

**MANAGEMENT OF FUNGAL DISEASES OF CAPSICUM  
(*Capsicum annuum* L.) UNDER PROTECTED CULTIVATION**

By  
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(2015-11-107)

**THESIS**

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Kerala Agricultural University




**DEPARTMENT OF PLANT PATHOLOGY  
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2017

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I hereby declare that the thesis entitled “**Management of fungal diseases of capsicum (*Capsicum annuum* L.) under protected cultivation**” is a bona fide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellow ship or other similar title, of any other university or society.

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Date: 30-08-2017

  
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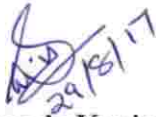


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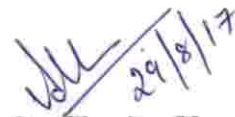
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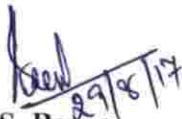
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*Deepa Pawar*

*Dedicated to  
my beloved  
parents and  
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# *Introduction*

## 1. INTRODUCTION

Capsicum (*Capsicum annuum* L.), also known as bell pepper, sweet pepper, shimla mirch, and green pepper is one of the highly remunerative annual herbaceous vegetable crops, belonging to family Solanaceae. It is rich in vitamin A, vitamin C and minerals like calcium, magnesium, phosphorus, and potassium (IIHR 2012). Originated in Mexico and Central America, it is being cultivated worldwide, especially in temperate region of Central and South America and European continents as well as in India and China. India contributes to one fourth of world production of capsicum with an average production of 268mt/ha per annum, with an average productivity of 12.6t/ha (Indian Agristat.2014-2015). Andhra Pradesh, Karnataka, are leading producers of capsicum followed by Maharashtra, Tamil Nadu, Himachal Pradesh, and hilly areas of Uttar Pradesh, in the country (Sreedara *et al.*, 2013).

Recently capsicum consumption is increasing in India, which is attributed to the increasing demand by urban consumers. However, its productivity is very low in India compared to western countries. The crop being a temperate one it can be grown round the year, under protected structures, where temperature and relative humidity (RH) can be manipulated. Capsicum requires day temperature of 25-30°C and night temperature of 18-20°C with RH 50-60 per cent. If temperature rises above 35°C or falls below 12°C fruit setting is affected (IIHR 2012). Production of capsicum is also constrained by many diseases, pests and disorders that reduce fruit quality and yield. Damage can also be caused by a wide range of major biological agents that cause damage including fungi, bacteria, viruses, insects, nematodes, birds, and mammals. The most important diseases caused by fungi are powdery mildew, anthracnose, *Cercospora* leaf spot, stem and fruit rot and damping off.

Use of chemical fungicides for management of fungal diseases leads to health hazards and environmental pollution, which in turn lead to increased vulnerability of crops to further infection by the pathogens. It may also lead to development of

resistant strains of pathogens. Moreover, crop failure due to diseases results in heavy economic losses especially in poly house cultivation, which requires very high input cost. Hence precise disease management with emphasis on biocontrol is essential for assuring profitable farming in protected structures.

In this context, the present study was under taken with the objective; to assess the incidence and severity of fungal diseases of capsicum under protected cultivation and to formulate an eco-friendly management practice against the diseases. The study included the following experiments.

1. Assessment of incidence and severity of fungal diseases of capsicum under protected and open conditions in farmers' fields.
2. Characterization of major fungal pathogens of capsicum under polyhouse and rain shelter conditions.
3. Management of fungal diseases of capsicum under poly house and rain shelter.
4. Enumeration of phylloplane microflora of capsicum under protected condition, as affected by foliar treatments.
5. Study the survival of bio control agents on phylloplane of capsicum under protected condition.



# *Review of Literature*

## 2. REVIEW OF LITERATURE

Capsicum (*Capsicum annum* L.) is one of the most popular fruit vegetables belonging to family Solanaceae grown throughout the world. It is rich in Vitamin A, Vitamin C and minerals like calcium, magnesium, phosphorus, and potassium. Owing to its economic importance as a high value vegetable crop, production of capsicum under protected condition has become popular in urban and semi urban areas of the country. However, cultivation of capsicum in poly/net house is facing many production constraints like new pests, diseases, micro nutrient deficiencies and so on (IIHR 2012). The crop suffers from many diseases caused by fungi, and bacteria, and viruses among which fungal diseases are the most prevalent, such as powdery mildew (*Leviellula taurica* (Lev.) Arnaced), anthracnose and fruit rot (*Colletotrichum capsici*) Syd. Butler and Bisby, stem and fruit rot (*Fusarium solani* (Mart.) Sacc.) are major fungal diseases affecting capsicum under polyhouse. These diseases cause significant yield loss up to 50 percent (Dubey and singh.,2012).

Use of synthetic fungicides is not acceptable owing to increasing concern of food safety due to various undesirable effects they cause. Moreover, it is reported that only 0.1per cent of the chemicals used for crop protection reach target pathogen, leaving 99.9per cent in the environment causing hazardous effects on non-target organisms (Pimenttel 1995). Effective biological management of fungal diseases of capsicum by *Trichoderma viride* and *Pseudomonas fluorescens* has been reported by Ngullie *et al.*, (2010). However it may not be economic, if disease is severe. Hence a bio intensive method is suggested for effective and economic management of diseases in protected cultivation (Jeffries and Koomen, 1992).

The literature collected for study are presented below under the following headings

## **2.1. Protected cultivation of vegetables**

## **2.2 Diseases of capsicum under protected cultivation**

### **2.2.1 *Cercospora* leaf spot**

### **2.2.2 Anthracnose and fruit rot**

### **2.2.3 Powdery mildew**

### **2.2.4 Stem and fruit rot**

## **2.3 Management of fungal diseases of capsicum**

### **2.3.1 Management by chemical control**

### **2.3.2 Management by biological control**

## **2.4 Effect of fungicides on no-target microflora**

## **2.5 Survival of biocontrol agents on leaf surface**

## **2.1. Protected cultivation of vegetables.**

Protected cultivation or greenhouse cultivation is the most contemporary approach to raise horticultural crops and has spread extensively all over the world in the last few decades. Protected cultivation also known as Controlled Environment Agriculture (CEA) is highly productive, conservative of water and land and also protective to the environment (Jensen, 2002). About 130 countries in the world are engaged in greenhouse vegetable production commercially. The world scenario depicts the area under protected cultivation to be nearly 6,93,787 hectares while total estimated area under greenhouse vegetable production in the world is 4,73,466 ha (Hickman, 2016). Presently around 25,000 ha are under protected structures, in our country out of which the area under vegetable crops is about 2,000 ha. So far, 1108 poly houses of size 40-400 m<sup>2</sup> are actively engaged in crop production in Kerala and

the major vegetables being cultivated in poly houses are salad cucumber, capsicum, yard long bean, bhindi and tomato. Studies conducted in Kerala Agricultural University at the Precision Farming Development Centre (PFDC), Thavanur, revealed that cultivation of vegetable crops in kitchen garden under rain shelter ensured year round production of vegetables needed for a family (Menon, 2010). The cultivation of crops such as tomato, salad cucumber, chilies, cowpea, cauliflower and capsicum in rain shelter in a rotational manner gave very high yield compared to cultivation in open field.

Greenhouse capsicum production is a lucrative industry in itself though it is more laborious than growing tomatoes. Despite the fact that it is a valuable crop with excellent prospects, it is slow growing, needs a high temperature to develop, fruit set occurs in periods and fruits are harvested in flushes. Different coloured varieties of capsicum, viz. red, yellow, green and black are high in demand at fast food restaurants for variety of food preparations, extraction of natural colours and preparing oleoresins and oils. They may also be used for producing paprika which is used for colouring foods, flavoring and in sauces (Burt 2005). The coloured varieties rich in Vitamin A, Vitamin C and antioxidants along with processed products have very well added to the market value of capsicum and fetch a premium price in the international market. Even though the major damage caused to capsicum is through thrips, mites and fungal pathogens like *Pythium*, *Rhizoctonia*, *Botrytis*, powdery mildew, *Fusarium* wilt and *Cercospora* leaf spot, (Sabir and Singh ,2012) also cause extensive damage to the crop.

According to Indian Agristat (2014-15), area under capsicum cultivation in India comes around 21 ha, while production and productivity are 268MT and 12.67mt ha<sup>-1</sup> respectively. However, in Kerala the area of capsicum cultivation is only 0.06 ha, with production of 0.61t and productivity 10.17mtha<sup>-1</sup>.

## 2.2 Diseases of capsicum under protected cultivation

Greenhouse vegetable crops are vulnerable to various pests and diseases. Since poly houses are enclosed structures, it provides favorable environment for rapid multiplication of plant pathogens. Once the inocula enter in to the structures, spread is very fast because of absence of natural enemies due to enclosed atmosphere. The spores of airborne pathogens like powdery mildew, downy mildew and gray mold are produced in large quantities, under humid conditions. They are released most readily when humidity drops. The most frequently occurring air born fungal pathogens in protected structures are species of *Alternaria*, *Botrytis*, *Cladosporium*, *Didymella*, *Erysiphe*, *Fulvia*, *Leveillula*, *Phoma*, *Phytophthora*, *Pseudoperonospora*, *Puccinia*, *Sclerotinia*, *Septoria* and *Sphaerotheca* and common fungal diseases, that thrive under the polyhouses include grey mold (*Botritis cinerea*), tomato late blight (*Phytophthora infestans*), tomato leaf mold (*Cladosporium fulvum*syn. *Fulviafulva*) and powdery mildew in capsicum (*Leveillula taurica*) roses and various other crops (Yucel *et al.*, 2013).

The major diseases affecting capsicum in protected structures are powdery mildew (*Leviellula taurica* (Lev.) Arnaced), Cercospora leaf spot (*Cercospora capsici*) (Heald and F.A wolf) anthracnose and fruit rot (*Colletotrichum capsici*) (syd). Butler and Bisby, and *Pythium aphanidermatum* stem and fruit rot (*Fusarium solani* (mart). Sacc). These cause significant yield loss up to 50 percent (Dubey and singh, 2012).

### **2.2.1 *Cercospora* leaf spot**

*Cercospora* leaf spot leads to serious losses in capsicum production when crop is grown under protected structure. The disease is observed world- wide, but it is more severe in tropical and sub-tropical regions where warm humid condition prevails (AVRDC, 2004).

#### **2.2.1.1 Symptomatology and epidemiology of *Cercospora* leaf spot**

Initially disease appears on younger leaves. The spots are circular with a light gray center and reddish-brown margin, which enlarges up to 1cm diameter. Later the spots become tan with a dark ring on the margin and yellowish halo under high humid atmosphere. Concentric zonations are seen in the spots, resulting in a frog-eye appearance. The tissues at the center of the lesion dry up and often drop out to form short holes. When numerous spots occur on the foliage, the leaves turn yellow and may drop or wilt. Defoliation is often very severe. Spots also develop on stems and petioles but they are oblong rather than circular, fruit are not affected. The fungus survives in or on seed and as tiny black stromata in old affected leaves in the soil, spores will survive in infected debris for at least one season. Foliar infection occurs by direct penetration. The spores require water for germination and penetration of the host, however, heavy dew appears to be sufficient for infection. The disease is most severe during period of warm temperature during the day and excessive moisture (either from rain or overhead irrigation). Growth of the pathogen is limited if the temperature is  $<5^{\circ}\text{C}$  and  $>35^{\circ}\text{C}$ . The pathogen is spread by splashing water, wind driven rain, wind, or implements tools, workers, and by leaf to leaf contact (AVRDC 2004; Suresh, 2013).

### **2.2.1.2 Characteristics of *Cercospora capsici***

Mycelium consists of smooth, branched, septate, hyaline hyphae, Conidiophores are erect, slightly divergent, sub cylindrical, unbranched, geniculate, pale brown, smooth, and thick-walled. Conidiogenous cells are terminal as well as intercalary; Conidia are hyaline, solitary, subcylindrical, very slightly curved, sub-acute to obtuse, thin-walled, smooth, base obconical to round. Sexual stage was not observed. Colonies are smooth to slightly irregular, erumpent, even to slightly uneven margin. On V8 medium the fungus produces colonies which are smoky black-centered with slightly whitish margin, slow-growing, do not cover whole plate after even one month, with less sporulation. Also with on PDA, the colonies are of medium dark with white margin, moderately growing, cover whole plate within 20 days, also with much less sporulation, Red color pigmentation is clearly visible on the inverted plate, indicating the presence of cercosporin toxin. In potato dextrose broth the fungus grows ball-like appearance with loose mycelium on outer surface. Optimal temperature for growth is 25°C (Meghvasi *et al.*, 2013).

### **2.2.2 Anthracnose and fruit rot**

Anthracnose and fruit rot is one of the most important diseases of bell pepper (*Capsicum annuum*) in tropical and subtropical region. In mild to warm climate and rainy seasons, fruit production losses can reach as high as 100 per cent when adequate control measures are not applied (Alves *et al.*, 2015). The pathogen is distributed in North America, Europe, Africa, American Saman, Australia, Federal states of Micronesia, Fiji, Vanuater (Mckenzie, 2013).

#### **2.2.2.1 Symptomatology and epidemiology of anthracnose and fruit rot**

Disease symptoms may occur either during the crop development in the field or after harvest, but only the fruit exhibits symptoms. Infection begins with the

appearance of small, circular, depressed lesions that rapidly expand and have no defined diameter. When relative humidity is high, the formation of a pink or orange conidial mass may be observed (Lopes and Avila, 2003). Environmental factors play a major role in the development of disease epidemics. The relationships among rainfall intensity, duration and crop geometry and dispersal of inocula possibly lead to different level of disease severity (Dodd *et al.*, 1992). The effect of temperature often interact with other factors, such as leaf surface wetness, however, appear to have the most direct influence on the host. Generally, infection occurs during warm, wet weather. Atmospheric temperature around 27°C and high humidity (a mean 20%) are optimum for anthracnose and fruit rot disease development (Robert *et al.*, 2001; Harp, 2008).

#### **2.2.2.3 Characteristics of *Colletotrichum capsici***

On PDA the fungus produce white mycelium initially which later turns arial mycelia and whitish gray. In slide culture appressoria appear dark brown, spherical, ovate or obclavate, smooth walled. Acervuli are dark brown to black and conspicuous for their dark setae (Shenoy *et al.*, 2007). Conidiomata, acervuli, producing conidia in a slimy grey-white matrix, many setae, conidia 17-26x3-4µm in size aseptate, smooth-walled, hyaline, curved, tapered towards both ends, ends pointed (Mckenzie ,2013).

#### **2.2.3 Powdery mildew**

On chilli (*Capsicum annum*, *C. frutescens*) powdery mildew *Leveillula taurica* is more widely distributed than an any other crop. Distributed in Asia, Mediterranean region, but pepper is the only solanaceous host on which the powdery mildew has been recorded in sub-sharan countries in Africa, as well as in many regions of south and Central America, and of Australia. (Palti, 2013).



### 2.2.3.1 Symptomatology and epidemiology of powdery mildew

The symptoms of powdery mildew begin as discolored spots on the upper surface of leaves as white-gray to purple growth of the causal organism; similar to downy mildew is observed on the lower side of the leaf. Infected areas dry up and turn black. When infection is severe the disease causes defoliation up to 100 per cent. No disease symptoms were observed on stem and fruits. Infection usually progress from the older to younger leaves and both seedlings and mature plants are affected. When the atmospheric temperature is 25°C, and the relative humidity is 85per cent, the disease development is very fast (Ondieki, 1971). However, leaf shedding was more pronounced at a lower humidity of 50per cent. For each leaf shedding at higher humidity, three leaves shed at the lower humidity. The powdery mildew causes extensive losses, both directly and also indirectly, when leaf shedding exposes and fruits to the sun leading to sunscald (Palti, 2013).

