Fusarium</u> <u>solani</u> overgrown by <u>Trichoderma</u> viride



Plate 13. Overgrowth. <u>Alternaria solani</u> overgrown by <u>Trichoderma</u> viride

	Pathogens tried			
Phylloplane fungi (test fungus)	<u>Fusarium</u> solan	Alternaria solani		
Botryodiplodia theobromae	B	В		
Pestalotia palmarum	c	ć		
Phoma sp.	C	Ά		
Trichoderma viride	В	В		
Aspergillus niger	В	В		
Aspergillus flavus	В	В		
Aspergillus terreus	D	В		

Table 8 : Type of reaction of fruit rot pathogens of brinjal with the phylloplane fungi.

Type	of	reaction	
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- A Homogenous free intermingling between organisms
  - B Overgrowth-pathogens overgrown by mycoparasite
  - C Cessation of growth at line of contact
  - D Clear zone of inhibition

#### 4.4.1.3. Chilli

The type of reaction between fruit rot pathogens and the phylloplane fungi is presented in Table 9.

The pathogens viz., <u>Fusarium solani</u> and <u>Colletotrichum</u> capsici were found to be overgrown by <u>Trichoderma viride</u> (Plate 14 and 15) and <u>Aspergillus niger</u> while cessation of growth at line of contact was noticed with <u>Phoma</u> sp., <u>A</u>. <u>flavus</u> and <u>A. terreus</u>.

Mode of antagonism of  $\underline{T}$ . <u>viride</u> towards  $\underline{F}$ . <u>solani</u> was by coiling and penetration while for <u>C</u>. <u>capsici</u> it was by penetration alone.

Table 9 :Type of reaction of fruit rot pathogens of chilli with the phylloplane fungi.

	Pathogens tried			
Phylloplane fungi (test fungus)	<u>Fusarium</u> <u>solani</u>	Colletotrichum capsici		
Botryodiplodia theobromae	c	в		
<u>Pestalotia</u> palmarum	C	В		
Phoma sp.	c	с		
Trichoderma viride	В	B		
Aspergillus niger	В	В		
Aspergillus flavus	С	С		
Aspergillus terreus	с	с		

Type of reaction : B - Overgrowth-pathogen overgrown by mycoparasite

C - Cessation of growth at line of contact



Plate 14. Overgrowth. <u>Fusarium</u> <u>solani</u> overgrown by <u>Trichoderma</u> <u>viride</u>

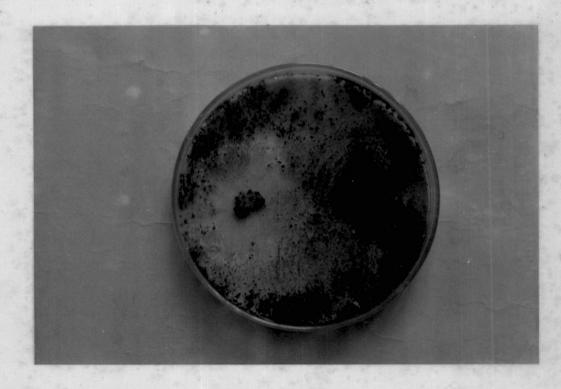


Plate 15. Overgrowth. <u>Colletotrichum</u> <u>capsici</u> overgrown by <u>Trichoderma</u> <u>viride</u>

## 4.5. Effect of selected mycoparasites with storage pathogens of Tomato, Brinjal and Chilli

4.5.1. Tomato

# 4.5.1.1 Infected by F. solani

The effect of <u>T</u>. <u>viride</u> applied as a biological antogonist to fruits infected by <u>F</u>. <u>solani</u> is given in Table 10a, Plate 16 and Fig.4. In control samples, fruits started rotting by the 4th day (26 percent) and continued to rot. Ninety percent damage was noticed by 8<sup>th</sup> day and the fruits were completely rotten by 12th day. In <u>Trichoderma</u> treated fruits rotting eventhough started by 4th day, recorded only 55% damage by 16th day.

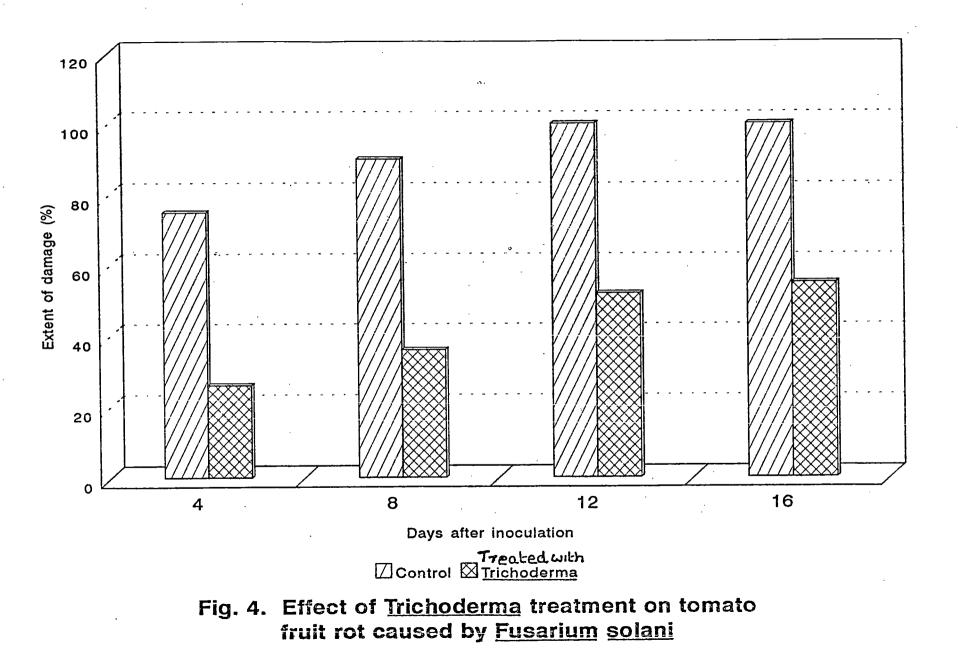
4.5.1.2. Infected by R. solani

The effect of <u>T</u>. <u>viride</u> applied as a biological antagonist to fruits infected by <u>R</u>. <u>solani</u> are given in Table 10b, plate 17 and Fig.5. In control samples the extent of damage was 87 percent by fourth day and the damage was complete (100 percent) by eighth day. Whereas in <u>Trichoderma</u> treated fruits, the extent of damage was 22 percent by fourth day and continued to increase. Only 60 percent damage was noticed on the 16th day.