### 2.2.3.2 Characteristics of *Leveillula taurica*

The endophytic intercellular mycelium, which develops chiefly in the host's mesophyll tissue, but may also extend to the cells of the palisade tissue, consists of irregular septate hyphae that may be sinuous or straight, and are up to 10µm in width (Tramier, 1963). This mycelium in many hosts is most abundant and is immediately below the epidermis of the lower leaf surface. In certain hosts, varicose granulations, are also observed in conidiophores, are sometimes apparent in the intercellular hyphae. In some hosts, hyphae arising from intercellular mycelium emerge through stomata together with the conidiophores (Rostam, 1983). Two types of conidia are generally recognized in *Leveillula taurica*. Primary conidia, those formed first on the conidiophores, usually have tapering tips, while secondary conidia, those formed subsequently, and have more rounded tops. The two types of conidia are similar in size (Palti, 2013). On pepper, Clerk and Ayesu-offri (1967) have described primary conidia as pyriform while secondary conidia were cylindrical.

Kurtet *al.*, (2004) have characterized the shape of primary conidia formed on pepper as “boat-like” and secondary conidia as elliptical.

Rostam (1983), has remarked that conidial chains are more often observed on plants grown under shelter (polyhouse), and less commonly on plants growing in the open. Attachment of mature conidia to conidiophores is so weak, that conidial chains disperse at the slightest movement of air. Cleark and Ayesu-affe (1967), observed germination of conidia on the surface of pepper leaves, and determined that conidia do not germinate as long as they are attached to conidiophores and that spores born in chains germinated just as freely as single spores, and the position of the spore in the chain had no effect on its germination potential. The length of conidiophores decreased as water stress increased, and the percentage of branched conidiophores decreased greatly, from 17.5 per cent in non-stressed to only 2.5 per cent in severely stressed plants (Carsae and Clerk, 1985).

#### **2.2.4 Stem and fruit rot of capsicum**

The distribution of *Fusarium oxysporum* is known to be cosmopolitan. The disease is most destructive in warm climate and warm, sandy soils of temperate regions. It can become severe only in green house (Agrios, 2004).

##### **2.2.4.1 Symptomatology and epidemiology of stem and fruit rot of capsicum**

Stem and fruit rot is the most important disease caused by *F. oxysporum* in sweet pepper plants. The disease first appears as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward curling) of the older leaves. At the seedling stage, plants may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant. Browning of the vascular tissue is a strong evidence of stem and fruit rot

in sweet pepper. The pathogen is primarily spread over short distances by irrigation water and contaminated farm equipment's. The fungus can also be spread over long distances either in infected transplants or in soil. Although the fungus can sometimes infect the fruit and contaminate its seed, the spread in fungus by way of the seed is very rare (Fletcher, 1994).

The fungus invades the plant with its sporangial germ tube or mycelium by penetrating the plant's roots. It can be infected directly through the root tips, through wounds or at the formation point of lateral roots. Once inside the plant, the mycelium grows through the root cortex intercellular. When the mycelium reaches the xylem, it invades the vessels through the xylem pits (Agrios, 2004). Due to the growth of the fungus within the plant's vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaves' stomata to close, the leaves wilt, and the plant eventually dies. At this point that the fungus invades the plant's parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly. The resulting spores which on their turn act as new inoculum for further spread of the fungus (Jarvis *et al.*, 1994).

#### **2.2.4.2 Characterization of *Fusarium* sp.**

In solid medium, such as PDA (Potato Dextrose Agar), the different special forms of *F. oxysporum* can have varying appearances. In general, the mycelium first appears white, and then may change to a variety of colors ranging from violet to dark purple-according to strain or special form of *F. oxysporum*. If sporodochia are abundant the culture may appear cream or orange in color (Smith *et al.*, 1988).

*F. oxysporum* produce three types of asexual spores, microconidia, macroconidia and chlamydospores. Microconidia are one or two celled and are the types most and frequent produce by the fungus under all conditions. It is also the type of spore most frequently produced within the vessels of infected plants. Macroconidia are three to five celled, gradually pointed and curved towards the ends. These spores

are commonly found on the surface of plant killed by this pathogen as well as in sporodochia like groups. Chlamydospores are round, thick-walled spores, produced either terminally or intercalary on older mycelium or in macroconidia, these spores are either one or two celled (Romberg and Davis, 2007).

### **2.3 Management of fungal diseases of capsicum**

Fungal diseases of capsicum are managed through chemically, biologically and biocontrol agents to reduce the yield loss and increase the production.

#### **2.3.1 Management by chemical methods**

Effective management of fungal diseases of capsicum in open as well as in protected structures by chemical fungicides has been reported by several workers. Ondieku (1971) reported that benomyl was found to be effective against powdery mildew of capsicum. Smith *et al.*, (1999) suggested that tebuconazole (folicur) and propiconazole (tilt) provided excellent control of powdery mildew of capsicum. Chaudhary *et al.*, (2015) used nine different fungicides including systemic and contact against powdery mildew. Results showed that out of nine, four were very effective to control the powdery mildew which includes hexaconazole, difenoconazole, propiconazole, triadimefon. The percent disease severity was the least and the yield was the highest in hexaconazole. Integrated disease management for the control of powdery mildew (*Leveillula taurica*) of bell pepper has been evaluated and it was found that sequential spray of tebuconazole and difenconazole is effective for control powdery mildew in bell pepper (Manojkumar *et al.*,2008). Sulphur, hexaconazole, dinocap, azoxystrobin are recommended for control of powdery mildew of capsicum under protected cultivation (IIHR,2012).

Hadden and Black (1989) suggested that field application of chlorothalonil and maneb is effective for the control of anthracnose and fruit rot in bell pepper. The chemical fungicides generally recommended for controlling anthracnose and fruit rot

disease are based on copper compounds, dithiocarbamates, benzimidazole and triazole compounds. However efficacy of new chemicals like strobilurins based fungicides (azoxystrobin, pyraclostrobin) for management of disease has been reported Waller.(1992).Damage caused by anthracnose in bell pepper can be reduced when integrated management measures are adopted, including use of healthy seeds of known origin and application of registered fungicides (Azevedo *et al.*,2006).Experiment conducted in green house and field for management of anthracnose and fruit rot of chill showed that application of propiconazole gave a dramatic reduction of the disease (Gopinath *et al.*,2006). Azoxystrobin was highly efficient for management anthracnose and fruit rot, powdery mildew in chilli was reported by (Anand *et al.*, 2010).

Solel (1970) reported the efficacy of benomyl and benzimidazole for the control of *Cercospora* leaf spot. According to Khunti *et al.*, (2005) all the triazole fungicides performed better as compare to conventional fungicides against *Cercospora* leaf spot. They found that minimum disease intensity and higher yield is obtained by application of hexaconazole, penconazole and tridemorph for control of the disease.

Song *et al.* (2004), studied the effect of fungicides for control of stem and fruit rot in tomato, and found that, prochloraz and carbendazim were the most effective fungicides with low toxicity. Nel *et al.*, (2007) showed that benomyl and the dimethylation fungicides significantly reduced the disease severity of *Fusarium* wilt when applied as a root dip treatment. Chlorothalonil, mancozeb and carbendazim are recommended for control of *Cercospora* leaf spot (IIHR 2012).

### 2.3.2 Management by biological methods

Owing to the public concern on food safety issues, efficacy of biological control agents is being tested against most of crop pests especially in poly house (Vukovic *et al.*, 2012). Use of environmentally friendly biological control agents can more effectively control the soil borne phyto pathogens (Nam *et al.*, 1988; Park, 1989; Saleem *et al.*, 2000). One of the most studied commercial biocontrol agents is *Trichoderma*. The most commonly used biocontrol agents for the management of diseases include *Trichoderma* spp. and fluorescent Pseudomonads. *Trichoderma* isolates are known for their ability to control foliar pathogens (Elad and Freeman, 2002). According to Panpatte *et al.*, (2014), the mechanism of biological control by *P. fluorescens* against foliar pathogens include antibiotic production, competition, HCN production and elicitation of a disease-resistance response, *i.e.*, induced systemic resistance (ISR).

Kokalis- Burelle *et al.*, (2002) conducted field trials to evaluate tomato and pepper transplants amended with formulations of several plant growth promoting rhizobacteria (PGPR) with soil solarization. Results showed that there was significant reduction in *Fusarium* colonies in pepper roots, at 45 days after planting compared to the untreated control. Mao *et al.*, (1998) evaluated various biocontrol agents for soil borne disease control in pepper and tomato. It was found that seed treatment with *Gliocladium virens* (GI-3) and *Burkholderia cepacia* (BC-F) reduced damping off in bell pepper.

Kiss (2003) reported *Ampelomyces* sp as a potential biofungicide for control of powdery mildew compared to other fungicides. Dubey *et al.*, (2007) reported *Trichoderma viride* and *T. harzianum* as the best antagonists for growth inhibition of several soil and seed borne plant pathogen. Sahi and Khalid (2007) studied *in vitro* biological control of *Fusarium oxysporum* causing wilt in *capsicum annum*. They reported that *Trichoderma* species have potential to suppress the colony growth of

*Fusarium oxysporum* incitant of stem and fruit rot in sweet peppers. Manojkumaret al.,(2008) conducted field evaluation of eight biocontrol agents against powdery mildew of bell pepper, and out of them, two biocontrol agents ie *Ampelomyces quisqualis* and *verticillium lecanigave* minimum percent disease incidence.Seangnak.et. al., (2013) reported the use of antagonisitic. *Streptomyces* spp. against chili wilt disease caused by *Fusarium oxysporum* sp *capsici*.

### 2.3.3Soil solarisation

Soil solarization is a cost-effective and environmentally safe nonchemical soil disinfestation method that, under appropriate conditions, can ensure an effective control of a wide range of pathogens, weeds and pests. Increase in soil temperature by solarization is sufficiently high and prolonged to cause irreversible damage to pathogens (Fenoll et al.,2010).Therefore, this technique is particularly suitable for the Mediterranean climate, where the occurrence of high summer temperature can ensure effective control of fungi, nematodes and weeds (Comprubi et al.,2007).

Soil solarization is done by covering moistened soil with transparent polyethylene which allows the sun's radiant energy to pass in to the soil where most of this energy is trapped and not reflected back owing to the change in the wave length on reflection and thus increasing the soil temperature (Katan, 1981).This increase in soil temperature in the presence of moisture is the mechanism by which soil borne diseases are controlled in solarized soil. Greenberger et al.,(1987) reported that solarized soil is frequently more suppressive &less conducive to certain soil borne pathogens than non-solarized soil, soil solarization has also been successfully combined with the fungal biocontrol agents *Trichoderma harzianum*, and *Talaromyces flavus*, which were added to the soil or planting material. Even though, this technique is more effective with direct sun light, it has been reported that inside green house also it produces significantly higher soil temperature and can therefore be effective in control of soil born plant pathogens (Elmore et al.,1997) Candido et

*al.*, (2008) conducted soil solarization in greenhouse for a period of 79 days and reported that difference in temperature between solarized and non-solarized soil was on an average 7.1, 6.4, and 6°C respectively, at 10, 20, 30 cm depth respectively. The maximum temperature was 47.3°C in solarized soil and 39.4°C in non-solarized soil at 10 cm depth, moreover, soil solarization improved soil structure and increased the availability essential plant nutrients and further increase plant growth, harvestable yield, and crop quality. The growth response of plants in solarized soil is a well-documented phenomenon and has been verified both in green house experiments and under field condition (Candido *et al.*, 2008).

Fungal diversity of any soil depends on a large number of factors such as pH, organic content and moisture (Rangaswami and Bagyraj, 2005). Gaddeyya *et al.*, (2012) reported that on isolation of soil microflora from polyhouse soil isolated 173 fungal colonies of 15 fungal species were obtained by serial dilution method, study conducted by Jasuja *et al.*, (2013) revealed that soil solarization in polyhouse effectively reduced the soil microflora *viz*, fungi, bacteria and actinomycetes. Changes in the population of soil-borne micro-organisms during and after soil solarization are attributed to increased soil temperature, thus leading to disease suppression and enhanced plant growth. The common bacteria, *Staphylococcus aureus*, *Esherichia coli*, *Pseudomonas aeruginosa*, *Bacillus anthracis* and *Bacillus substilis* are predominant species in the soil samples, and common fungi include *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma sp*, *Fusarium oxysporum*, and *Rhizopus sp*.

## 2.4 Phylloplane microflora

The phylloplane microbial communities present on the leaf surface, including fungi, bacteria, and actinomycetes have been shown to perform important ecological functions and be both beneficial and harmful to their host plant. In agricultural and horticultural crops, some phylloplane microbes have antagonistic properties against



plant pathogens while few may themselves be pathogens also. Some others can influence the crop physiology. Understanding the influence of agricultural practices on phylloplane microflora is important in order to create the best conditions for crop development. Blakeman (1985) reported that the activity of both saprophytes and pathogens on leaves is dependent on the microclimatological conditions at the plant surface as well as on the chemical environment. Bacteria, yeasts, and filamentous fungi may form resident populations on leaves. For enumerating the phylloplane micro flora, microorganisms are usually washed off the leaf surface in a wash buffer (Morris *et al.*, 1997), which is then inoculated onto artificial growing media and incubated. Dilution ranges can be used to control the density of microorganisms on the plates. Phylloplane microorganism populations can be described in terms of population density and species richness. The density of culturable microorganisms is commonly described as the number of colony forming units (cfu) per unit of sample (Bakker, 2004).

The use of foliar pesticides to control diseases can major disruption of phylloplane microorganism populations, often reducing the number and diversity of organisms. This cause a negative effect on naturally occurring biological control, which in some occasions make the plants more susceptible to other disorders (Bosshard *et al.*, 1987). Moreover, the activity of fungicides is affected by naturally occurring compounds on the leaf, interacting synergistically or antagonistically with the fungicides and this gives an indirect indication of pesticide residue residing in the foliage and on fruits (Dik, 1991), Gokulapalan (1998) reported the harmful effect of plant protection chemicals on epiphytic microflora on rice. Elad *et al.*, (1996) studied establishment of an active *Trichoderma* population on the phylloplane and effect on grey mould (*Botrytis cineria*).

## 2.5 Survival of bio control agents on leaf surface

The fungal biocontrol agents *T. viride* survive mainly on the hyphae of the pathogen present on the leaf surface or else it may get transferred to resting spores where, bacterial biocontrol agent *P. fluorescens* survive on surface wetness brought about by a thin film of water on leaf surface or by leaf exudates (Hirano and Upper 2000). Survival of Trichoderma strains may be quantified by serial dilution method on semi selective medium (Elad and Kirshner, 1993) however, morphologically it is not possible to distinguish between isolates in a mixture. Cirvilleri *et al.* (1999) reported that the population densities of *P. fluorescens* strains decline within 30 days after inoculation on plant species such as pepper, tomato, eggplant, and strawberry. Freeman *et al.*, (2004) who studied the variability of the *Trichoderma* strains the variability of the Trichoderma strains T-39, T-105, T-161 and T-166 application. Furthermore, survival of the strains declined undetectable levels after two weeks incubation

The survival of *P. fluorescens* and *T. viride* sprayed on rose leaves was assessed by taking leaf samples at 5,15,30,45,60 and 75 days after foliar application. Leaf samples (1g) were transferred to a test tube containing 100ml of sterile water and after thorough shaking, the population of *P. fluorescens* and *T. viride* in suspension was established by dilution plating, using King's B medium for *P. fluorescens* and TSM for *T. viride* (Elad and Freeman,2002). *P. fluorescens* and *T. viride* multiplied on rose leaves and suppressed the pathogen population. Because it modified the leaf habitat by depleting the pathogen and did not allow the multiplication of *Diplocarpon rosae* (Karthikeyan *et al.*, 2006)

# *Materials & Methods*

### 3. MATERIALS AND METHODS

The present study on "Management of fungal diseases of capsicum (*Capsicum annuum* L.) under protected cultivation" was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2015-2017. The details of materials used and techniques adopted for the investigation are described below.

#### 3.1 Assessment of incidence and severity of fungal diseases of capsicum under protected structures in farmers' field

A survey was conducted during August-February 2016-2017 by selecting polyhouses where capsicum is grown at Vellanikkara, Thanniyam, Elanad, Manalur and Puranattukara, in Thrissur, Chithaliin Palakkad, Plamootikada and Neyyattinkara in Thiruvananthapuram. Incidence and severity of fungal diseases of capsicum in poly houses were assessed using standard score chart and procedures (Plate 1). The major meteorological factors such as temperature and relative humidity influencing the crop and pathogen prevailing in the structure and open condition were recorded.

In present survey, diseases *viz.* powdery mildew, leaf spot, fruit rot and stem rot and fruit rot on capsicum were observed in farmers' poly houses. Per cent disease incidence (PDI) was calculated using the formula suggested by Wheeler, (1969).

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of plants infected} \times 100}{\text{Total number of plants}}$$

Percent disease severity (PDS) was calculated, using 0-5 scale (Suresh, 2013) Table 3.1, for leaf spot (Plate 3) and 0-9 scale (Mayer and Dator, 1986) Table-3.2, for powdery mildew (Plate 2) as furnished below. In each polyhouse, five locations were selected and ten plants were observed at random in each location. Total of five leaves two each from bottom, middle and one from the top of the plant were observed.

**Table 3.1 Score chart for leaf spot capsicum    Table 3.2 Score chart for powdery mildew capsicum**

<b>Score</b>	<b>Per cent leaf area infected</b>
0	No infection
1	Below 10 per cent infection
2	>10-25per cent infection
3	>25-50 per cent infection
4	>50-75 per cent infection
5	Above 75 per cent infection

<b>Score</b>	<b>Per cent leaf area infected</b>
0	No infection
1	Below 1per cent infection
3	<1-10 per cent infection
5	>10-25 per cent infection
7	>25-50 per cent infection
9	>50 per cent infection

Per cent disease severity (PDS) was calculated using the following formula (Wheeler, 1969)

$$\text{PDS} = \frac{\text{Sum of all disease ratings} \times 100}{\text{Total number of leaves observed} \times \text{Maximum disease score}}$$

### **3.1.1 Correlation analysis of incidence and severity of fungal diseases of capsicum with meteorological factors**

Data collected during the survey was subjected to correlation analysis using SPSSv16.0 data editor to assess the influence of meteorological factors on incidence and severity of fungal diseases in protected structures.

### **3.2 Symptomatology, isolation and characterization of the pathogens**

Infected plant samples with symptoms of leaf spot, stem and fruit rot, powdery mildew and fruit rot were collected separately from different locations during the survey (Plate 1). Symptoms of each disease were carefully observed and compared with the photographs and description available in the literature. The specimens were also subjected to examination under microscope and the characters of pathogens associated were studied. Pathogens were isolated from the infected specimens. Samples were washed under tap water, pressed on a blotting paper to remove excess water and small bits were taken from infected area of the leaf along with some healthy area. The bits were surface sterilized with one per cent sodium hypochlorite solution and then washed in three changes of sterile water. The bits were then placed on Potato Dextrose Agar (PDA). Hyphae ramifying from the leaf bits on PDA were sub cultured to fresh mediated plates and incubated under room temperature. The cultures were purified by hyphal tip method and maintained for further studies. The cultural and morphological characters of the isolated fungi were studied.

### **3.2.1 Pathogenicity**

The pathogenicity of the organisms associated with different diseases was tested by artificial inoculation on healthy plant parts. Healthy leaves, twigs and fruits collected from the field were washed under tap water and wiped with 70per cent ethyl alcohol. The leaves /twigs/fruits were inoculated with actively growing seven to eight day old culture of pathogen after giving pin pricks (Plate 8). Piece of moist cotton was placed over inoculated area and the inoculated leaves/twigs/fruits were kept in humid chamber and observed daily for the appearance of symptoms. Plant parts on which pin pricks were given and piece of moist cotton was placed over the area served as control. The symptoms developed on inoculated area were observed, and compared with the symptoms produced on natural infection. The pathogens were reisolated from the infected area, and the characters were studied and thus proved the Koch's postulates.

### **3.2.2 Cultural and morphological characterization of pathogens**

The cultural and morphological characters of the isolated pathogens *viz.*, colour, rate and pattern of growth, conidial and conidiophore characters were studied.

### **3.2.3 Molecular characterization**

Purified cultures of the pathogens isolated from leaf spot, fruit rot, and stem and fruit rot were sent to Rajiv Gandhi Center for Biotechnology Thiruvananthapuram, for molecular characterization. The ITS sequences (Plate 11) obtained were blasted in NCBI web site, similarity of sequences was studied and the pathogens were identified.

### 3.3 *In vitro* evaluation of bio control agents and fungicides against powdery mildew of capsicum

*In vitro* evaluation of treatments against powdery mildew was carried out using the method suggested by Tenhovirta, (2012) as (Plate 12). The treatment details are given below.

**Table 3.3** Treatments details of *in vitro* evaluation against powdery mildew of capsicum

Treatments	Foliar spray
T1	<i>Trichoderma viride</i> ( <i>asperellum</i> ) (20 g.L <sup>-1</sup> )
T2	<i>Pseudomonas fluorescens</i> (20g.L <sup>-1</sup> )
T3	Kocide (0.2per cent)
T4	Mancozeb (0.2 per cent)
T5	Tebuconazole (0.1 per cent)
T6	Difenoconazole (0.05 per cent)
T7	Control

Healthy leaves of capsicum were collected from the field, washed thoroughly with tap water, and foliar treatments were applied using hand sprayer. The treated healthy leaves were placed on moist filter paper in Petri plates. Conidia of *Leveillula taurica* from infected leaves were then gently brushed on to the treated leaves in the Petri plates. The inoculated leaves were incubated at room temperature. Lesions were measured and leaf area infected (LAI) was calculated. Inoculated untreated leaves served as control.



### **3.4 Field experiment for management of fungal diseases of capsicum under protected cultivation**

Field experiments were conducted under rain shelter of size 200m<sup>2</sup> and polyhouse of size 300m<sup>2</sup> both having gable type roof, constructed in North-South direction in the Department of Plant Pathology, College of Horticulture, Vellanikkara(Plate13). The experiments were carried out during June to February, 2016-2017 to evaluate different treatments for the management of fungal diseases of capsicum under protected condition. The details of the experiment are as follows.

Design : RBD

Treatments: 7

Replication: 3

Plot size : 3X1.0m<sup>2</sup>

Spacing : 0.5X0.5 m<sup>2</sup>

Variety : Indra

Season : October to January (2016-17)

**Table 3.4 Treatment details of field experiments**

Treatment	Description	Concentration (%)		
T1	Soil solarisation+ soil application of <i>Trichoderma viride(asperellum)</i> (as per POP)	2%		
T2	T1+Seed treatment and foliar spray with <i>Pseudomonas fluorescens</i>	2%		
T3	T1+Seed treatment with carbendazim+ mancozeb +foliar spray with copper hydroxide	0.2%		
T4	T1+Seed treatment with carbendazim +mancozeb+ foliar spray with mancozeb	0.2%		
T5	T1+Foliar spray with tebuconazole	0.1%		
T6	T1+Foliar spray with difenoconazole	0.05%		
T7	Untreated Control			
<b>Fungicides used for the experiment</b>				
Sl. No	Chemical name	Trade name	Contact/systemic	Concentration (%)
1	Carbendazim (12%) + Mancozeb (63%)	Saaf 50WP	Systemic	0.2%
2	Copper hydroxide	Kocide	Contact	0.2%
3	Mancozeb	Indofil M 45	Contact	0.2%
4	Tebuconazole	Folicur 25EC	Systemic	0.1%
5	Difenoconazole	Score 25WP	systemic	0.05%
<b>Biocontrol agents used for the experiment</b>				
Description			Concentration (%)	
<i>Trichoderma viride(asperellum)</i> (KAU)			2%	
<i>Pseudomonas fluorescens</i>			2%	
Cowdung+Neemcake + <i>Trichoderma viride(asperellum)</i>			1kg/m <sup>2</sup>	

### **3.4.1 Preparation of nursery**

Nursery was raised during July –September 2016, in protrays using soil less medium, consisting of coir pith, perlite and vermiculate in 3:1:1 proportion by weight. Capsicum seeds treated as per technical program were sown one seed per cavity. Per cent seed germination was recorded.

### **3.4.2 Field preparation**

Field experiments were conducted in polyhouse and rain shelter simultaneously (Plate 13). Land inside the structure was ploughed and thoroughly prepared into beds of size 3.0x1.0 m<sup>2</sup>. In treatments T1 to T6 the beds were subjected to soil solarization for 90 days, then polythene sheets were removed, and seedlings were transplanted at a spacing of 0.5m x 0.5m and 3-4cm depth. Fertigation and irrigation were carried out using drip irrigation system.

#### **3.4.2.1 Soil solarization**

Beds of 3x1m<sup>2</sup> size and 10 cm height were taken, irrigated using rose can and the beds were perfectly leveled. Transparent polythene sheet of 150 gauge thickness was stretched and spread over the beds so that it was placed touching the surface and there were no air pockets in between. The sides of the polythene sheet were sealed by putting soil (Plate 14). Soil solarization was carried out during June-August for a period of 90 days in protected structures. Soil thermometers were installed at 10cm depth (Plate 14) in solarized and non-solarized beds in polyhouse and soil temperature was recorded at 7:30 am and 2:30 pm daily during the period of solarization.

### 3.4.2.2 Enumeration of soil microflora

Soil samples were collected from inside polyhouse and rain shelter. Then it was thoroughly mixed and using quadrant method, the quantity was reduced to 100g. It was then air dried and filtered using the sieve of mesh size 2mm. Enumeration of soil microflora *viz*, fungi, bacteria and actinomycetes was done using serial dilution and plating technique. The sample (1g) was added to 99ml sterile water, shaken for one minute and serially diluted upto  $10^{-6}$  dilution. For isolation of fungi, bacteria and actinomycetes  $10^{-3}$ ,  $10^{-6}$  &  $10^{-5}$  dilutions were plated on Martin's Rose Bengal Streptomycin Agar, Nutrient Agar, and Ken knight's Agar respectively. Microbial suspension (1ml) of the respective dilution was pippered into sterile Petri plate and 15ml of molten cooled medium was added. Three replications were kept for each sample. The plates were then incubated at room temperature, and fungal, bacterial and actinomycetes colonies were counted at 24<sup>th</sup>hrs, 48<sup>th</sup>hrs and on 7<sup>th</sup> day respectively. The population of microflora was expressed as number of cfu g<sup>-1</sup> of dry soil.

### 3.4.3 Management of fungal diseases of capsicum under protected cultivation

The effectiveness of selected plant protection chemicals, biocontrol agents as per Table. 3.4 was tested against fungal diseases of capsicum under protected condition. Application of treatments was given at the onset of the disease. Subsequent sprays were given at 15 days interval. Systemic fungicides were sprayed three times, and contact fungicides and biocontrol agents were applied two times. Disease severity of cercospora leaf spot was recorded using 0-5 scale (Table3.1) before spraying and 15 days after each spraying. From each plot, four plants were selected at randomly, and a total of five leaves from each plant were observed. Based on the per cent leaf area infected, disease severity was calculated as described in 3.1.

#### **3.4.3.1 Meteorological parameters**

Temperature and relative humidity inside the polyhouse and rain shelter were recorded at 7.30 am and 2.30pm daily during the experiment using temperature and moisture meter which are permanently installed in the structures. Correlation analysis was performed between major meteorological factors and disease severity using SPSS v16.0 data editor. Per cent disease severity in control, recorded at 15 days interval and temperature and relative humidity during the week prior to the date of observation of the disease were utilized for the analysis.

#### **3.4.3.2 Biometric observations**

Biometric observations such as plant height, days to flower, days to harvest, were recorded and tabulated to know the effect of treatment on these parameters (Plate 15 and 16)

#### **3.4.3.3 Economic analysis**

Total cost incurred and total returns per plot ( $3m^2$ ) were calculated separately for the treatments. The benefit: cost ratio was calculated at market price (Rs.50kg<sup>-1</sup>) of capsicum.

#### **3.4.3.4 Incidence of other diseases and pests**

Incidence of pests and other diseases of capsicum in polyhouse and rain shelter was observed (Plate 17)

### **3.5 Enumeration of phylloplane microflora of capsicum**

The methodology adopted by Elad and Kirshner (1993) was used for enumeration of phylloplane microflora. The phylloplane microflora (fungi, bacteria and actinomycetes) of the crop was estimated using serial dilution plating of leaf

washings to know the changes due to the treatments. Capsicum leaves were collected from all treatments in polyhouse before spray and at five days after each spray. Area of the leaf used for the study was measured by graph paper method. Then the leaf was cut into small pieces and added to 100ml sterile water, agitated well for one minute and 1ml of the leaf washing was plated on suitable media (Plate 18). *Viz* Martin's rose bengal streptomycin agar, nutrient agar and Kenkight's agar for fungi, bacteria and actinomycetes respectively. Population of the phylloplane micro flora was expressed as number of cfu cm<sup>-2</sup> area of leaf.

### **3.6 Survival of the *Pseudomonas fluorescens* on phylloplane of capsicum**

The population of biocontrol agent sprayed on leaves was estimated at periodical intervals of 5, 10, 15, days after spraying using serial dilution of leaf washings as in 3.7. Here, the medium used was King's B agar for isolation of *P. fluorescens*. Leaves were collected from field before and after treatment application at an interval of five days. For isolation of *P. fluorescens*, 10<sup>-6</sup> dilutions were used. The diluted leaf washings was plated and colonies of *P. fluorescens* were counted at 48h. of incubation. The Population was expressed as cfu cm<sup>-2</sup> area of leaf. (Plate 19).

### **3.7 Statistical analysis**

The data collected during experiments were analyzed using web agri-stat package (WASP) 2.0

# *Results*

## 4. RESULTS

The studies on "Management of fungal diseases of capsicum (*Capsicum annuum* L.) under protected cultivation" was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2015-2017. The study consisted of survey on incidence and severity of fungal diseases of capsicum in farmers' field and experiments under protected structures. Experiments to evaluate different treatments against fungal diseases of capsicum were conducted in poly house and rain shelter simultaneously. The survey was conducted during 2016-2017 by selecting poly houses where capsicum was being cultivated (Plate 1). During the survey, eight poly houses from three districts viz. Thrissur, Palakkad and Thiruvananthapuram were visited. The incidence and severity of fungal diseases of capsicum in protected structures were assessed using standard score charts (Tables 3.1 and 3.2) and procedures. The major meteorological factors such as temperature and relative humidity (RH) influencing the crop and the pathogens prevailing in the structures and open condition were also recorded using whirling psychrometer.

### 4.1 Assessment of incidence and severity of fungal diseases of capsicum under protected structures in farmers' field

Incidence of fungal diseases like powdery mildew, leaf spot, fruit rot and stem and fruit rot were noticed in the poly houses where capsicum was being cultivated. Per cent disease severity (PDS) for leaf spot varied from 2.97 to 5.36. Per cent disease severity for powdery mildew varied from 3.1 to 90.23. Incidence of leaf spot was found to be high during rainy season in Vellanikkara, Thannlyam and Manalur and the PDS were 3.8, 5.37 and 2.37 respectively. Incidence of powdery mildew was observed in all the poly houses except Vellanikkara. The disease severity was the highest in Plamootinkada (90.23) followed by Neyyatinkara (89.76) and Manalur (82.3). Incidence of Stem and fruit rot and anthracnose was observed only in



Vellanikkara and the per cent incidence was very less *i.e* 0.5 and 1 per cent respectively (Table 4.1).

Meteorological data was recorded in (Table 4.1.1) using whirling psychrometer and temperature and moisture meter. A wide variation was noticed in the major meteorological parameters among the polyhouses, which may be attributed mainly to the season, type of the structure, location *etc*. In general temperature and RH are higher inside the polyhouse compared to outside. There was no significant correlation between temperature and RH and the disease severity at the time of visit.