		Control		Treated with <u>Trichoderma</u> vin	<u>:ide</u>
Days after inocul- ation		Nature of damage	Extent of damage (in %)	Nature of Extend damage damage (in %)	e
4	profus myceli skin l	ped cracks, e dirty white al growth, oose,soft rot ped,exudation d.	75.00	No cracks, <u>Trichoderma</u> grown over rotten areas. Firm fruits, 26 no exudation	.00
8	myceli thicke exudat	widened, al cover med,high ion,unpleasant almost completely	90.00	No cracks, bluish green spore mass of <u>Trichoderma</u> , no 36 unpleasant odour or exudation.	.00
12	high e	te rotting, xudation pleasant	100.00	Little cracking,rotting increased. bluish spore 52 mass coverage increased, fruits continued to be firm, no foul smell and no exudation.	.00
16	•	te rotting flattened	100.00	Cracking increased 55 slightly, infected area widened. Bluish spore mass almost covered the fruit.	.00

Table 10a : Effect of <u>Trichoderma</u> treatment on tomato fruit rot cause by <u>Fusarium</u> <u>solani</u>

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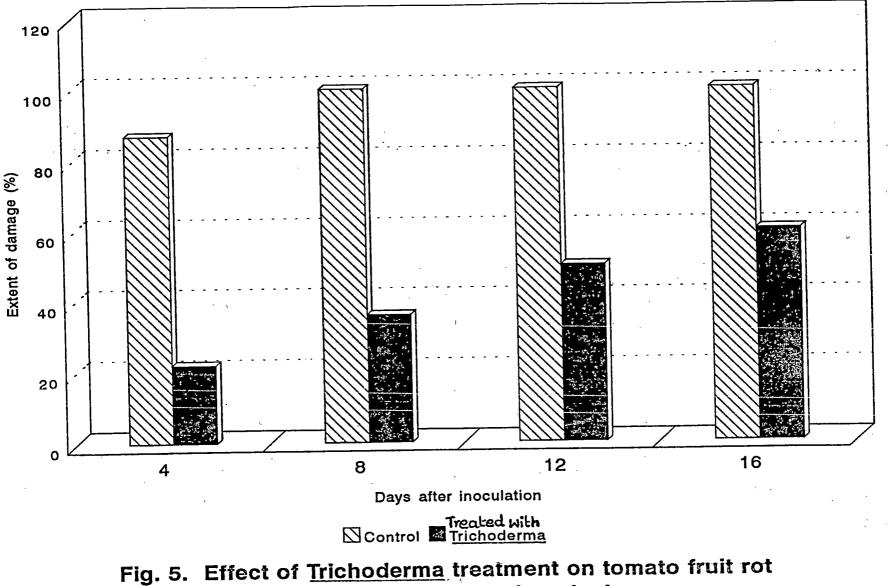


		Control	•	Treated with <u>Trichoder</u>	<u>ma viride</u>
	after ulation	Nature of damage	Extent of damage (in %)	Nature or	Extent of damage (in %)
4	and lit		87.00	Mycelial growth restricted to point of inoculation, fruits remained firm, no exudation.	22.00
8	Whole f: with my fully r		100.00	Bluish spore mass of <u>Trichoderma</u> over the mycelia of the pathogen. Infection noticed, but fruits continued to be firm without exudation.	36.00
12	and fla	udation of sap	100.00	Spore coverage increased, infection progressed. Fruits continued to be firm without exudation.	50.00
16	Fruits rotten	turned into mass	100.00	Infected area increase but fruits continued to be firm without any exudation.	ed. 60.00

Table 10b : Effect of <u>Trichoderma</u> treatment on tomato fruit rot cause by <u>Rhizoctonia</u> <u>solani</u>

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caused by <u>Rhizoctonia</u> <u>solani</u>

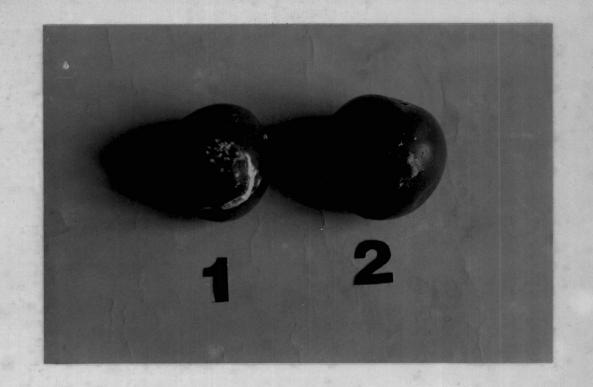


Plate 16. Biological control of <u>Fusarium</u> <u>solani</u> with <u>Trichoderma</u> <u>viride</u> - 4 days after treatment 1. Control 2. <u>Trichoderma</u> treated

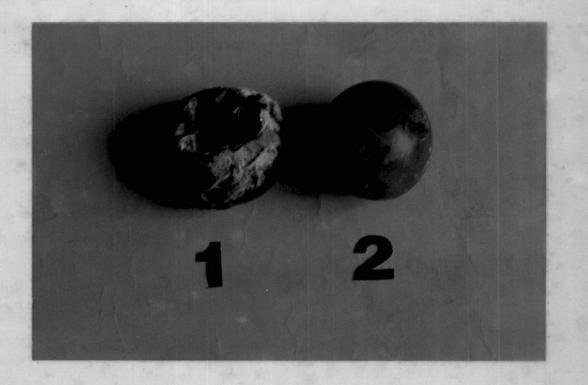


Plate 17. Biological control of <u>Rhizoctonia</u> <u>solani</u> with <u>Trichoderma</u> viride 8 days after treatment 1. Control 2. Trichoderma treated

### 4.5.2. Brinjal

## 4.5.2.1. Infected by F. solani

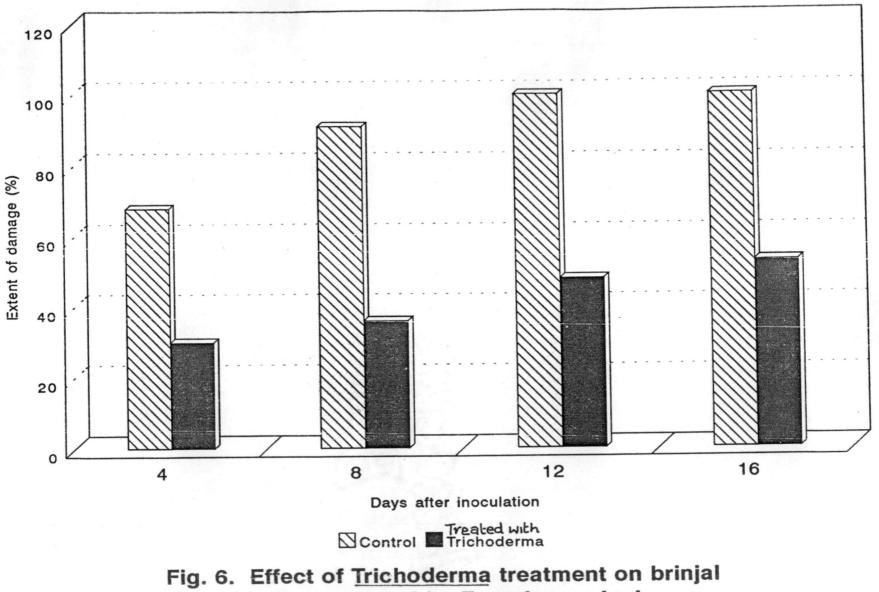
The effect of <u>T</u>. <u>viride</u> applied as a biological antogonist to brinjal fruits infected by <u>F</u>. <u>solani</u> is given in Table 11a, Plate 18 and Fig.6. In control samples, fruits showed severe symptoms of rotting by 4th day (68 percent) and resulted in complete rotting by 12th day where as in <u>Trichoderma</u> treated fruits, even though symptoms were visible by 4th day, it caused only 48 percent damage by 12th day.