**Table 4.1 Incidence and severity of fungal diseases of capsicum in farmers' poly house**

Polyhouse		Disease				Meteorological parameters			
Location	Period 2016-2017	PDS		PDI		Inside polyhouse		Outside polyhouse	
		(leaf spot)	(Powdery mildew)	(Fruit rot)	(Stem and fruit rot)	Temp (°C)	RH (%)	Temp (°C)	RH (%)
Vellanikkara	July	5.36	-	1	0.5	30.50	79.0	29.8	78.0
Thannlyam	Aug	3.8	7.2	-	-	33.80	81.0	32.7	80.0
Elanad	Aug		5.3	-	-	31.66	78.5	30.5	76.6
Manalur	Aug	2.97	82.3	-	-	24.50	82.0	23.0	81.0
Chithali	Sept		3.1	-	-	36	89.0	35.5	81.0
Poranattukara	Sept		9.6	-	-	33.33	78.3	31.1	76.0
Neyyatinkara	Feb		89.76	-	-	31	85.0	29.5	86.0
Plamootinkada	Feb		90.23	-	-	32	90.0	30.5	89.0

PDS-Per cent disease severity

**Plate1 Field visit for assessment of incidence and severity of fungal diseases of capsicum**



**Field visit**



**Poly house**



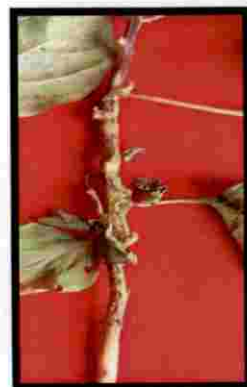
**Powdery mildew**



**Leaf spot**



**Fruit rot**



**Stem and fruit rot**

**Plate 2 Score chart for powdery mildew of capsicum**

**Abaxial**



0

1

3

5

7

9



**Adaxial**

**Plate 3 Score chart for leaf spot of capsicum**



0

1

2

3

4

## **4.2 Symptomatology of fungal diseases of capsicum**

Diseased specimens were brought to the laboratory and were observed under microscope by preparing slides from the infected tissues. Purified, cultures were also subjected examination under microscope to study the cultural and morphological characters of the fungal pathogens.

### **4.2.1 Symptomatology and characterization of powdery mildew of capsicum**

Initially, the symptoms of powdery mildew appeared as discolored spots on the upper surface of leaves, where later white-grey to purple powdery growth of the causal organism, was observed on the lower side of the leaf (Plate 4). Infected areas dried up and turned black. As infection became severe, the disease caused defoliation also (Plate 4). Symptoms were not observed on stem and fruits. Infection progressed from the older to younger leaves. The disease caused reduced growth of leaves and fruits led to low quality and quantity of the produce. At the later stage of infection, the leaves were completely covered with powdery growth of the pathogen. The powdery growth on the leaves was observed under the microscope, two type's conidia were observed. The primary conidia with tapering tips and of size of  $66 \times 8-12 \mu\text{m}$  (Plate 4) and the secondary conidia with rounded tips  $60-74 \times 14-2.9 \mu\text{m}$  (Plate 6). The conidia and conidiphores characters of the pathogen were similar to that of *Leveillula taurica*.

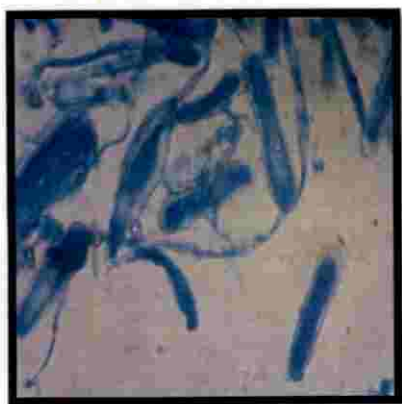
### **4.2.2 Symptomatology and characterization of leaf spot of capsicum**

The disease appeared as circular spots on leaves with a light grey center and a reddish-brown margin, the spots grew up to one cm in diameter. The disease was observed first on young leaves. Spots later became tan with a dark margin and a yellowish halo around the spots. Concentric zonations were observed on the lesions

**Plate 4 Symptomatology and characterization of powdery mildew of capsicum**



**Powdery growth on surface of the leaves**



**Primary conidia (400X)**



**Secondary conidia (400X)**



**Young germinating conidium of *Leveillula taurica* (400X)**

**Plate 5 Symptomatology and characterization of *Cercospora* leaf spot in capsicum**



**Cercospora leaf spot**



**Frog eye symptom**

**Cultural characterization of *Cercospora capsici***

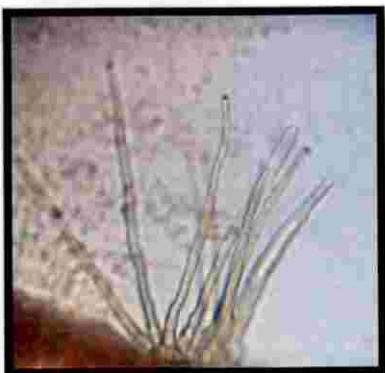


**Growth on PDA**

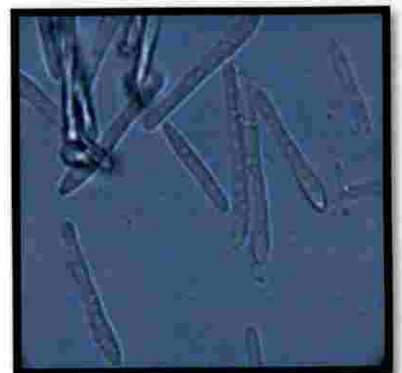


**Dark discoloration**

**Morphologic characterization of *Cercospora capsici***



**Conidiophores (400X)**



**Conidia (400X)**

(Plat 5) which had the typical “frog-eye” appearance. The center of lesions dried up and dropped out as the leaves got aged. Numerous spots were seen on the foliage and the leaves turned yellow and dropped off prematurely. Symptoms were not observed on the fruits. The fungus was isolated on PDA, which grew very slowly and the colony attained 4cm diameter only, at one and half months of growth. However sporulation was not observed in culture. Dark discoloration of the medium was observed when viewed from the bottom of the plate (Plate 5).The conidia and conidiophores present on the lesion on the infected leaves were subjected to observation under microscope. Conidiophores were erect, slightly divergent, sub cylindrical, unbranched, geniculate, pale brown, smooth and thick walled of the size 32-80X1-2 $\mu$ m (Plate 5).Conidia were hyaline, sub cylindrical, slightly curved of the size of 28-76X1-1.5 $\mu$ m(Plate 5).The cultural and morphological characters of the fungus were similar to that of *Cercospora capsici*.

#### **4.2.3 Symptomatology and characterization of fruit rot of capsicum**

Symptoms first appeared on mature fruits as small, water soaked, yellow or dark sunken lesions which rapidly expanded. The lesions enlarged up to 3to 4 cm in diameter and coalesced (Plate 6). Fully expanded lesions were dark red to light tan in color with varying amounts of visible dark acervuli bearing spore mass on the lesions. On PDA (Potato Dextrose Agar), the fungus grew as white mycelium later becoming grey-green with age. Conidia were aseptate, and hyaline, curved and tapered towards both ends with the size of 17-26X3-4 $\mu$ m (Plate 6). The cultural and morphological characters of fungus were similar to that of *Colletotrichum capsici*.

#### **4.2.4 Symptomatology and characterization of stem and fruit rot of capsicum**

The disease first appeared as slight vein clearing on the outer portion of the younger leaves, followed by epinasty (downward curling) and yellowing of the lower leaves. Formation of adventitious roots, wilting of leaves and young stems,

**Plate 6 Symptomatology and characterization of fruit rot of capsicum**



**Symptoms on fruit**



**Growth on PDA**



**Conidia(400X)**

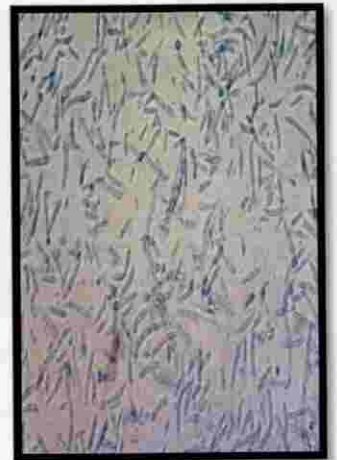
**Plate 7 Symptomatology and charcterisation of stem and fruit rot**



**Symptoms on stem**



**Growth on PDA**



**Micro and  
macro conidia (400X)**



defoliation, marginal necrosis of remaining leaves, were observed and finally entire plant wilted and dried up (plate 7). On isolation the fungus associated with the disease produced white mycelium on PDA, which later changed to violet to dark purple in color (Plate 7). When observed under the microscope two types of conidia were found, which were similar to the micro and macro conidia of *Fusarium* sp. (Plate 7). The microconidia are two celled with the size of 5-6.4X3-3.4 $\mu$ m and macroconidia are five celled of the size 15-28.5X3.2-5.4 $\mu$ m. The cultural and morphological characters of the fungus were similar to that of *Fusarium* sp.

#### **4.2.5 Pathogenicity test**

The Pathogenicity of fungi associated with fruit rot, leaf spot and stem and fruit rot were tested by artificial inoculation on healthy plants parts. Fungi associated with various symptoms observed during the survey, where isolated and inoculated on healthy plant parts *ie*, fruits and leaves and twigs (Plate 8). In the case of fruit rot symptoms were observed on fruit and leaves after five days of inoculation. The symptoms were similar to those observed in natural infection; however, the lesions were darker than those observed in field. Symptoms were not observed on leaves during the study. But on artificial inoculation, lesions were formed on leaves also. However the size of lesion was smaller compared to lesions on fruits (Plate 9). In case of stem and fruit rot, symptoms were produced after six days of inoculation on twigs and fruits. On twigs there was rotting and discoloration of the entire twig and on fruits, the symptoms began as dark decaying spots. Later it enlarged and formed rotten lesions, on which white mycelial growth of the fungus was observed. In the case of leaf spot, very small dark spots were produced after eight days of inoculation. However, typical symptoms as in natural infection were not noticed.

#### **4.2.6 Molecular characterization of pathogen**

Purified cultures of pathogens isolated from Cercospora leaf spot (CEC), fruit rot (COC) and stem and fruit rot (FUS) were sent to Rajiv Gandhi Centre for

**Plate 8 Pathogenicity test for fungal diseases of capsicum.**



**Stem**



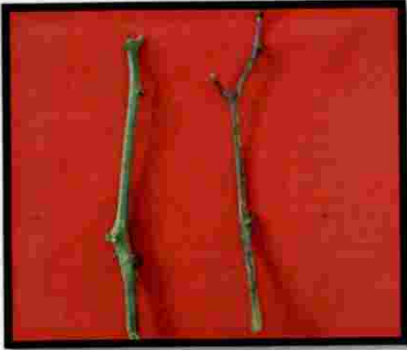
**Fruit**



**Leaves**

**Plate 9 Symptoms on artificial inoculation**

**A. Stem and fruit rot**



**Control**

**Inoculated**



**White mycelium on the stem**

**B. Fruit rot**



**Control**

**Inoculated**



**Small circular spot**

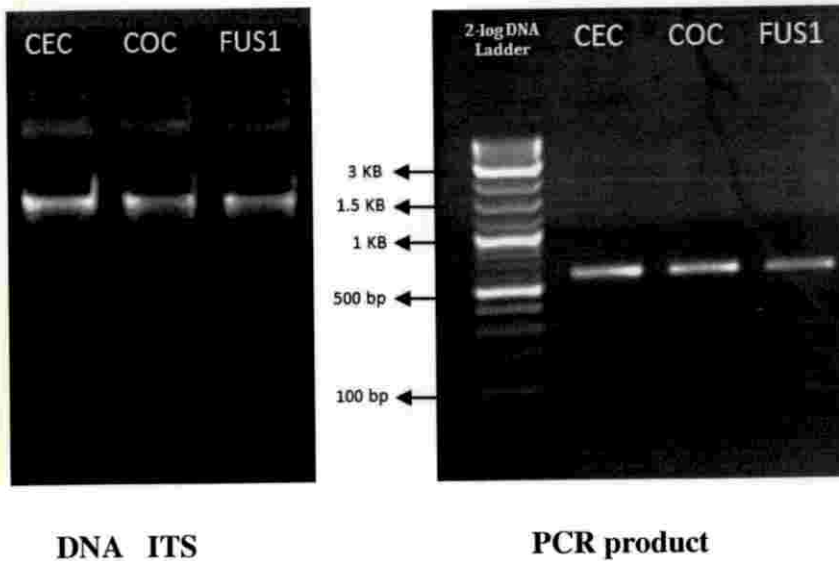
**C. Leaf spot**



**Inoculated**

**Control**

## Plate 10 Molecular characterisation of pathogens



## Plate 11 sequencing analysis

### III Sequences

```
>SR877-CEC-ITS
AGGGATCATTACTGAGTGAGGGCCCTTCGGGCTCGACCTCCAACCCTTTGTGAACACAACCTTGTGCTTCGGGGGCGACCC
TGCCGTTTTGACGGCGAGCGCCCGGAGGCCTTCAAACACTGCATCTTTGCGTCGGAGTTTAAAGTAAATTAAACAAAAC
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GTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTGGTATTCCGAGGGGCATGCCTGTTTCGAGCGTCATTTCAACC
TCAAGCCTCGCTTGGTATTGGGCGCCGCGGTGTTCCGCGCGCCTCAAAGTCTCCGGCTGAGCTGTCCGTCTCTAAGCGTT
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```

```
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AATGCGATAAGTAAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGG
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AGGTAGTGGCGGACCCCTCTCGGAGCCTCTTTCGCTAGTAACTTTGCTCTCGCATTTGGGATTCGGAGGGACTCTAGCCG
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```

```
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TAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACCTCTGAGTAAAACCATAAATAAATCAAA
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAATGCGATAAGTAAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAAC
CCTCAAGCCCCGGGTTTGGTGTGGGGATCGGCGAGCCCTTGCGGCAAGCCGGCCCGAAATCTAGTGGCGGCTCTCGCT
GCAGCTTCCATTGCGTAGTAGTAAACCCCTCGCAACTGGTACGCGGCGCGGCCAAGCCGTTAAACCCCAACTTCTGAAT
GTTGACCTCGGATCAGGTAGGAATACCCGCTGAACCTTAA
```

CEC-*Cercospora capsici*

COC-*Colletotrichum capsici*

FUS-*Fusarium* sp.

Biotechnology Thiruvananthapuram, for molecular characterization (Plate10). The ITS sequences (Plate11) obtained were blasted in NCBI web site, similarity of sequences was studied. Based on sequence similarity the identity of the pathogens causing leaf spot, fruit rot and stem and fruit rot was confirmed as *Cercospora capsici*, *Colletotrichum capsici*, and *Fusarium* sp. respectively.

#### 4.3 *In vitro* evaluation of fungicides and biocontrol agents on powdery mildew of capsicum

Prior to the incidence of diseases, the fungicides and biocontrol agents were evaluated *in vitro* to study their efficiency against powdery mildew of capsicum. Data presented in Table-4.2 and Fig. 4.1 indicates that T5-tebuconazole (0.1per cent) is the most effective fungicide to control powdery mildew followed by T6-difenoconazole (0.05per cent) and *Pseudomonas fluorescens* also recorded better results comparative to other treatments (Plate 12).

**Table 4.2 *In vitro* evaluation of bio control agents and fungicides on powdery mildew of capsicum**

Treatments	Per cent leaf area infected (LAI)		
	2DAS*	4DAS	6DAS
T1- <i>Trichoderma viride</i> ( <i>asperellum</i> ) (2 per cent)	10.2 <sup>c</sup>	11.1 <sup>c</sup>	11.9 <sup>ab</sup>
T2- <i>Pseudomonas fluorescens</i> (2 per cent)	4.7 <sup>b</sup>	5.8 <sup>b</sup>	8.7 <sup>b</sup>
T3-Copper hydroxide(0.2per cent)	9.08 <sup>c</sup>	10.2 <sup>c</sup>	13.2 <sup>c</sup>
T4-Mancozeb(0.2 per cent)	11.3 <sup>c</sup>	12.5 <sup>c</sup>	13.6 <sup>c</sup>
T5-Tebuconazole(0.1 per cent)	0 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>
T6-Difenconazole(0.05 per cent)	1.5 <sup>a</sup>	1.74 <sup>ab</sup>	2.17 <sup>a</sup>
T7-Untreated control	11.2 <sup>c</sup>	13 <sup>c</sup>	14.3 <sup>c</sup>
CV	26.03	33.98	24.12
CD	3.13	4.669	3.867

DAS Days after spraying

61

**Plate 12-*In vitro* evaluation of treatments against powdery mildew**



**Hand spraying**



**Placing infected leaf on the treated leaf**



**Incubation**



**Control**

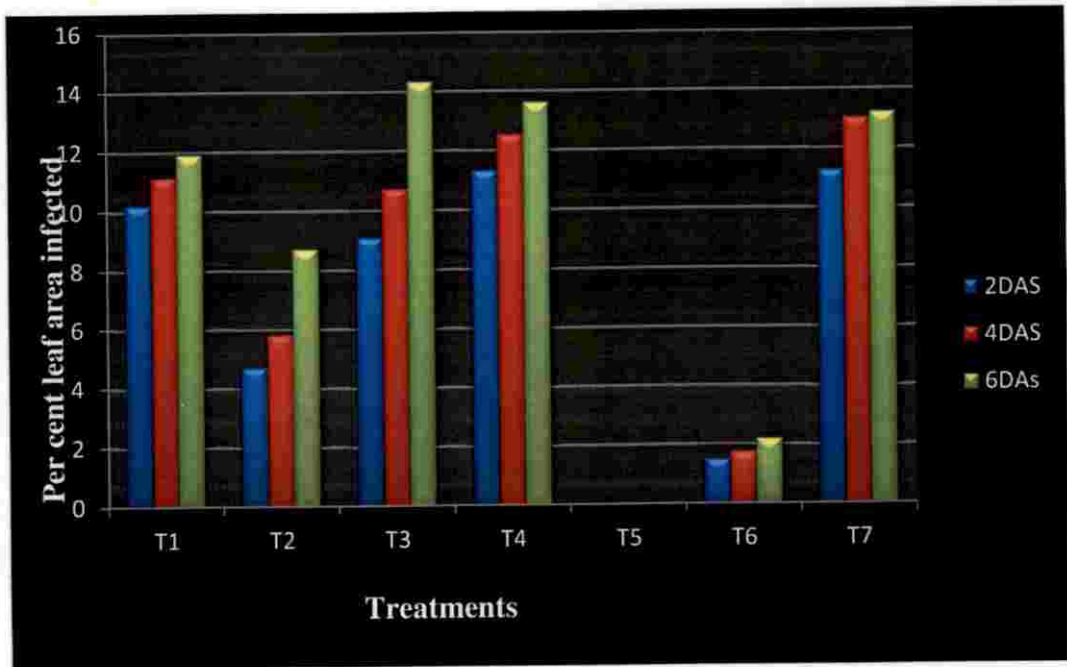
**Treated**



**Control**

**Treated**

**Fig. 4.1.** *In vitro*-evaluation of treatments against powdery mildew of capsicum



T1-*Trichoderma viride* (*asperellum*) (2%)

T2- *P. fluorescens* (2%)

T3-Copper hydroxide (0.2%)

T4-Mancozeb (0.2%)

T5-Tebuconazole (0.1%)

T6-Difenoconazole (0.05%)

T7-Control

#### 4.4 Management of fungal diseases of capsicum under protected condition

Field experiments were conducted simultaneously in polyhouse (300m<sup>2</sup>) and rain shelter (200m<sup>2</sup>) during June -February of 2016-2017 to evaluate different treatments for management of fungal diseases of capsicum (Plate13).

##### 4.4.1 Soil solarisation

In all treatments except T7 (control) soil solarization was carried out for period of 90days before transplanting. Soil thermometers were installed at 10cm depth in solarized and non-solarized beds in polyhouse (Plate 14) and soil temperature was recorded at 7:30 am and 2:30 pm daily during the period of solarization in Table 4.3.

**Table 4.3 Soil temperature of solarized and non-solarized soil in polyhouse and rain shelter**

Std week	Soil temperature at 10cm depth							
	Polyhouse				Rain shelter			
	7:30am		2:30pm		7:30am		2:30pm	
	S	NS	S	NS	S	NS	S	NS
21	31.21	29.85	36.07	34.24	31.50	29.50	34.00	31.75
22	31.57	30.21	35.10	33.95	29.50	27.50	33.00	32.00
23	31.28	30.14	35.57	33.71	31.00	28.00	36.50	32.00
24	30.85	30.14	35.00	33.35	32.00	28.00	37.00	34.50
25	30.71	30.50	34.78	33.55	32.50	29.00	36.50	34.00
26	30.04	28.18	34.78	29.18	31.00	30.00	35.50	34.50
27	30.05	29.57	35.92	29.57	32.00	30.50	35.50	33.00
28	29.16	28.50	35.07	35.33	31.00	29.00	34.50	34.00
29	29.21	28.07	33.85	31.64	32.00	31.00	36.00	34.00
30	31.14	29.42	35.21	33.92	32.50	30.00	34.00	32.00
31	30.31	29.12	35.62	33.50	30.00	28.50	35.50	31.00
32	30.71	29.85	35.83	35.00	32.50	31.00	37.00	33.50
33	28.64	28.35	34.28	31.83	30.00	28.50	35.50	32.00

\*average temperature of seven days      S-Solarized soil      NS-non solarized soil



**Plate 13 Protected structures for field experiments**



**Poly house**



**Rain shelter**

**Plate 14 Soil solarization and installation of soil thermometers in protected structures**



**Poly house**



**Rain shelter**

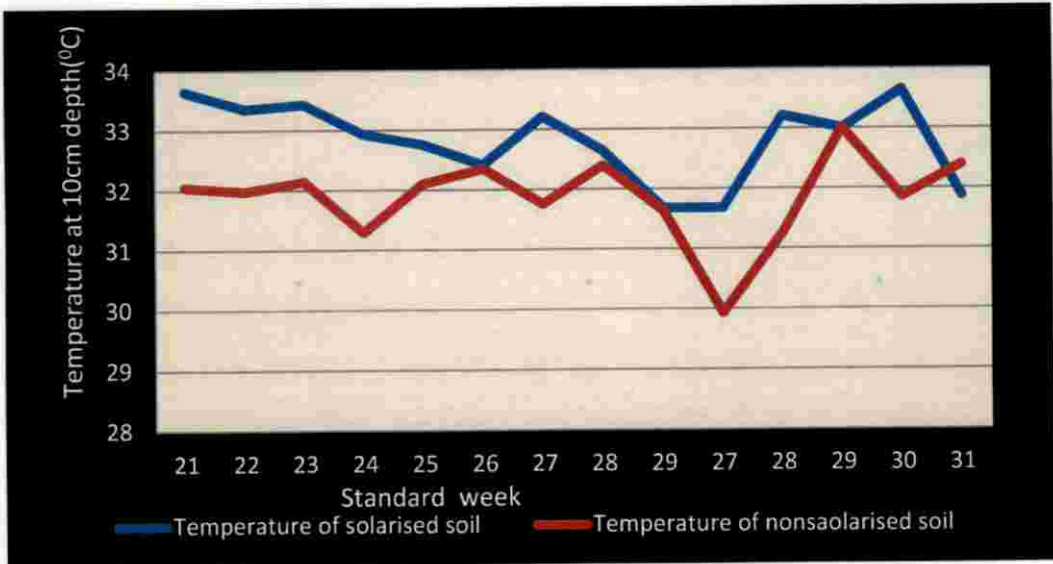


**Solarized plot**

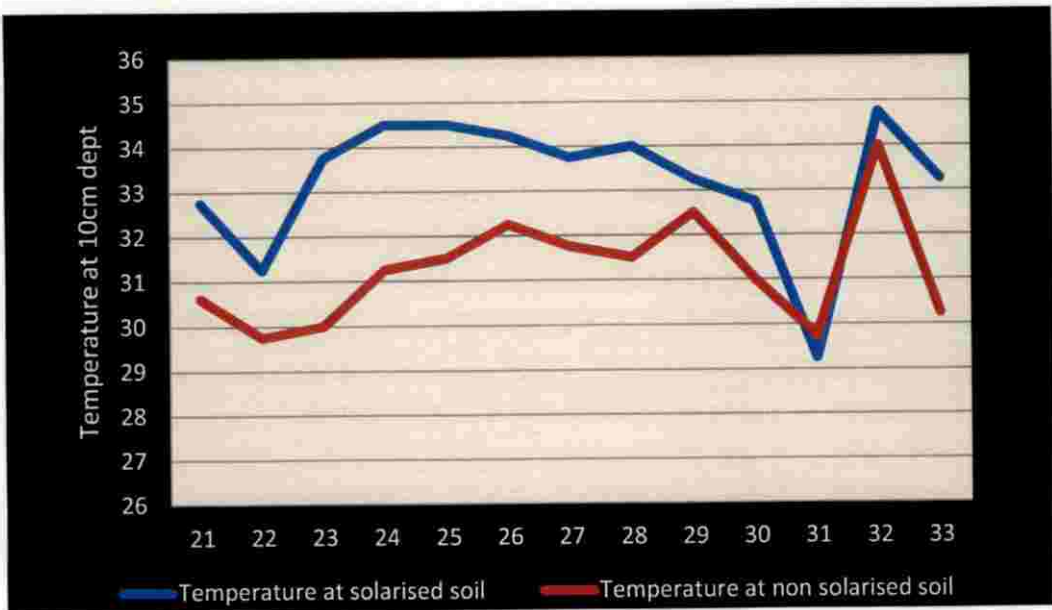


**non solarized plot**

**Fig. 4.2. Soil temperature in solarized and non-solarized soil in polyhouse**



**Fig. 4.3. Soil temperature in solarized and non-solarized soil in rain shelter**



Data on soil temperature of both solarized and non-solarized beds during the period of solarization are presented in Table-4.3. In general, soil temperature was found to be high in polyhouse compared to rain shelter, and in both the temperature in solarized beds was higher than non-solarized beds. In polyhouse there is an increase in soil temperature up to 6.35°C in solarized beds over non-solarized beds at 2:30pm, at which, temperature ranged from 33.85-36.07°C in solarized beds, whereas in non-solarized beds from 29.18-35.33°C (Fig.4.2). At 7:30am, soil temperature in solarized beds inside the polyhouse varied from 28.64-31.57°C, whereas in non-solarized bed it ranged from 28.18-29.85°C (Table 4.3).

In rain shelter, there was an increase of soil temperature up to 4.5°C in solarized beds over non-solarized beds at 2:30pm. During this period temperature varied from 33-35.5°C in solarized beds, whereas in non-solarized beds from 31-34.5°C (Fig.4.3). At 7:30am, soil temperature in rain shelter varied from 29.5-32.5°C, whereas in non-solarized beds from 31-34.5°C.

#### **4.4.2 Enumeration of soil microflora in polyhouse and rain shelter**

The population of fungi, bacteria and actinomycetes in solarized and non-solarized soil was estimated in polyhouse and rain shelter. Results are presented in (Table 4.4). During the period of solarisation, there was reduction in the population of soil microflora such as fungi, bacteria and actinomycetes in all the plots, in poly house. But in rain shelter the population of fungi and actinomycetes were less and that of bacteria was more in solarized plots compared to non-solarized plots.

**Table.4.4 Population of soil microflora in solarized and non-solarized soil in polyhouse and rain shelter**

Soil microflora	Polyhouse		Rain shelter	
	Solarized plot	Non-solarized plot	Solarized plot	Non solarized plot
Fungi ( $\times 10^3 \text{cfu g}^{-1}$ )	13.3*	13.66	3.33	4.66
Bacteria ( $\times 10^6 \text{cfu g}^{-1}$ )	0.66	11.33	6.00	2.66
Actinomycetes( $\times 10^5 \text{cfu g}^{-1}$ )	1.66	1.33	2.66	3.00

cfu - colony forming units \* Mean of three replication

#### 4.4.3 Analysis of soil nutrients

Soil samples collected from polyhouse and rain shelter (Table 4.5) were analyzed for soil reaction, electrical conductivity and major and minor nutrients.

**Table 4.5 Soil nutrient status inside polyhouse and rain shelter**

Parameter tested	Poly house solarized soil	Poly house non-solarized soil	Rain shelter solarized soil	Rain shelter non-solarized soil
pH	6.3	6.2	6.4	6.1
Electric conductivity (dSm <sup>-1</sup> )	0.014	0.042	0.057	0.031
Total nitrogen (%)	0.14	0.23	0.13	0.16
Total phosphorus (%)	0.17	0.14	0.20	0.13
Total potassium (%)	0.13	0.11	0.130	0.09
Calcium (ppm)	0.56	0.46	0.76	0.37
Magnesium(ppm)	0.36	0.46	0.48	0.39
Iron(ppm)	5.16	5.01	5.37	4.69
Manganese (ppm)	796.0	824.0	861.0	725
Zinc(mg/kg)	155.2	138.0	203.6	103.6
Copper(ppm)	55.0	53.0	65.8	52.4

In poly house as well as in rain shelter, all soil nutrients were higher in solarized soil except for total nitrogen, iron, zinc and manganese. It was found that soil reaction and EC are slightly higher in solarized soil compared to non in both the structure.

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#### **4.5 Management of fungal diseases of capsicum under polyhouse and rain shelter**

The effectiveness of plant protection chemicals, and biocontrol agents were tested against the fungal diseases of capsicum under polyhouse and rain shelter. The crop was raised during August to February 2016-17. Foliar application of treatments was given at the onset of *Cercospora* leaf spot. Subsequent sprays were given at 15days interval. Disease severity was recorded leaf spot using 0-5 scale (Table 3.1) for *Cercospora* leaf spot and 0-9 scale for powdery mildew (Table3.2) before spraying and 15days after each spraying.

##### **4.5.1 Effect of treatments on seed germination of capsicum**

Effect of seed treatment on seed germination was studied and it was found that, treatment with *Pseudomonas fluorescens*, gave 100 per cent germination whereas, the per cent germination was only 92 in seed treated with carbendazim+ mancozeb and 80 in control (Table 4.12 and 4.13)

##### **4.5.2 Effect of treatments on management of *Cercospora* leaf spot and yield of capsicum in polyhouse**

Results of the field experiment for management of fungal diseases of capsicum in poly house are presented in Table 4.6. Incidence of *Cercospora* leaf spot was noticed 77 days after planting in poly house. Before spray there was no significant difference in disease severity among the treatments. There was a gradual increase in the disease until the second foliar treatment, however, subsequent to the second foliar application, the disease severity was reduced. All the treatments were found to be superior to control in reducing rate of increase of the disease compared to control. Lowest disease severity was recorded in T4 (mancozeb) at all the stages of observation and per cent reduction over control was 52.51 per cent after the second spray followed by T5 (tebuconazole) which showed 36.83 per cent reduction in

disease severity after last spray. However, treatments T4 and T5 were on par after each spraying.

Among biocontrol agents, soil solarization with *Trichoderma viride* (*asperellum*) along with seed treatment and foliar spray with *Pseudomonas fluorescens* (T2), gave the best result in the reduction of *Cercospora* leaf spot which accounted for 40.51 per cent over control. T2 is followed by T1 (soil solarization with soil application of *Trichoderma viride* (*asperellum*)) where the reduction was 39.98 per cent. Treatments T1 and T2 were found to be on par with chemical treatments T4 and T5, after second treatment.

Effect of treatments was reflected on yield of capsicum also and all the treatments recorded higher yield than control. Even though the lowest PDS was recorded in T4 (mancozeb), mean yield was found to be more in treatment T5 and T6 with an increase of 70.31 per cent and 58.96 per cent respectively over control. However, mean yield was found to be on par with the treatment T4.