## 4.5.2.2. Infected by A. solani

Similar results were also recorded with <u>T</u>. <u>viride</u> in brinjal fruits infected by <u>A</u>. <u>solani</u> (Table 11b). The extent of damage was 48 percent in control samples on the fourth day after inoculation. This continued to increase and complete destruction of the fruit was noticed by twelth day whereas in <u>Trichoderma</u> treated fruits, the extent of damage was 44 percent by fourth day and by sixteenth day it recorded about 90 percent damage.

	Control		Treated with Trichoderma virid	
Days after inocul- ation	Nature of damage	Extent of damage (in %)	Nature or	Extent ( damage (in %)
4	White mycelia was visible. Infection rapidly increased, brownish lesions developed	68.00	Mycelial growth restricted,fruits remained firm	30.00
8	Mycelial coverage increased, fruit became soft and exudation noticed.	91.00	Little mycelial growth, fruits continued to be firm. No exudation	36.00
12	Complete destruction of the fruit, exudation increased	100.00	Fruits continued to be firm. Bluish spore mass of <u>Trichoderma</u> was visible over infected areas. No exudation.	a 48,00
16	Complete destruction of the fruit, exudation maximum	100.00	Fruits continued to be firm, infection slightly increased, Bluish spore mass was noticed to be scattered all over the surface of fruits.	

Table 11.a : Effect of Trichoderma treatment on brinjal fruit rot cause by Fusarium solani



fruit rot caused by Fusarium solani



Plate 18. Biological control of <u>Fusarium solani</u> with <u>Trichoderma</u> viride 8 days after treatment 1. Control 2. Trichoderma treated

	Cont	rol	Treated with T	richoderma
Days after inocul- ation	Nature of damage	Extent of damage (in %)	damage	Extent of damage (in %)
4	Blackish grey oval lesion observed. Fruits became soft, centre of lesions got depressed	48.00	Trichoderma was found to be overgrown by the pathogen. Brown lesion extended. Brown exudation noticed.	
8	Rotted area largely advanced towards pedical.Centre became black brown fruits, almost fully rotten with exudation.	83.00	Fruits became soft, ooze increased	78.66
12	Complete destruction of fruits, exudation <b>t</b> was maximum	100.00	Fruits became soft, almost complete destruction of fruits.Centre of the lesion depressed, profuse exudation noticed.	87.00
16	Complete destruction of fruits, fruits fruits flattened	100.00	Fruits became soft, almost complete destruction of fruits, with profuse exudation.	91.00

Table 11.b : Effect of <u>Trichoderma</u> treatment on brinjal fruit rot caused by <u>Alternaria solani</u>

#### 4.5.3. Chilli

## 4.5.3.1. Infected by F. solani

The fruits inoculated with <u>F. solani</u> showed severe symptoms of rotting (87 percent) by 8th day and resulted in almost complete rotting by 12th day. <u>Trichoderma</u> treated fruits also showed similar results, <u>Trichoderma</u> treatment showed no disinct reduction of fruit rot by <u>F. solani</u> (Table 12a).

## 4.5.3.2. Infected by C. capsici

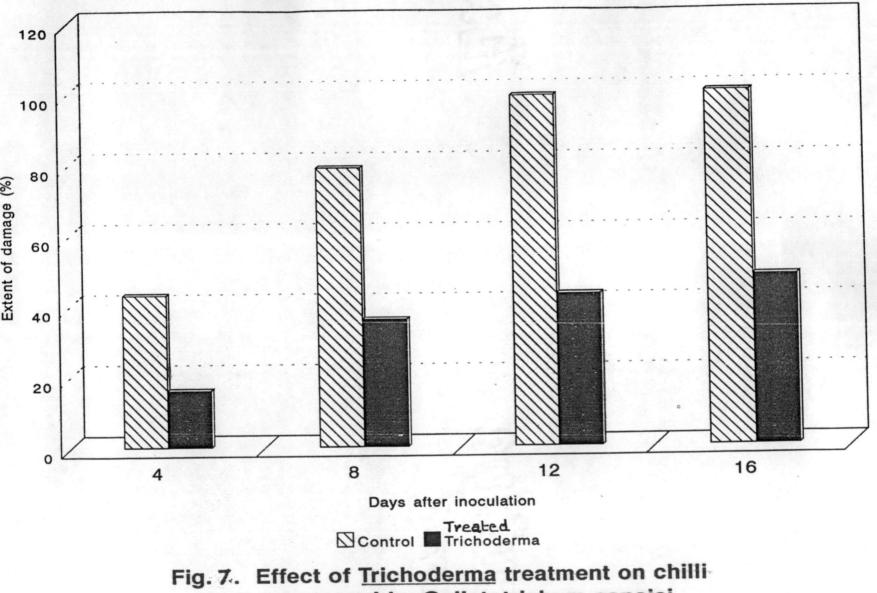
But for <u>Colletotrichum</u> rot, <u>Trichoderma</u> treatment gave good results. Fruits inoculated with <u>C. capsici</u> showed severe rotting (79 percent) during 8th day and complete rotting by 12th day. But in <u>Trichoderma</u> treated fruits, fruit rot infection was 16 percent after 4th day. This continued to record increase and by 16th day caused 50 per cent damage. (Table 12b, Plate 19 and Fig. 7).

	Contr	ol	Treated with Trichode:	rma viride
Da <b>y</b> s after inocul- ation	Nature of damage	Extent damage (in %)	of Nature of damage	Extent of damage (in %)
4	Yellowish brown discolouration exudation noticed	49.00	Yellowish discolour- ation. Exudation noticed	46.00
8	Lesion size increased considerably,white mycelial growth, and profuse exudation with rotten smell	87.00	Lesion sized increased, white mycelial growth and profuse exudation with a rotten smell.	84.00
12	Whole fruit covered with fungal growth almost complete rotting.	96.00	Whole fruit covered with fungal growth, rotting almost completed and with maximum exudation.	96.00
16	Whole fruit disinte- grated into a pulpy mass with rotten smell	100.00	Complete destruction of fruits,with high exudation and rotten smell	100.00

Table 12a. : Effect of <u>Trichoderma</u> treatment on chilli fruit rot caused by <u>Fusarium solani</u>

	Co	ntrol	Treated with Trichoderma viride			
Days after inocul- ation	Nature of damage	Extent o damage (in %)	of Nature of damage	Extent of damage (in %)		
4	Deep brown oval lesions	43.00	Discolouration highly reduced	16.00		
8	Black small acervuli seen, deep brown lesions developed	79.00	Discolouration slowly advanced, no acervuli formation	36.00		
12	Whole fruit blackened with acervuli and became shrunken	99.00	No acervuli noticed discolouration noticed. Fruits not shrunken.	43.00		
16	Complete coverage of fruit with acervuli, became a black shrivelled mass	100.00	No acervuli,discolour- ation continued. Fruits continued to be firm. Not shrunken.	48.00		