#### **4.5.3 Effect of treatments on management of *Cercospora* leaf spot and yield of capsicum in rain shelter**

Results of the field experiment for management of fungal diseases of capsicum in rain shelter are presented in Table 4.7. Incidence of *Cercospora* leaf spot was noticed 82 days after planting in rain shelter. Before spray, there was no significant difference in disease severity among the treatments. There was a gradual increase of the disease till the second foliar spray however, all the treatments at all the stages of observation were found to be superior to control in reducing rate of increase of the disease. Lowest disease severity was recorded in T4 (mancozeb) at all the stages of observation and the per cent reduction over control was 43.20 after second spray. Treatment T4 was followed by T5 (tebuconazole) which showed 22.65 per cent reduction in disease severity after the last spray. However, treatment T4 and T5 were on par after each spraying.

Among the biocontrol agents seed treatment with *Trichoderma viride* (*asperellum*) along with foliar spray with *Pseudomonas fluorescens* (T2) gave the best result in reducing *Cercospora* leaf spot, which accounted for 30.06per cent reduction over control. T2 is followed by T1 (soil solarization and soil application of *Trichoderma viride* (*asperellum*)) where the reduction was 29.25per cent. Treatment T1 and T2 were found to be on par with chemical treatment T4 and T5



**Table 4.6 Effect of treatments on management of *Cercospora* leaf spot and yield of capsicum in polyhouse**

Treatment	Per cent disease severity (PDS)						Mean yield(kg plot <sup>-1</sup> )	Percent increase over control
	Before treatment	After first treatment	Per cent reduction over control	After second treatment	Per cent reduction on over control	Mean yield(kg plot <sup>-1</sup> )		
T1-S+SA of <i>Trichoderma viride</i> ( <i>asperellum</i> ) (as per POP)	2.93*	10.66 <sup>cd</sup>	14.23	8.00 <sup>ab</sup>	39.98	26.8 <sup>ef</sup>	8.67	
T2-T1+ST and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	0.96	7.86 <sup>ab</sup>	36.76	7.93 <sup>ab</sup>	40.51	32.13 <sup>cd</sup>	30.29	
T3-T1+ST with carbendazim 12%+mancozeb 63% (@2g.kg <sup>-1</sup> ) +foliar spray with copper hydroxide (0.2%)	2.00	10.00 <sup>bc</sup>	19.54	12.73 <sup>c</sup>	4.5	29.73 <sup>de</sup>	20.55	
T4-T1+STwith Carbendazim12%+mancozeb63% (@2g.kg <sup>-1</sup> ) + foliar spray with mancozeb (0.2%)	1.46	7.66 <sup>a</sup>	38.37	6.33 <sup>a</sup>	52.51	35.96 <sup>bc</sup>	45.82	
T5-T1+Foliar spray with tebuconazole (0.1%)	1.33	7.68 <sup>ab</sup>	38.21	8.42 <sup>ab</sup>	36.83	42.0 <sup>a</sup>	70.31	
T6-T1+Foliar spray with difenconazole	1.33	10.66 <sup>cb</sup>	14.23	9.46 <sup>b</sup>	29.0	39.2 <sup>ab</sup>	58.96	
T7-Untreated control	1.50	12.43 <sup>d</sup>		13.33 <sup>c</sup>		24.66 <sup>f</sup>		
CV	58.9	13.68		17.75		8.28		
CD	NS	2.33		2.98		4.83		

\*Mean of three replications, S-Soil Solarization, SA- Soil Application, ST - Seed Treatment.

**Table 4.7 Effect of treatments on management of *Cercospora* leaf spot and yield of capsicum in rain shelter**

Treatments	Per cent disease severity (PDS)				After second treatment	Per cent reduction over control	Mean yield(kg plot <sup>-1</sup> )	Percent increase over control
	Before treatment	After first treatment	Per cent reduction over control	Per cent reduction over control				
T1-S+SA of <i>Trichoderma viride</i> ( <i>asperellum</i> ) (as per POP)	5.46	15.33 <sup>b</sup>	2.66	11.46 <sup>ab</sup>	29.25	24.66 <sup>c</sup>	10.78	
T2-T1+ST and foliar spray with <i>Pseudomonas fluorescens</i> (20%)	3.73	8.86 <sup>a</sup>	43.74	11.33 <sup>ab</sup>	30.06	32.57 <sup>ab</sup>	46.31	
T3-T1+ST with carbendazim 12% + mancozeb 63% (@2g.kg <sup>-1</sup> ) + foliar spray with copper hydroxide (0.2%)	7.53	14.80 <sup>b</sup>	6.03	15.00 <sup>bc</sup>	7.40	31.20 <sup>b</sup>	40.16	
T4-T1+ST with carbendazim 12%+ mancozeb 63% (@2g.kg <sup>-1</sup> ) + foliar spray with mancozeb (0.2%)	4.60	8.96 <sup>a</sup>	43.11	9.20 <sup>a</sup>	43.20	34.10 <sup>ab</sup>	53.18	
T5-T1+Foliar spray with tebuconazole (0.1%)	3.60	9.00 <sup>a</sup>	42.85	12.53 <sup>abc</sup>	22.65	42.00 <sup>a</sup>	63.07	
T6-T1+Foliar spray with difenconazole (0.05%)	4.13	11.40 <sup>ab</sup>	27.61	13.80 <sup>bc</sup>	14.81	34.60 <sup>ab</sup>	55.43	
T7-Untreated control	7.60	15.75 <sup>b</sup>		16.20 <sup>d</sup>		22.26 <sup>c</sup>		
CV	35.76	4.50		17.52		8.69		
CD	NS	21.07		3.98		4.76		

Mean of three replications, S- Soil Solarization, SA- Soil Application ST-Seed Treatment

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#### 4.5.4 Effect of treatments on management of powdery mildew and yield of capsicum in poly house

Data presented in Table 4.8 depict the efficacy of various treatments against powdery mildew of capsicum in polyhouse. Incidence of powdery mildew was noticed 180 days after transplanting in poly house. But the first spray was given on 78<sup>th</sup> day after transplanting, subsequent to the incidence of *Cercospora* leaf spot. Hence all the treatments were applied prophylactic in the case of powdery mildew. Initially the per cent disease severity was zero in T5 followed by T4 (0.53per cent), and T6 (5.05 per cent), whereas T4, T5 and T6 are on par. Per cent reduction over control was 100 per cent in T5. After the 2<sup>nd</sup> spray per cent disease severity was again zero in T5 followed T4 (8.63per cent). Per cent reduction over control was 100 per cent in T5 and 82.11 per cent in T4. After 3<sup>rd</sup> spray also per cent disease severity in T5 and T6 was zero and 14.25per cent respectively. Per cent reduction over control was 100per cent in T5, and 83.23 per cent in T6, which showed that systemic fungicides, tebuconazole and difenconazole are highly effective for control of powdery mildew of capsicum.

Among biocontrol agents, seed treatment with *Trichoderma viride* (*asperellum*), along with foliar spray with *Pseudomonas fluorescens* (T2) gave the best result in the reduction of powdery mildew, which accounted for 68.90 per cent over control. Treatment T2 was found to be on par with the chemical treatment T6 also.

Effect of treatments was reflected on yield of capsicum also and all the treatments recorded higher yield than control. Mean yield was found to be the highest in the treatment T5 followed by T6 with per cent increase of 70.31 and 58.96 per cent respectively over control.

#### 4.5.5 Effect of treatments on management of powdery mildew and yield of capsicum in rain shelter

Data presented in Table 4.9 shows the efficacy of various treatments against powdery mildew of capsicum in rain shelter. Incidence of powdery mildew was noticed 116 days after transplanting in rain shelter. But the first spray was given on 84<sup>th</sup> day after transplanting, subsequent to the incidence of *Cercospora* leaf spot. Hence all the treatments were applied prophylactic in the case of powdery mildew. After initial spray, disease severity was the lowest in T5 (0.36 per cent) followed by T6 (0.55 per cent) and they were on par. The per cent reduction over control was 98.2 and 97.2 per cent in T5 and T6 respectively. After the 2<sup>nd</sup> spray per cent disease severity was 5.03 and 17.73 per cent in T5 and T6 respectively. Per cent reduction over control was 94.2 per cent in T5 and 79.5 per cent in T6. However after the 3<sup>rd</sup> spray the per cent disease severity in T5 was 6.47 per cent, where as in T6 was 8.24 per cent. Per cent reduction over control was 78.44 in T5 and 72.55 in T6. It showed that systemic fungicides, tebuconazole and difenconazole are effective for control of powdery mildew in rain shelter

Among biocontrol agents, seed treatment with *Trichoderma viride*(asperellum), along with foliar spray with *Pseudomonas fluorescens* (T2) gave the best result in the reduction of powdery mildew, which accounted for 57.46 per cent reduction over control followed by T1 (Soil solarization and soil application of *Trichoderma viride*(asperellum)) where reduction was 57.06 per cent over control. Treatment T1 and T2 found to be on par with chemical treatment T5 and T6 also.

The effect of the treatments was reflected on yield of capsicum also and all the treatments recorded higher yield than control, Mean yield was found to be the higher in treatment T5 followed by T6 with per cent increase of 63.07 and 55.43 per cent respectively over control.

#### **4.5.6 Meteorological parameters**

Temperature and relative humidity inside the poly house and rain shelter was recorded at 7:30 am and 2:30 pm daily during experiment (Table 4.10). Temperature was found to be higher in polyhouse compared to rain shelter. In poly house the temperature varied from 23.07-32.42°C at 7:30am and 32.22-39.18°C at 2:30pm, relative humidity inside the polyhouse varied from 46.61-72 per cent at 7:30am and 55.42-95.56per cent at 2:30pm. In rain shelter, temperature varied from 21.02-32.9°C at 7:30am and 29.04-36°C at 2:30pm, and the relative humidity varied from 46.8-91.9 per cent at 7:30am and 63.14 to 94 per cent at 2:30pm

**Table 4.8 Effect of treatments on management of powdery mildew and yield of capsicum under polyhouse**

Treatment	Per cent disease severity(PDS)						Mean yield(kg plot <sup>-1</sup> )	Percent increase over control
	Initial treatment	Per cent reduction over control	After second treatment	Percent reduction over control	After third treatment	Per cent reduction over control		
T1-S+SA of <i>Trichoderma viride</i> ( <i>asperellum</i> ) (as per POP)	8.26 <sup>ab</sup>	44.19	24.80 <sup>d</sup>	48.31	83.83 <sup>d</sup>	13.76	26.8 <sup>ef</sup>	8.67
T2-T1+ST and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	4.13 <sup>ab</sup>	72.09	14.06 <sup>b</sup>	70.86	26.43 <sup>b</sup>	68.90	32.13 <sup>cd</sup>	30.29
T3-T1+ST with carbendazim12% +mancozeb 63% (@2g.kg <sup>-1</sup> ) +foliar spray with copper hydroxide (0.2%)	12.54 <sup>b</sup>	15.27	22.54 <sup>cd</sup>	53.29	79.16 <sup>d</sup>	6.87	29.73 <sup>de</sup>	20.55
T4-T1+STwith carbendazim12%+mancozeb 63% (@2g.kg <sup>-1</sup> ) + foliar spray with mancozeb (0.2%)	0.53 <sup>a</sup>	96.41	8.63 <sup>b</sup>	82.11	52.26 <sup>c</sup>	38.51	35.96 <sup>bc</sup>	45.82
T5-T1+Foliar spray with tebuconazole. (0.1%)	0.00 <sup>a</sup>	100	0.00 <sup>a</sup>	100	0.00 <sup>a</sup>	100	42.00 <sup>a</sup>	70.31
T6-T1+Foliar spray with difenconazole	5.05 <sup>ab</sup>	65.87	15.05 <sup>bc</sup>	68.8	14.25 <sup>ab</sup>	83.23	39.2 <sup>ab</sup>	58.96
T7-Untreated control	14.80 <sup>c</sup>		48.26 <sup>e</sup>		85.00 <sup>d</sup>		24.66 <sup>f</sup>	
CV	66.31		22.51		16.77		8.28	
CD	7.77		7.63		14.53		4.83	

\*Mean of three replications, S-Soil Solarization, SA- Soil Application, ST -Seed Treatment

**Table 4.9 Effect of treatments on management of powdery mildew and yield of capsicum under rain shelter**

Treatment	Per cent disease severity							Per cent reduction over control	After third treatment	Per cent reduction over control	Mean yield(kg plot <sup>-1</sup> )	Percent increase over control
	Initial treatment	Per cent reduction over control	After second treatment	Percent reduction over control	After third treatment	Per cent reduction over control	After third treatment					
T1-S+SA of <i>Trichoderma viride</i> ( <i>asperellum</i> ) (as per POP)	3.39 <sup>ab</sup>	83.06	63.16 <sup>cd</sup>	27.23	12.77 <sup>abc</sup>	57.06	24.66 <sup>c</sup>	10.78				
T2-T1+ST and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	3.88 <sup>ab</sup>	80.61	24.71 <sup>b</sup>	71.53	12.89 <sup>abc</sup>	57.46	32.57 <sup>ab</sup>	46.31				
T3-T1+ST with carbendazim 12%+mancozeb %63 (@2g.kg <sup>-1</sup> ) +foliar spray with copper hydroxide (0.2%)	9.77 <sup>b</sup>	51.19	76.23 <sup>cd</sup>	12.17	19.77 <sup>bc</sup>	34.14	31.20 <sup>b</sup>	40.16				
T4-T1+STwith carbendazim 12%+mancozeb 63% + (@2g.kg <sup>-1</sup> ) + foliar spray with mancozeb (0.2%)	5.22 <sup>ab</sup>	73.92	58.77 <sup>c</sup>	32.29	15.22 <sup>bc</sup>	49.00	34.1 <sup>ab</sup>	53.18				
T5-T1+Foliar spray with tebuconazole. (0.1%)	0.36 <sup>a</sup>	98.20	5.03 <sup>a</sup>	94.20	6.47 <sup>a</sup>	78.44	36.3 <sup>ab</sup>	63.07				
T6-T1+Foliar spray with difenconazole	0.55 <sup>a</sup>	97.2	17.73 <sup>b</sup>	79.57	8.24 <sup>ab</sup>	72.55	34.6 <sup>ab</sup>	55.43				
T7-Untreated control	20.02 <sup>c</sup>		86.80 <sup>c</sup>		30.02 <sup>d</sup>		22.26 <sup>c</sup>					
CV	67.49		15.88		31.58		8.69					
CD	7.41		13.43		8.46		4.76					

Mean of three replications, S-Soil Solarization, SA- Soil Application, ST - Seed Treatment



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Table 4.10 Meteorological data during the period of experiments in poly house and rain shelter

Std week*	Meteorological parameter							
	Polyhouse			Rains helter				
	7:30am		2:30pm	7:30am		2:30pm		
Temp(°C)	RH (%)	Temp(°C)	RH (%)	Temp(°C)	RH (%)	Temp (°C)	RH (%)	
23	29.2	70.5	35.97	78.54	28.35	91.4	35.48	91.7
24	29.91	71.25	36.9	78.58	28.47	91.9	36	92.57
25	30.04	71.48	36.32	79.37	29.7	91.5	34.8	80.48
26	30.5	70.94	36.16	78.61	29.14	89.5	36.21	92.54
27	32.42	71.5	35.24	78.54	32.9	91.4	34.3	92.54
28	31.14	71.52	34.54	78.5	33	90.07	35	91.64
29	31.78	70.62	35.78	78.85	32.68	91	34.91	94.1
30	32.71	74.4	34.78	77.5	29.5	90.8	33.92	94
31	30.5	76	36.25	74.4	27.5	88.5	31.75	90.5
32	31.28	75.57	35.07	80	30.07	79.55	34.88	81.57
34	30.92	77	35	81.21	30.01	78.3	35.85	79.85
34	31.78	71.85	34.42	75.14	30.85	77.15	33.64	77
35	31.2	75.4	35.5	80.2	29.7	81.22	35.68	79.8
36	28.98	75	33.95	84.42	27.6	78.51	33.64	77
37	23.3	67.42	30.1	94	21.02	66.83	30	92.42
38	23.4	65.14	30.5	95.42	21.92	63	30.05	93.85
39	23.4	66.57	30.6	95.14	21.3	64.8	29.51	94.5
40	23.8	67.57	30.04	95.85	21.12	66.66	29.04	94.42
41	23.65	64.28	33.05	93.92	22.45	67.0	32	91
42	23.58	72	34	95	23.4	67.8	31.24	93.14
43	26	66	35.33	95.66	22.2	69.8	34.45	92.5
44	23.92	66.14	34.78	95.42	22.3	64.8	31.57	94.57
45	25.57	65.2	34.5	92.14	22.2	69.8	32.35	80.28
46	25.14	68.85	34.02	84.71	21.9	68.6	32.35	80.28
47	23.91	63.57	33.57	71.85	21.7	53	32.65	63.14



48	26.28	66.42	34.72	72.42	22.01	54.16	32.65	63.14
49	27.35	67.85	35.78	74.57	22.01	56.33	33.91	70.14
50	23.2	62	32.64	81.85	21.8	61.66	31.64	82.14
51	23.68	62.3	32.22	85.35	21.48	54.16	31.8	82.85
52	23.28	46.61	33.57	55.42	23.42	47	34.78	63.71
1	23.07	48	34.14	58.14	22.85	47.16	34.14	64.14
2	25.54	49.9	36.61	65	23.54	46.8	35.75	76.57
3	25.57	58.85	37.28	66	22.04	52.83	35.6	83.21
4	26.71	64	39.11	79.35	24.78	58.91	35.6	83.14
5	28.91	66	36.07	83.5	27.24	63.25	35.57	82.21
6	29.3	67	37.8	84	29.3	68	36.7	85
7	28.78	68	37.34	85	27.9	67	37.5	86

\* weekly mean

**Table 4.5.7 Correlation analyses of severity of fungal diseases of capsicum with major meteorological parameters**

Correlation analysis was performed utilizing the data collected during the field experiments (Table 4.11). It was found that there is significant positive correlation between severity of *Cercospora* leaf spot and temperature in poly house (Fig.4.4).Where as in rain shelter, the disease severity was showing significant positive correlation with RH (Fig.4.5).

However in the case of powdery mildew it was found that there is significant positive correlation between disease severity and temperature in poly house (Fig.4.6). Severity of powdery mildew was not significantly correlated with RH in poly house, and also with temperature in rain shelter (Fig.4.7).

**4.11 Correlation analysis of severity of fungal diseases with major meteorological parameters**

Meteorological parameter	Correlation coefficient			
	Cercospora leaf spot		Powdery mildew	
	Poly house	Rain shelter	Poly house	Rain shelter
	PDS	PDS	PDS	PDS
RH	0.425	0.997**	-0.638	0.63
Temperature	0.967*	0.245	-0.867*	-0.376

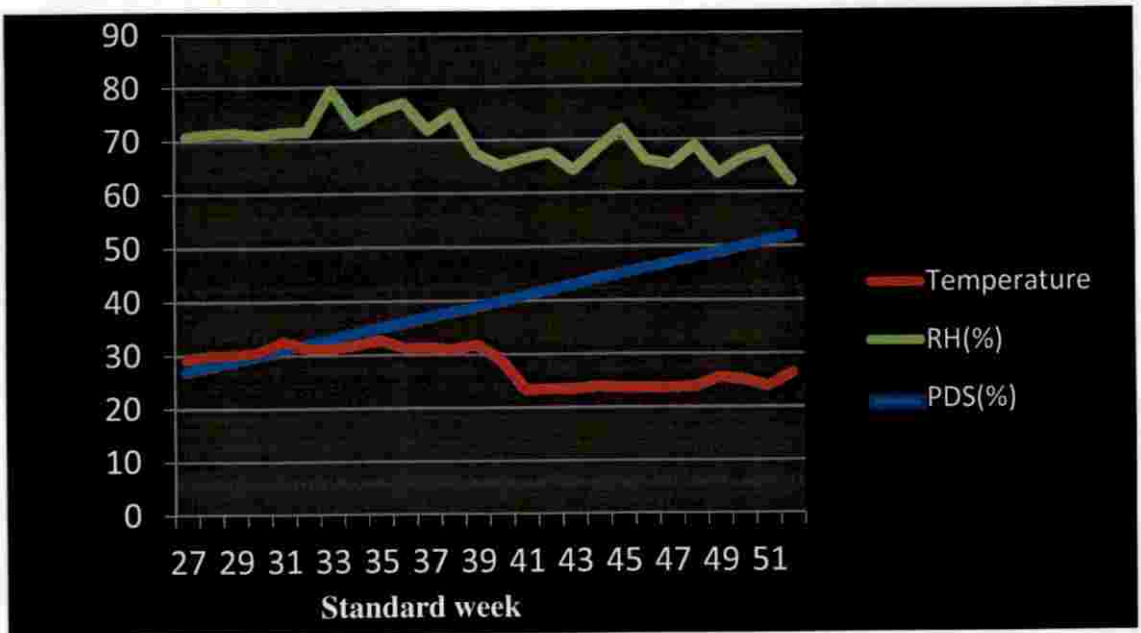
PDS-Per cent disease severity

RH- relative humidity

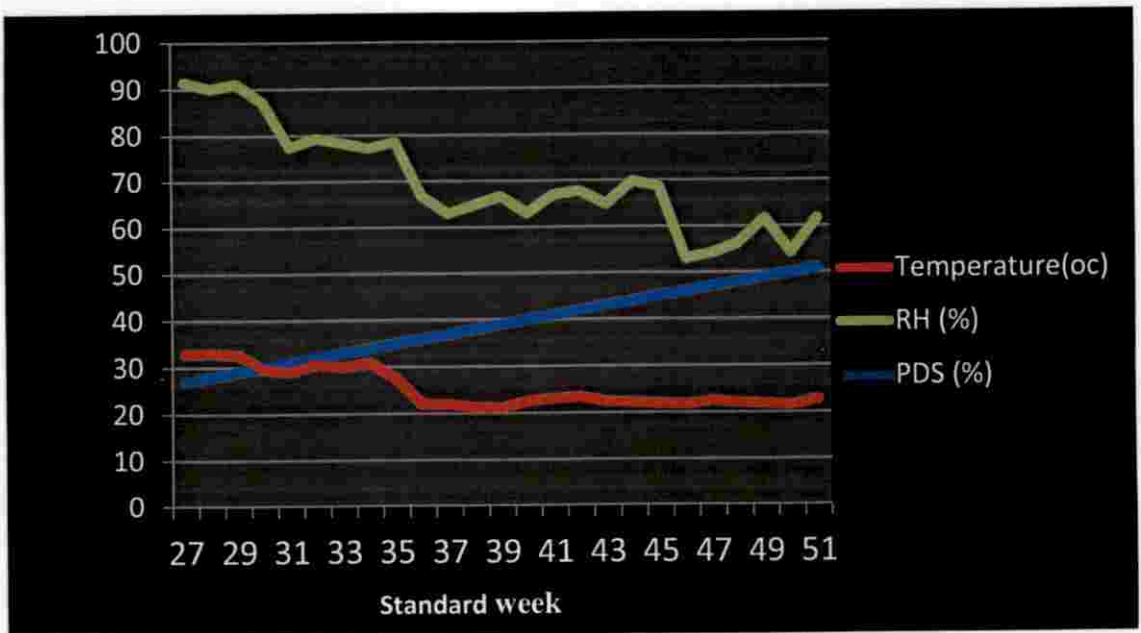
\*Correlation is significant at 0.05 level

\*\*Correlation is significant at 0.001 level

**Fig.4.4. Influence of meteorological parameters on severity of *Cercospora* leaf spot in poly house**



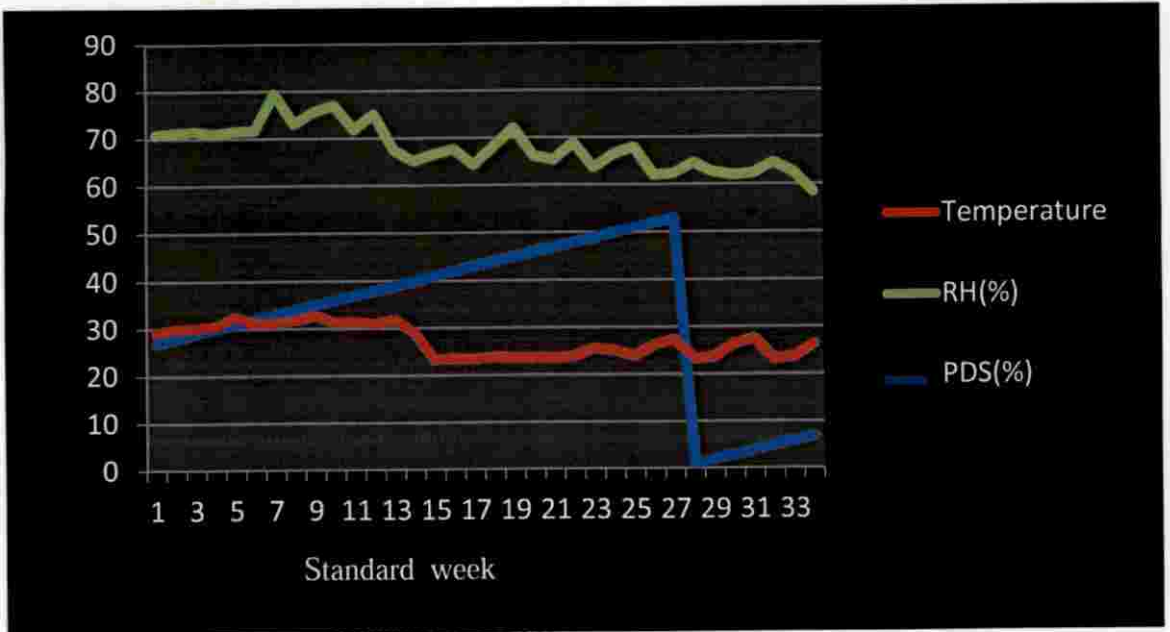
**Fig.4.5. Influence of meteorological parameters on severity of *Cercospora* leaf spot in rain shelter**



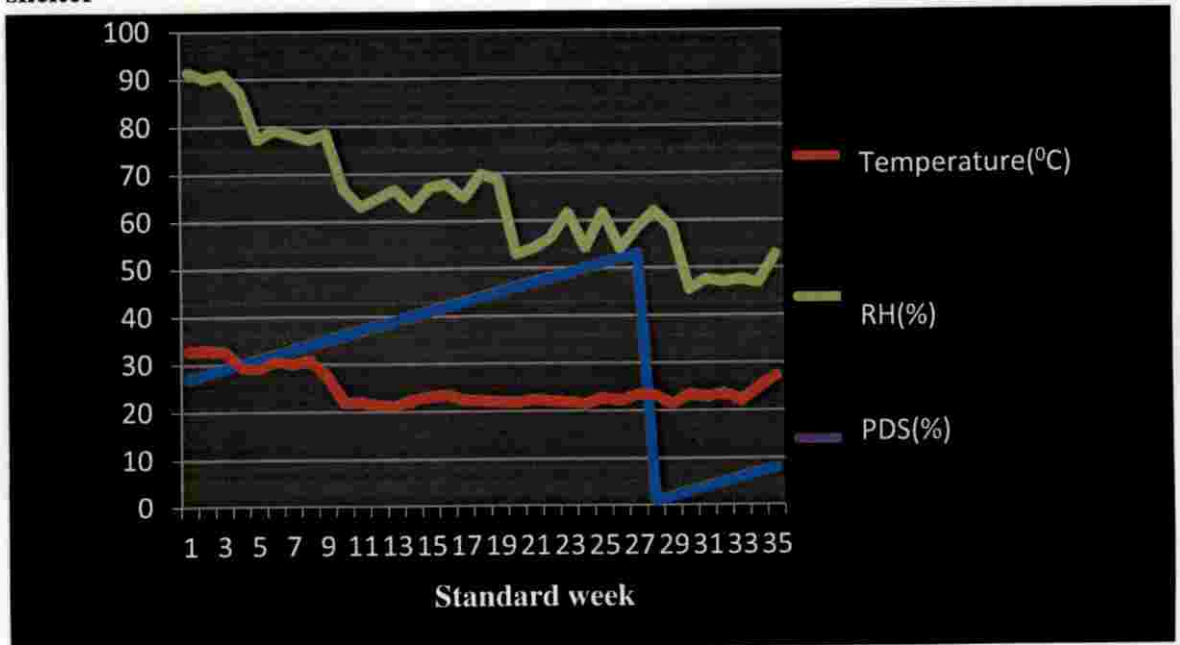
RH-Relative humidity

PDS-Per cent disease severity

**Fig.4.6. Influence of meteorological parameters on severity of powdery mildew in poly house**



**Fig.4.7. Influence of meteorological parameters on severity of powdery mildew in rain shelter**



**RH-Relative humidity**

**PDS-Per cent disease severity**

#### **4.5.8 Effect of treatments on the biometric characters**

Biometric parameters such as height of the plant, days to flowering, days to fruiting, days to harvest, height of the plant were recorded during field experiments (Plate 15 and 16) Effect of seed treatment on seed germination was also observed (Table 4.12 and 4.13).

#### **4.5.9 Effect of treatment on biometric characters of capsicum in poly house**

In polyhouse experiment (Table 4.12) effect of seed treatment on seed germination was studied and it was found that, treatment with *Pseudomonas fluorescens* gave 100 per cent germination whereas, the per cent germination was only 92 per cent in seed treatment with carbendazim 12%+mancozeb 63% and 80 per cent in control. There was significant difference in all the biometric parameters such as height of the plant, days to flowering, and days to fruiting and days to harvest. Highest plant height was observed in T2 and the lowest in T7. Moreover there was slight earliness in flowering and fruiting in T3, T1 and T6, compared to other treatments. The highest yield was found in T5 followed by T6 (Plate 15).

**Table 4.12 Effect of treatments on biometric characters of capsicum in poly house**

Treatment	Biometric characters*					
	PSG	Height of the plant (cm)	Days to flowering	Days to fruiting	Days to harvest	Yield (kg/plot)
T1- S+SA of <i>Trichoderma viride(asperellum)</i> (as per POP)	90	50.37 <sup>b</sup>	61.66 <sup>a</sup>	68.0 <sup>ab</sup>	101 <sup>b</sup>	26.8 <sup>ef</sup>
T2- T1+ST and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	100	86.20 <sup>a</sup>	78.33 <sup>b</sup>	78.66 <sup>bc</sup>	96 <sup>b</sup>	32.13 <sup>cd</sup>
T3 -T1+ST with carbendazim 12% +mancozeb63% (@2g.kg <sup>-1</sup> ) +foliar spray with copper hydroxide (0.2%)	92	61.09 <sup>b</sup>	58.33 <sup>a</sup>	71.33 <sup>a</sup>	91.66 <sup>b</sup>	29.73 <sup>bc</sup>
T4 -T1+STwith carbendazim 12%+mancozeb63% (@2g.kg <sup>-1</sup> ) + foliar spray with mancozeb (0.2%)	86	44.66 <sup>b</sup>	80.33 <sup>bc</sup>	86.66 <sup>de</sup>	104.0 <sup>a</sup>	35.96 <sup>bc</sup>
T5- T1+Foliar spray with tebuconazole. (0.1%)	92	52.93 <sup>b</sup>	87.33 <sup>c</sup>	93.33 <sup>e</sup>	76.66 <sup>a</sup>	42.00 <sup>a</sup>
T6- T1+Foliar spray with difenconazole (0.05%)	86	55.48 <sup>b</sup>	63.00 <sup>a</sup>	75.66 <sup>abc</sup>	90.3 <sup>ab</sup>	39.2 <sup>ab</sup>
T7-Control	80	45.66 <sup>b</sup>	74.33 <sup>b</sup>	85.33 <sup>abc</sup>	104.3 <sup>b</sup>	24.66 <sup>f</sup>
CV	-	18.56	6.54	7.04	8.41	8.28
CD	-	18.70	8.29	10.01	14.2	4.83

S-Soil Solarization SA-Soil Application ST-Seed Treatment PSG-Per cent seed germination

#### 4.5.10 Effect of treatment on biometric characters of capsicum in rain shelter

In rain shelter experiment (Table 4.13),effect of seed treatment on seed germination was studied and it was found that, treatment with *Pseudomonas fluorescens* gave 100 per cent germination whereas, the per cent germination was only 92 per cent in seed treatment with carbendazim +mancozeb and 80 per cent in control. There was no significant difference among the treatments with regard to any of the biometric parameters except yield. Yield was the highest in T5 followed by T6 (Plate 16).