Table	12.b	:Effect	of Trichoderma	treatment	on	chilli fruit rot	caused
		by Coll	etotrichum caps	ici			



fruit rot caused by Colletotrichum capsici

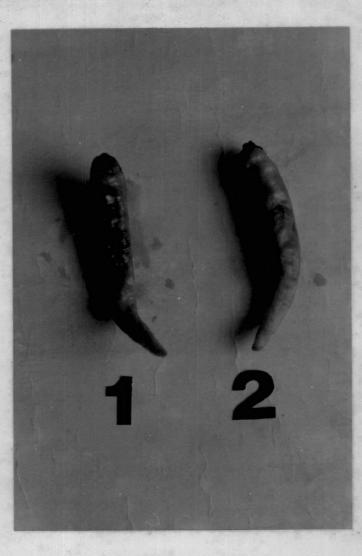


Plate 19. Biological control of <u>Colletotrichum</u> <u>capsici</u> using <u>Trichoderma</u> <u>viride</u> 1. Control 2. <u>Trichoderma</u> treated

# DISCUSSION

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#### 5. DISCUSSION

Fungi are mainly responsible for the spoilage of vegetables during transit and storage. The common fungi associated with the storage rot of solanaceous vegetables include species of Alternaria, Phytophthora, Rhizoctonia, Mucor and Fusarium, These fungi are all soil borne pathogens and Colletotrichum. hence can be assumed to be carried over from the soil at the time of harvest. Transit and storage atmosphere may also help in the deposition of fungal spores on them. The spores deposited on the surface of these vegetables may remain in the latent form and may not usually show symptoms. The injuries sustained during harvest and transit plays a key role in the development of symptoms during storage. The symptom also varies depending upon climatic factors and storage practices.

Among the storage rots of tomato, soft rot by <u>Furasium</u> spp. occupy a prominent position. This included <u>F</u>. <u>nivale</u> <u>F</u>. <u>equiseti</u>, <u>F</u>. <u>solani</u>, and <u>F</u>. <u>roseum</u> (Thakur and Yadav, 1971; Khanna and Chandra, 1976; Garg and Gupta, 1979; Thomas <u>et al</u>., 1981). In the present study also storage rot due to <u>F</u>. <u>solani</u> and <u>F</u>. <u>oxysporum</u> was found to be very common.

Storage rot due to <u>Alternaria</u> <u>solani</u> also occupied an important position. Almost all the samples yielded <u>Alternaria</u>. Similar observations were also recorded by Rao (1965).

The role of <u>Phytophthora palmivora</u> and <u>Rhizoctonia solani</u> in initiating soft rots were also spectacular. Both of them caused complete rotting within 2-3 days during rainy months and is in agreement with the findings of Critopoulos (1954) who has recorded <u>P. capsici</u> as the most vigorous type.

Zygomycetous fungi like <u>Mucor hiemalis</u>, <u>Choanephora</u> <u>cucurbitarum</u> and <u>Rhizopus</u> <u>stolonifer</u> were also found to be associated.

<u>Mucor hiemalis</u> and <u>C</u>. <u>cucurbitarum</u> recorded in the present study are new reports even though rotting due to <u>M</u>. <u>mucedo</u> and <u>M</u>. <u>piriformis</u> (Moline and Kuti, 1984) and <u>Rhizopus</u> spp. viz., <u>R</u>. <u>stolonifer</u>, <u>R</u>. <u>arrhizus</u>, <u>R</u>. <u>nigricans</u> and <u>R</u>. <u>oxyzae</u> (Sonoda <u>et al.</u>, 1982) were already reported.

The other fungi which have recorded considerable damage in the present study included <u>Aspergillus</u> spp. viz., <u>A. niger</u> and <u>A. flavus</u> followed by <u>Geotrichum candidum</u>, <u>Curvularia lunata</u> and <u>Cladosporium</u> sp.. These fungi are all known pathogens of tomato and initiated fruit rot under storage conditions (Golan, 1980; Bartz, 1980; Narain and Rout, 1981).

Fruit rot due to <u>Phoma</u> sp. was also common eventhough the fungus is reported to have caused fruit rot in cold climate and stemblight under relatively warm conditions (Singh, 1985).

Similarly <u>Geotrichum</u> sp. was also recorded in certain cases to have caused minor damage. <u>G. candidum</u> is well known in initiating sour rots on ripe and over ripe vegetables at warm and humid conditions (Moline, 1984; Sharma and Sumbali, 1993).

Brinjal fruits also suffer great losses due to post harvest infection. As in the case of tomato considerable rotting due to <u>Fusarium solani</u> has been recorded throughout the study. Mehta and Mehta (1989) has recorded the extent of rotting by <u>F. oxysporum</u> and <u>F. moniliforme</u> to be maximum under storage conditions.

Soft rot due to <u>Rhizopus nigricons</u> was encountered as a new pathogen eventhough rotting by <u>Rhizopus</u> <u>stolonifer</u> has been reported already (Sharma and Sumbali, 1993).

<u>Phomopsis</u> <u>vexans</u> also caused considerable damage under storage conditions. Pawar and Patel (1957) has recorded severe blighting under storage conditions eventhough John (1991) has recorded severe blighting of the stem and fruit under field conditions from Vellayani. Hence it can be assumed that the pathogen might have been carried from the field to the store house.

Soft rot by <u>Aspergillus niger</u> and <u>Botrytis cinerea</u> recorded in the present study has already been reported by Kumar <u>et al</u>. (1986) and Sharma and Sumbali (1993). <u>Phytophthora palmivora</u>, <u>Pythium aphanidermatum</u> and <u>Rhizoctonia solani</u> were also recorded to induce soft rot during humid climatic conditions.

<u>Rhizopus nigricans and P. palmivora</u> recorded in the present study are new reports on brinjal. The wound parasite, <u>Botryodiplodia</u> <u>theobromae</u> was also reported irrespective of season.

Anthracnose disease of Chilli caused by Colletotrichum gloeosporioides is recorded to cause considerable damage both under field and storage conditions in various parts of India (Ramakrishnan and Wilson, 1968; Thind and Jhooty, 1984). It is prevalent throughout the year and is common on semi ripe and ripe The disease development is maximum during the humid fruits. In the present study also, symptoms were recorded seasons. throughout the year with maximum development during humid The symptoms eventhough initiated in the field might seasons. have continued to develop during transit and storage (Singh, In certain cases the necrotic areas have extended and 1985). covered the entire length of the fruit. This is facilitated through the action of toxins produced by fungus under the suitable environmental conditions (Sharma and Sharma, 1969).

<u>Fusarium</u> spp. viz., <u>F. oxysporum</u> and <u>F. solani</u> have also caused considerable damage and is reported from different localities in India (Sharma <u>et al</u>. 1980; Sharma and Sumbali, 1993). Symptoms generally started from the stylar end as small lesions and further development is favoured by high humidity (Dasgupta and Mandal, 1989).

The other fungi associated with soft rots included <u>Mucor</u> <u>hiemalis</u>, <u>Cladosporium</u> sp., <u>Phytophthora</u> <u>capsici</u>, <u>Phoma</u> sp., <u>Curvularia lunata</u> and <u>Penicillium italicum</u>.

<u>Mucor hiemalis</u> and <u>Penicillium italicum</u> recorded in the present study are new reports on Chilli.

Successful infection also depends upon the age of the vegetable. Age exerts a pronounced influence on the rotting pattern. But this factor could not be evaluated as the age of the vegetables collected from the local areas could not be clearly ascertained.

Further studies with inoculation trials have indicated the role of wounds or injuries in initiating infection. Injuries serve as a prerequisite for successful colonization by pathogens. The study thus clearly indicated the importance of avoiding injury to vegetables during harvest, transit and storage.

Another important aspect is the transmission of diseases through seeds. Seeds extracted from rotted samples serve as the source of primary infection in the field. Majority of the fungi associated with spoilage wase already reported to be seedborne (George, 1992). Thus the study highlighted the significance of seed borne transmission in vegetable crops.

Growth and sporulation of fungi are in general influenced by environmental factors like relative humidity, rainfall and temperature of the atmosphere.

Studies on the effect of environmental factors and fungal incidence have revealed that fungi like <u>P. palmivora</u>, <u>R. solani</u>, <u>M. hiemalis</u> and <u>C. cucurbitarum</u> to be highly specific and associated with low temperature accompanied by high relative humidity and rainfall. A relative humidity of above 90 percent coupled with a temperature range of 24 to 30°C favoured infection by <u>Phytophthora</u> sp. (Singh, 1985). In the present study also high mean relative humidity of 88 percent and rainfall of 14-24mm coupled with low temperature occurred during the period of July and August.

In the case of <u>Alternaria</u> rotting also, 100 percent relative humidity with a temperature of 28°C was found to be favourable and the ratio of disease development was proportional to the

humidity level (Mehta <u>et al.</u>, 1975). But in the present study damage due to <u>A. solani</u> was recorded throughout the year except during January and February when the relative humidity and maximum temperature ranged from 70-91 percent and  $25-33^{\circ}$ C respectively.

The incidence of <u>Geotrichum</u> was noticed from January to September, when the temperature and mean relative humidity ranged from 28 to 33°C and from 70 to 91 percent respectively. Bartz (1980) reported <u>Geotrichum</u> infection to be severe during shipment in Florida at 80 percent relative humidity and at a temperature range of 25-27°C. Abundant moisture followed by warm dry weather helped in th rapid development of disease (Singh, 1985).

Studies on <u>Aspergillus</u> rot caused by <u>A</u>. <u>flavus</u> showed that infection appeared in its most severe form during January, February, March, April and December while rotting due to <u>Fusarium</u> sp. was noticed throughout the year irrespective of range in temperature or relative humidity. In a similar study Khanna and Chandra (1976) recorded infection by <u>A</u>. <u>flavus</u> to be severe during October and November. Similarly <u>Fusarium</u> rot was noticed only during January and February in Allahabad.

In the case of brinjal also <u>Fusarium solani</u> and <u>Alternaria</u> <u>solani</u> caused maximum rotting and were observed throughout the study irrespective of the variations in relative humidity and temperature. Mehta and Mehta (1989) has also made similar observations and recorded maximum rotting at room temperature.

<u>Phomopsis vexans</u> emerged as an important pathogen and caused from considerable damage from April to December. The relative humidity and temperature also showed wide variation which ranged from 75-95 percent and 25-33°C respectively. This is in confirmity with the findings of Pawar and Patel (1957) who have recorded a temperature of 25°C and relative humidity about 75 percent as optimum for its growth and sporulation.

In the present study <u>Phytophthora palmivora</u>, <u>Pythium</u> <u>aphanidermatum</u> and <u>Rhizoctonia solani</u> were found to be associated during the rainy periods of the year. A relative humidity of above 87 percent was recorded at 7 AM from June to September. The temperature also ranged from 25-30°C during this period. Rainfall was also maximum during this period.

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Among the several fungi associated with the spoilage of Chilli, <u>Colletotrichum capsici</u> emerged as the most common pathogen irrespective of the variations in mean relative humidity (73-90 percent) or temperature (25-33°C) eventhough Das gupta and

Mandal (1989) reported that <u>Colletotrichum</u> rotting was favoured by high relative humidity.

Fusarium rot caused by <u>F. solani</u> was also prevalent throughout the year. The fungus is recorded on both ripe and green chillies with lesions starting from the stylar end (Das <u>gupta</u> and Mandal, 1989). They have suggested that the disease is favoured by high relative humidity.

Fruit scab by <u>Cladosporium</u> was noticed only during the summer months when the maximum temperature ranged from 30-33°C. Mandal and Dasgupta (1980) has also recorded <u>C. tenuissimum</u> during summer and partial rotting under humid conditions.

In general, it was observed that relative humidity and temperature are the two important factors responsible for the development of fungal rots in stored vegetables. Relative humidity is in turn influenced by the amount of rainfall received.

The quantitative and qualitative composition of the phylloplane microflora of crop plants varied with their age and growing conditions. Healthy leaves in general carried more saprophytic organisms compared to young and unhealthy leaves. The leaves of field grown crops are covered by a relatively dense popualation of microorganisms including spores and hyphae,

yeasts, bacteria, actinomycetes and pollen grains. It is also to be noted that the leaf surface microorganisms are under the great influence of host, variety, environment, age of the plants, leachates etc. But detailed investigations on the above foliar factors could not be taken up and study was concentrated only on isolation and identification of fungi associated with the the three solanaceous crops viz., tomato, brinjal and chilli. The isolated were utilised for furthur studies in the fungi so selection of a suitable antagonist against storage pathogens.

A comparative glance at the fungi isolated revealed that brinjal crop harboured a rich crop of fungi (26 nos) followed by and chilli (19). It is interesting to note that tomato (21) fungi like <u>Pestalotia palmarum, Aspergillus niger, A</u>. terreus, flavus, Botryodiplodia theobromae, nigricans, Rhizopus Α. Trichoderma viride and Phoma sp. were common to all three host While certain other fungi were exclusively associated plants. with one host only. For example, Acremonium sp., Clasterosporium Rhizoctonia solani, oryzae, Helminthosporium flagellatum, Choanephora cucurbitarum and Penicillium wortmanil were isolated from tomato while Aureobasidium sp., Cylindrocladium scoparium and Aspergillus spp. comprising of A. alliaceus, A. aculeatus, restrictus, A. panamensis, A. niger, A. flavus, A. terreus, <u>A</u>. A. ochraceous and A. ustus were restricted to brinjal.

Similarly fungi like <u>Aspergillus tamarii</u>, <u>F. tricinctum</u>. <u>Cladosporium</u> sp., <u>Curvularia lunata</u> and <u>Phoma</u> sp. were recorded from chilli.

Host specificity is already known from the work of Singh and Sinha, 1962. Host specificity among other factors is known to be controlled by the physical nature of the surface and the chemical nature of exudates.

Distribution of microorganisms in relation to host species, weather changes, leaf maturity, and air currents are all well known. Along with this meteorological factors such as atmospheric temperature, humidity and rainfall **ve**re also important in influencing the quality of microorganisms (Gregory, 1957ond1961).

The selected phylloplane fungi/test fungi were utilised for antagonistic studies with the major fruit rot pathogens in <u>in vitro</u> studies with dual cultures. Very good antagonistic activity (overgrowth of the pathogen by the test fungus) was shown by <u>B</u>. <u>theobromae</u>, <u>T</u>. <u>viride</u>, <u>Phoma</u> sp., <u>A</u>. <u>niger</u>, <u>A</u>. <u>flavus</u> and <u>A</u>. <u>terreus</u>. But <u>Aspergillus</u> spp. were not considered for further studies because of their possible role in the production of the potent toxin viz., aflatoxin (Kulik and Holciday, 1969).

The study was limited to <u>Trichoderma</u> <u>viride</u> alone because of its potential antagonistic activity against a variety of pathogens (Dennis and Webster, 1971b; Elad <u>et al</u>., 1980; Das, 1986; Gokulapalan, 1989).

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Several reports on the occurrence of micro organisms antagonistic to plant pathogens are available. Isolates of fungi, yeasts and bacteria have been reported to be antagonistic to a wide range of pathogens and are successfully utilised against soilborne, foliar and post harvest diseases of vegetables and fruits (Weindling, 1932; Ghewande, 1987; Janisiewiez, 1988).

The mechanism of mycoparasitism is based on the studies with dual cultures in synthetic media. But the effect of nutrition or the physiology of hyperparasitism has not been studied.

The mode of mycoparasitism has been observed to differ among Boosalis (1954 and 1956) heave observed hyphal Thus fungi. penetration of <u>R</u>. solani by <u>Penicillium</u> sp. and penetration followed by collapse and disintegration in P. vermiculatumsolani combination. But disintegration due to alteration in <u>R</u>. the pH of the medium or due to nutrient improverishment (Newhook, also 1976) has also been suggested. It was Skidmore, 1951: that penetration was made possible through the suggested disintegration of host cell walls by the action of antibiotics

(Baigent and Ogawa, 1960; Brame and Flood, 1983) or due to lysis by enzymatic action (Jones <u>et al.</u>, 1974). In similar studies suppression or growth inhibition by <u>T. pseudokoningii</u> in <u>Botrytis</u> <u>cinerea</u> (Tronsmo and Raa, 1977) is also reported.

In certain cases in addition to competition for nutrients, space and mechanical obstructions, physical factors and size of the host hyphae were also suggested to be involved in penetration (Durrell, 1966; Huang and Hoes, 1967; Dwivedi and Arora, 1978).

Huang (1978) has observed direct hyphal contact resulting in collapse or disintegration of cells in the hyperparasitism of sclerotiorum and <u>Sclerotinia</u> catenulatum on Gliocladium In similar studies Pathak <u>et</u> <u>al</u>. (1981)Fusarium spp.. recorded hyphal parasitism by coiling, penetration, rupture of the host hyphae and ramification inside the host when Rhizopus nigricans was hyper parasitised by Fusarium oxysporum f.sp. lycopersici.

Fravel (1988) has postulated the role of toxic or inhibitory metabolites in addition to parasitism, competition for nutrients or space, or mechanical obstructions.

In the present study, the role of <u>Trichoderma</u> as a biological antagonist was established against the major pathogens of tomato (<u>F. solani</u> and <u>R. solani</u>), brinjal (<u>F. solani</u> and <u>A. solani</u>) and chilli (<u>F. solani</u> and <u>C. capsici</u>).

A variety of antagonists has been already recognised for the biological control of fruit rot pathogens. Thus Botrytis rot of strawberry and apple has: been successfully controlled through harzianum T. pseudokoningii т. and application of the (Tronsmo and Dennis, 1977; Tronsmo and Ystaas, respectively Antagonistic yeast and bacteria were also found to be 1980). effective against citrus fruit rot caused by Diplodia natalensis Penicillum expansum as well as Alternaria alternata and and Rhizopus stolonifer decay of tomatoes (Chalutz et al., 1988).

The results of the present study thus clearly indicated the possibilities of utilising <u>T</u>. <u>viride</u> as a biocontrol agent for combating the major fruit pathogens of tomato viz., <u>F</u>. <u>solani</u> and <u>R</u>. <u>solani</u>. It can also be successfully utilised against fruit rot of brinjal caused by <u>F</u>. <u>solani</u> and the common anthracnose disease of chilli caused by <u>C</u>. <u>capsici</u>.

# SUMMARY

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#### 6. SUMMARY

The fungi commonly associated with the decay of solanaceous vegetables viz., tomato, brinjal and chilli under storage conditions were isolated and identified.

<u>Mucor hiemalis</u> and <u>Choanephora cucurbitaram</u> as well as <u>Rhizopus nigricans</u> and <u>Phytophthora palmivora</u> are new reports on tomato and brinjal respectively. Similarly <u>Mucor hiemalis</u> and <u>Penicillium italicum</u> are new reports on chilli.

Based on the frequency of occurrence and extent of damage <u>Fusarium solani</u> was selected for all the three crops along with <u>Rhizoctonia solani</u>, <u>Alternaria solani</u> and <u>Colletotrichum capsici</u> for tomato, brinjal and chilli respectively. These fungi were used for further studies.

Studies on the occurrence of fungal pathogens and their correlation with weather parameters have showed minimum spoilage during the dry periods of the year viz., January, February and March. The fungal population was found to be correlated with low temperature and high relative humidity and rainfall.

The phylloplane studies revealed maximum fungal population on brinjal followed by tomato and chilli. The fungi included common pathogens and saprophytes.

Fungi like <u>Botryodiplodia</u> theobromae, <u>PestalotiOpsispalmarum</u>, <u>Phoma</u> sp., <u>Trichoderma</u> <u>viride</u>, <u>Aspergillus</u> spp. viz., <u>A</u>. <u>niger</u>, <u>A</u> <u>flavus</u> and <u>A</u>. <u>terreus</u> were common to all the three crops and hence were selected for <u>in vitro</u> studies against the selected fruit rot pathogens respectively.

The mechanism of action of  $\underline{T}$ . <u>viride</u> towards the pathogens was found to be through coiling, penetration and disintegration of hyphae.

The role of <u>Trichoderma</u> as a biological antagonist against the common fruit rot pathogens was studied. It was observed that <u>T. viride</u> was effective in reducing the fruit rot of tomato caused by the major pathogens viz., <u>F. solani</u> and <u>R. solani</u> by about 50 percent upto 12 days of storage under artificial inoculated conditions. Similar results were also obtained with <u>F. solani</u> on brinjal and <u>C. capsici</u> on chilli.

The study thus highlighted the possibility of utilising <u>Trichoderma</u> as a biocontrol agent against the major storage disease of tomato, brinjal and chilli.

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\* Originals not seen

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# APPENDICES

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#### APPENDICES

#### APPENDIX-I

### Potato dextrose agar

Potato	200.0 g.
Dextrose	20.0 g.
Agar	20.0 g.
Distilled water	1000 ml.

#### APPENDIX-II

Peptone dextrose agar with rosebengal and streptomycin

Dextrose	10.0 g.
Peptone	5.0 g.
Pottassium dihydrogen	phosphate 1.0 g.
Magnesium Sulphate	0.5 g.
Agar	20.0 g.
Distilled Water	1000 ml.
Streptomycin	0.3 ml. per 100 ml. of cooled medium
Rose bengal	l part in 30,000 parts of the medium
рн	6.8

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#### APPENDIX-III

Anova for percentage of infection of tomato stored after 4 days of inoculation.

Source	df	SS	MSS	F Value
Treatment	7	3896.961	556.709	* *
Error	16	796	49.85	11.190
 Total	23	4692.961		

Anova for percentage of infection of tomato stored after 8 days of inoculation.

Source	df	SŞ	MSS	F Value
Treatment	7	2108.5	301.214	* *
Error	16	1370	85.625	3.518
	23	3478.5		

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Anova for percentage of infection of tomato stored after 12 days of inoculation.

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Source	df	SS	MSS	F Value
Treatment	7	1475.625	210.804	* *
Error	16	635.328	39.708	5.309
Total	23	2110.953		

Anova for percentage of infection of tomato stored after 16 days of inoculation.

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Source	df	SS	MSS	F Value
Treatment	7	1005.625	143.6607	* *
Error	16	478	29.875	4.809
 Total	23 .	1483.625		

df	SS	MSS	F Value
7	280.958	40.137	* *
16	276	17.25	2.327
23	556.958	24.216	
	7 16	7 280.958 16 276	7         280.958         40.137           16         276         17.25

Anova for percentage of infection of brinjal stored after 8 days of inoculation.

Source	df	SS	MSS	F Value
Treatment	7	2116.997	302.428	* *
Error	16	668.836	41.802	7.235
Total	23	2785.833	121.123	

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Anova for percentage of infection of brinjal stored after 12 days of inoculation.

Source	df	SS	MSS	F Value
Treatment	7	4889.958	698.565	* *
Error	16	1104	69	10.124
Total	23	5993.958	260.607	

Anova for percentage of infection of brinjal stored after 16 days of inoculation.

Source	df	SS	MSS	F Value
Treatment	7	7667.291	1093.899	* *
Error	16	1570.667	98.167	11.143
Total	23	9227.958	401.216	
	.cant at 5%	level		

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#### APPENDIX-V

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Anova for percentage of infection of chilli stored after 4 days of inoculation.

df	SS	MSS	F Value
7	641.333	171.048	* *
16	556	40.083	4.267
23	1197.333	52.058	5
	7 16	7 641.333 16 556	7         641.333         171.048           16         556         40.083

Anova for percentage of infection of chilli stored after 8 days of inoculation.

Source	df	SS	MSS	F Value
Treatment	7	11022.29	1571.76	* *
Error	16	3684.668	230.29	6.83
 Total	23			
* * Signifi	cant at 5%	level		

Anova for percentage of infection of chilli stored after 12 days of inoculation.

df	SS	MSS	F Value
7	22517.667	3217.095	* *
16	1244.333	77.771	41.366
23	23764	1033.217	
	7	7 22517.667 16 1244.333	7         22517.667         3217.095           16         1244.333         77.771

Anova for percentage of infection of chilli stored after 16 days of inoculation.

Source	df	SS	MSS	F Value
Treatment	7	18135.167	2590.738	* *
Error	16	630.667	39.417	65.727
Total	23	1.8765.833	815.906	

### APPENDIX-VI

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## WEATHER DATA OF 1993 FROM JANUARY TO DECEMBER

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inte in a suite ann an Anna an Stàitean			Temperatu	ure (°C)	Relative	e Humidit	y(%)	Rainfall
Month	Week	Days	Max	Min At	7.22AM	At 2.221	PM Mean	(mm)
	1	1-7	30.4	18.6	91.1	55.1	73.1	0.00
January	2	8-14	30.4	21.1	93.3	62.0	77.65	0.00
	3	15-21	29.9	20.7	93.7	68.8	81.25	0.00
	4	22-28	30.7	21.7	93.3	58.7	83.71	0.00
	5	29-31	30.23	20.63	93.66	63.67	78.67	0.00
2	1	1-7	31.33	19.44	90.85	49.57	70.04	0.00
February	2	8-14	30.68	20.41	91.57	59.14	75.36	0.00
	3	15-21	31.51	23.22	87.42	73.43	80.43	0.00
	4	22-28	31.34	21.85	92.00	74.00	83.00	2.80
3	1	1-7	32.14	22.27	89.85	61.28	75.57	0.00
March	2	8-14	32.74	21.92	89.26	59.86	74.85	0.00
	3	15-21	32.53	24.03	91.00	67.26	79.13	0.00
	4	22-28	32.64	24.05	87.05	66.34	76.70	18.50
	5	29-31	32.20	24.00	75.33	65.00	70.17	0.00

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			Temperatu	re (°C)	Relative	e Humidit	y(%)	Rainfall
Month	Week	Days	Max	Min At	7.22AM	At 2.22P	M Mean	(mm)
4	1	1-7	31.97	24.6	82.57	72.00	77.29	0.00
April	2	8-14	32,33	24.33	90.57	73.00	81.79	6.35
	3	15-21	32.62	20.76	90.14	73.57	81.85	12.50
	4	22-28	33.27	25.40	75.00	78.43	76.71	1.80
	5	29-30	33.00	25.05	93.50	84.50	89.00	2.30
5	1	1-7	33.34	25.93	89.14	78.57	83.86	0.00
May	2	8-14	28.69	26.01	92.14	7 <b>8.7</b> 1	85.43	21.00
	3	15-21	31.97	24.70	85.71	72.41	79.07	9.00
	4	22-28	31.34	23.75	92.71	76.43	84.57	8.40
	5	29-31	29.76	24.17	88.67	77.00	82.84	36.30
6	1	1-7	29.70	23.49	91.29	73.57	82.43	40.53
June	2	8-14	29.49	23.74	91.85	76.57	84.21	12.27
	3	15-21	30.25	22.04	91.57	79.43	85.50	5.86
	4	22-28	30.42	24.60	90.42	93.97	82.71	7.63
	5	29-30	29.30	23.00	87.00	75.00	81.00	28.90
7	1	1-7	28.71	22.71	92.29	88.14	90.22	29.00
July	2	8-14	29.46	22.47	89.86	82.00	85.93	13.53
	3	15-21	28.47	22.95	90.14	84.71	80.35	7.53
	· <b>4</b>	22-28	28.20	22.96	92.57	68.14	80.36	3.63
	5	29-31	28.67	25.20	94.33	83.00	88.67	4.88

			Temperatu	re ( <sup>o</sup> C	) Relative	Humidity	7(%)	Rainfall
Month	Week	Days	Max	Min	At 7.22AM	At 2.22PM	4 Mean	( mm )
8	1	1-7	25.20	23.43	91.71	78.57	85.14	5.00
August	2	8-14	29.71	24.05	92.57	73.43	83.00	1.20
1104 05 -	3	15-21	28.86	23.89	87.00	72.71	79.85	1.00
	4	22-28	29.50	23.49	92.14	89.88	91.01	4.00
	5	29-31	29.83	23.43	91.66	78.33	85.00	14.00
9	1	1-7	29.78	23.19	87.70	75.85	81.78	0.00
September		8-14	30.99	23.69	90.14	73.00	81.57	0.00
	3	15-21	30.97	27.13	L 86.00	73.57	79.78	33.00
	4	22-28	30.38	24.15	5 88.57	82.00	85.29	1.00
	5	29-30	30.20	23.0	5 87.00	81.00	84.00	14.93
10	 l	1-7	29.00	23.20	0 89.00	80.70	84.85	23.00
October	2	8-14	29.50	23.4	91.80	77.30	84.55	19.68
	3	15-21	30.40	23.3	0 91.10	74.60	82.85	13.30
·	4	22-28	30.60	23.1	0 87.40	77.00	82.20	19.20
	5	29-31	29.75	24.0	6 88.33	83.50	85.92	4.95
11	1	1-7	30.08	23.5	4 91.71	81.00	86.53	8.44
November	2	8-14	28.62	22.8	0 95.00	73.42	84.21	56.33
	3	15-21		23.2	4 91.57	80.00	85.78	7.65
	4	22-28		23.1	7 92.57	82.71	87.63	9.75
	5	29-30		27.2	5 83.00	73.50	78.2	5 0.00

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· <u> </u>			Temperature (°C) Relative Humidity(%) Rainfall						
Month	Week	Days	Max	Min At	7.22AM	At 2.221	M Mean		
12	1	1-7	29.41	23.51	95.00	78.57	86.79	8.10	
December	2	8-14	29.51	23.21	90.41	74.57	82.41	11.00	
	3	15-21	30.34	22.81	93.43	85.29	89.36	7.75	
,	4	22-28	30.14	22.72	89.42	77.71	83.56	8.80	
	5	29-31	29.33	23.00	92.33	80.00	86.17	0.00	

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## EXPLORATION OF THE FEASIBILITY OF BIOLOGICAL CONTROL OF POST HARVEST DISEASES OF SOLANACEOUS VEGETABLES

By

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ABSTRACT OF A THESIS Submitted in partial fulfilment of the requirement for the degree MASTER OF AGRICULTURE

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#### ABSTRACT

The fungi commonly associated with the spoilage of solanaceous vegetables viz., tomato, brinjal and chilli under storage conditions were studied for a continuous period of one year during 1993.

Tomato fruits were found to be damaged by <u>Aspergillus</u> <u>flavus</u>, <u>Fusarium solani</u>, <u>F. oxysporum</u>, <u>Colletotrichum</u> <u>gloeosporioides</u> and <u>Alternaria solani</u>. These fungi were present throughout the year, while <u>Rhizoctonia solani</u>, <u>Mucor hiemalis</u> and <u>Choanephora cucurbitarum</u> were seasonal in occurrence. <u>Mucor</u>. <u>hiemalis</u> and <u>C. cucurbitarum</u> recorded in the present study are new reports.

With brinjal the common pathogens included Alternaria solani, Fusarium solani, Colletotrichum gloeosporioides, Penicillium sp., Botrytis cinerea, Rhizopus nigricans, Curvularia lunata and Botryodiplodia theobromae. While Phomopsis vexans, Phytophthora palmivora, Pythium aphanidermatum and Rhizoctonia solani were confined to the rainy periods of the year.

<u>Rhizopus</u>. <u>nigricans</u> and <u>P</u>. <u>palmivora</u> recorded in the present study are new reports on brinjal.

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Studies with chilli yielded fungi like <u>Fusarium solani</u>, <u>F. oxysporum</u> and <u>Colletotrichum capsici</u> throughout the year. <u>Phutophtnora capsici</u> and <u>Mucor hiemalis</u> were recorded during the rainy seasons only.

Mucor hiemalis and Penicillium italicum recorded in the present study are new reports in chilli.

The nature and extent of damage caused by major pathogens were studied for all the three crops. Based on the frequency of occurrence and extent of damage two pathogens were selected for each crop for further in vitro studies. This included  $\underline{F}$ . solani and  $\underline{R}$ . solani for tomato and  $\underline{F}$ . solani and  $\underline{A}$ . solani for brinjal. For chilli, the pathogens selected included  $\underline{F}$ . solani and C. capsici.

Studies on the occurrence of fungal pathogens and their correlation with weather parameters have showed minimum spoilage during the dry periods of the year viz., January, February and March. A drift in the fungal flore was noticed with changing seasons. Low temperature coupled with high relative humidity and rainfall were found to be favourable for growth and sporulation of fungi. Fungi like Phytophthora, Pythium, Rhizoctonia and Mucor were found to occur during this period.

The phylloplane fungi associated with tomato, brinjal and The plants were raised in pots and chilli were studied. observations were recorded at fortnightly intervals for а continuous period of four months. Brinjal leaves harboured the maximum fungal population followed by tomato and chilli. The fungi included the common pathogens and saprophytes. Fungi like theobromae, Pestalotia palmarum, Phoma sp., Botryodiplodia Trichoderma viride, A: niger, A. flavus and A. terreus were common to all the three crops and were selected for vitro in studies, along with the common pathogens of the specific crops in search for a suitable antagonist.

Based on the above studies <u>B</u>. <u>theobromae</u>, <u>T</u>. <u>viride</u> and <u>Aspergillus</u> spp. viz., <u>A</u>. <u>niger</u>, <u>A</u>. <u>flavus</u> and <u>A</u>. <u>terreus</u> were selected as suitable antagonists. But only <u>T</u>. <u>viride</u> was utilised for further studies.

The mechanism of action of <u>Trichoderma</u> <u>viride</u> towards <u>Fusarium solani</u> was through coiling and penetration while with <u>R. solani</u> the inhibition was through coiling and disintegration of hyphae. Penetration and disintegration was found with <u>A. solani</u> while for <u>C. capsici</u> it was by penetration alone.

role of Trichoderma as a biological antagonist against The common fruit rot pathogens were studied. Fresh samples of the tomato, brinjal and chilli were collected and sprayed with the conidial suspension of <u>T</u>. viride, airdried and inoculated with their respective pathogens and the extent of damage recorded and with the control. It was observed that  $\underline{T}$ . viride was compared effective in reducing the fruit rot of tomato caused by the major pathogens viz., <u>F. solani</u> and <u>R. solani</u> by 52 and 50 percent respectively up to 12 days of storage under artificial inoculated Similar results were also conditions at room temperature. obtained with F. solani in brinjal (48 percent) and C. capsici in chilli (43 percent).

The study thus highlighted the effectiveness of utilising <u>Trichoderma</u> as a biological antagonist against the major storage pathogens of tomato, brinjal and chilli.