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**Plate 15 Various stages of the crop in polyhouse**



**Transplanting stage**



**One month after transplanting**



**Three month after transplanting**



**Flowering stage**



**Fruiting stag**



**Harvesting stage**

**Table 4.13 Effect of treatments on biometric characters of capsicum in rain shelter**

Treatments	Biometric characters*					
	PSG	Height of the plant (cm)	Days to flowering	Days to fruiting	Days to harvest	Yield(kg/plot)
T1- S+SA of <i>Trichoderma viride</i> ( <i>asperellum</i> ) (as per POP)	90	52.14	80.667	99.0	124	24.66 <sup>c</sup>
T2- T1+ST and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	100	61.66	95.66	116.6	130	32.57 <sup>ab</sup>
T3 -T1+ST with carbendazim12% +mancozeb63% (@2g.kg <sup>-1</sup> ) +foliar spray with copper hydroxide (0.2%)	92	61.20	94.66	112.0	119.3	31.20 <sup>b</sup>
T4 -T1+STwith carbendazim12%+mancozeb 63% (@2g.kg <sup>-1</sup> ) + foliar spray with mancozeb (0.2%)	86	53.08	103.0	123.3	140	34.1 <sup>ab</sup>
T5- T1+Foliar spray with tebuconazole. (0.1%)	92	65.47	98.3	110.6	146.0	36.3 <sup>ab</sup>
T6- T1+Foliar spray with difenconazole (0.05%)	86	63.71	98.6	116.6	136.66	34.6 <sup>ab</sup>
T7-Control	80	58.70	85.0	109.3	130	22.26 <sup>c</sup>
CV	-	25.67	8.52	7.76	11.30	8.69
CD	-	NS	NS	NS	NS	4.76

S-Soil Solarization SA-Soil Application ST-Seed Treatment PSG-Per cent seed germination

#### 4.5.11 Economic analysis

Economic analysis of the treatments was carried in case of field experiments. Benefit: Cost ratio was calculated at the market price *ie* Rs.50 kg<sup>-1</sup> for all the treatments.



**Plate 16 Various stages of the crop in rain shelter**



**Transplanting stage**



**One month after transplanting**



**Three month after transplanting**



**Flowering stage**



**Fruiting stag**



**Harvesting stage**

#### 4.5.12 Economic analysis of capsicum in poly house

Benefit: cost ratio of different treatments in polyhouse was calculated and presented in Table 4.14. At the market price for all treatments (Rs.50 kg<sup>-1</sup>) the highest B:C ratio was observed in treatment T5 (Soil solarization+ soil application of *Trichoderma*+ foliar spray with tebuconazole) i.e 2.18:1.

**Table 4.14 Economic analysis of capsicum in poly house**

Treatments	Benefit: Cost ratio			
	Total cost	Yield(kg /plot)	Benefit: Cost ratio @Rs. 50kg <sup>-1</sup>	
			Total return	B:C ratio
T1-S+SA of <i>Trichoderma viride(asperellum)</i> (as per POP)	824	26.8	1340	1.62:1
T2- T <sub>1</sub> + ST and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	898.4	32.13	1606.5	1.78:1
T3- T <sub>1</sub> +ST with carbendazim12%+ mancozeb 63% (0.2%) + foliar spray with copper hydroxide (0.2%)	946.5	29.73	1486.5	1.56:1
T4-T <sub>1</sub> +ST with carbendazim12%+ mancozeb63% (0.2%) + foliar spray with copper hydroxide (0.2%)	940	35.96	1798	1.91:1
T5-T <sub>1</sub> +Foliar spray with tebuconazole (0.1%)	959.5	42	2100	2.18:1
T6-T <sub>1</sub> +Foliar spray with difenconazole (0.05%)	948.3	39.2	1960	2.0:1
T7- Untreated control	815	24.66	1233	1.5:1

S- Soil Solarisation. ST- Seed Treatment SA-Soil Application

**Table 4.5.13 Economic analysis of capsicum in rain shelter**

Benefit: Cost ratio of different treatments in rain shelter was calculated and presented in Table 4.15. At the price of Rs.50kg<sup>-1</sup>for capsicum, the highest B:C ratio was observed in T5 (Soil solarization+ soil application of *Trichoderma*+ foliar spray with tebuconazole) *ie.* 1.93:1.

On comparing between polyhouse and rain shelter it is found that B:C ratio is more in all the treatments in polyhouse than those in rain shelter except (T2-T<sub>1</sub>+ Seed treatment and foliar spray with *Pseudomonas fluorescens*). However in both poly house and rainshester T5 was found to be highly economic cmpared to all other treatments.

**Table 4.15 Economic analysis of capsicum in rain shelter**

Treatments	Total cost	Yield(kg / plot)	Benefit: Cost ratio	
			Benefit: Cost ratio @Rs. 50kg <sup>-1</sup>	
			Total return (Rs)	B:C ratio
T1-S+SA of <i>Trichoderma viride (asperellum)</i> (as per POP)	820	24.66	1233	1.50:1
T2- T <sub>1</sub> + ST and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	890.5	32.57	1628.5	1.82:1
T3- T <sub>1</sub> +ST with carbendazim12%+ mancozeb63% (0.2%) + foliar spray with copper hydroxide (0.2%)	953	31.20	1560	1.63:1
T4-T <sub>1</sub> +ST with carbendazim12%+ mancozeb 63% (0.2%) + foliar spray with copper hydroxide (0.2%)	933.75	34.1	1705	1.82:1
T5-T <sub>1</sub> +Foliar spray with tebuconazole (0.1%)	939.5	36.3	1815	1.93:1
T6-T <sub>1</sub> +Foliar spray with difenconazole (0.05%)	938.6	34.6	1730	1.84:1
T7- Untreated control	793	22.26	1113	0.14:1

S- Soil Solarisation. ST- Seed Treatment SA-Soil Application

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#### 4.5.14 Incidence of other diseases and pests

Incidence of other diseases and pests were also recorded during the experiment. Major incidence of bacterial wilt (*Ralstonia solanacearum*) was noticed in polyhouse and rain shelter, and minor incidence of leaf curling, mosaic. Infestation of pests such as fruit borer (*Helicoverpa armigera*), aphids (*Aphis gossypii*), and mealy bug (*Phenacoccus parvus*) was recorded (Plate 17).

**Plate 17 Incidence of pest and disease other than fungal diseases in protected structure**



**Mealybug (*Phenacoccus parvus*)**



**Black aphid (*Aphis gossypii*)**



**Fruit borer (*Helicoverpa armigera*)**



**Bacterial wilt (*Ralstonia solanacearum*)**



**Leaf curling**

#### **4.6 Enumeration of phylloplane microflora under protected condition.**

The phylloplane microflora (fungi, bacteria, actinomycetes) of the crop was enumerated before and after each spray using serial dilution plating in both polyhouse and rain shelter (Plate18).

##### **4.6.1 Population of phylloplane fungi in polyhouse**

In Table 4.16, changes in fungal population due to foliar treatments are furnished. The first spray was given at five months after transplanting when there was incidence of leaf spot caused by *Cercospora capsici*. Before the spray there was more or less uniform phylloplane fungal population on the leaves, but after the first spray there was significant difference among the treatments at 1% level of significance. It was observed that, there was a drastic increase in the population of fungi during this period in control (T7). However, the population declined even in the control during second spray. After third spray population of fungi increased in T1, T5 and T6. The reduction in phylloplane fungal population was the most in the T6, T5 and T4. (Fig.4.8)

## Plate 18 Enumeration of phylloplane microflora of capsicum



Assessment of leaf area



Extraction of phylloplane microflora in sterile water



Media used

Fungi-Martin Rose Bengal Agar  
Actinomycetes-Ken knights Agar  
Bacteria-Nutrient Agar



Incubation of plates



Phylloplane fungi



Phylloplane bacteria



Phylloplane actinomycetes

**Table 4.16 Effect of foliar treatments on phylloplane fungi of capsicum in poly house**

Treatment	X 10 cfu /cm <sup>2</sup> of leaf area				Per cent reduction over control
	Before spray	After 1 <sup>st</sup> spray	After 2 <sup>nd</sup> spray	After 3 <sup>rd</sup> spray	
T <sub>1</sub> -Soil solarisation +soil application of <i>Trichoderma viride (asperellum)</i> (2%)	0.20 <sup>b</sup>	0.90 <sup>b</sup> <sup>c</sup>	1.22 <sup>a</sup>	1.90 <sup>a</sup>	-30.5
T <sub>2</sub> -T <sub>1</sub> +Seed treatment and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	0.39 <sup>a</sup>	1.13 <sup>b</sup>	0.59 <sup>b</sup>	1.18 <sup>c</sup>	10.60
T <sub>3</sub> -T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb 63% (0.2%) + foliar spray with copper hydroxide (0.2%),	0.18 <sup>b</sup>	0.70 <sup>cd</sup>	0.72 <sup>b</sup>	0.52 <sup>b</sup>	60.60
T <sub>4</sub> -T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb 63% (0.2%) +foliar spray with mancozeb (0.2%),	0.2 <sup>b</sup>	0.39 <sup>de</sup>	0.20 <sup>c</sup>	0.38 <sup>cd</sup>	71.281.0
T <sub>5</sub> -T <sub>1</sub> +Foliar spray with tebuconazole (0.1%) twice at 10 days interval,	0.28 <sup>ab</sup>	0.22 <sup>e</sup>	0.14 <sup>c</sup>	0.25 <sup>d</sup>	65.90
T <sub>6</sub> -T <sub>1</sub> +Foliar spray with difenconazole (0.05%)	0.17 <sup>b</sup>	0.12 <sup>e</sup>	0.02 <sup>c</sup>	0.45 <sup>c</sup>	
T <sub>7</sub> -Untreated control	0.15 <sup>b</sup>	2.3 <sup>a</sup>	0.18 <sup>c</sup>	1.32 <sup>b</sup>	

#### 4.6.2 Population of phylloplane bacteria in poly house

In Table 4.17 changes in bacterial population due to foliar treatments are furnished. In the case of bacteria also, before the treatment application the population was more or less uniform on the leaves, but after the 1<sup>st</sup> spray there was significant difference among the treatments. It was observed that there was drastic decrease in the population of bacteria during this period in all the treatments whereas, the population was drastically declined in treatments T5 and T6 tebuconazole and difenconazole respectively. After the 2<sup>nd</sup> spray bacterial population was the highest in T2 (8.21). After the 3<sup>rd</sup> spray there was drastic reduction in the phylloplane bacteria in T5 and T6. However, the data in Table 4.17 indicate differential effect of fungicide treatments on phylloplane bacteria. The contact fungicide (T3 and T4) and systemic



fungicides (T5 and T6) cause a sudden decline of bacteria but after the second spray, the effect is reversed as there is increase in population (Fig.4.9).

**Table 4.17 Effect of foliar treatments on phylloplane bacteria of capsicum in polyhouse**

Treatment	X 10 cfu/cm <sup>2</sup> of leaf area			After 3rd spray	Per cent reduction over control
	Before spray	After 1 <sup>st</sup> spray	After 2 <sup>nd</sup> spray		
T <sub>1</sub> -Soil solarisation +soil application of <i>Trichoderma viride</i> ( <i>asperellum</i> ) (2%)	1.65	1.06 <sup>a</sup>	6.98 <sup>a</sup>	0.49 <sup>b</sup>	34.66
T <sub>2</sub> -T <sub>1</sub> + Seed treatment and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	1.39	0.543 <sup>b</sup>	8.21 <sup>a</sup>	0.40 <sup>bc</sup>	46.6
T <sub>3</sub> -T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb63% (0.2%) + foliar spray with copper hydroxide (0.2%),	2.02	0.510 <sup>b</sup>	7.58 <sup>a</sup>	0.26 <sup>c</sup>	65.3
T <sub>4</sub> - T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb63% (0.2%) +foliar spray with mancozeb (0.2%),	2.11	0.443 <sup>b</sup>	0.64 <sup>b</sup>	0.05 <sup>d</sup>	93.33
T <sub>5</sub> -T <sub>1</sub> +Foliar spray with tebuconazole (0.1%)	1.52	0.17 <sup>c</sup>	0.48 <sup>b</sup>	0.36 <sup>bc</sup>	52
T <sub>6</sub> -T <sub>1</sub> +Foliar spray with difenconazole (0.05%)	0.50	0.35 <sup>bc</sup>	1.27 <sup>b</sup>	0.47 <sup>b</sup>	59.57
T <sub>7</sub> -Untreated control	1.38	1.14 <sup>a</sup>	2.34 <sup>b</sup>	0.75 <sup>a</sup>	

#### 4.6.3 Population of phylloplane actinomycetes in poly house

Data on population of phylloplane actinomycetes in poly house presented (Table 4.18). Before application of treatments as well as after the first and second spray, there was no significant differences in population of actinomycetes. But after the third spray, the population varied significantly. The population of actinomycetes decreased in the all treatments during the experiment. The reduction was more in fungicides. (Fig.4.10)

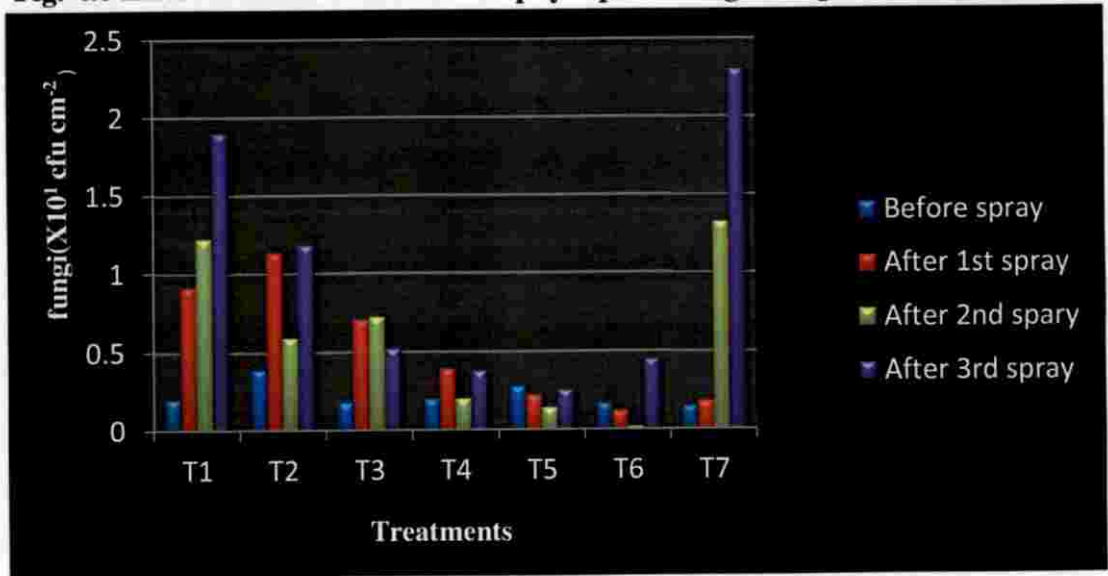
**Table 4.18 Effect of foliar treatments on phylloplane actinomycetes of capsicum in polyhouse**

Treatments	X10 cfu /cm <sup>2</sup> of leaf area				
	Before spray	After 1 <sup>st</sup> spray	After 2 <sup>nd</sup> spray	After 3 <sup>rd</sup> spray	Per cent reduction over control
T <sub>1</sub> -Soil solarisation +soil application of <i>Trichoderma viride</i> ( <i>asperellum</i> ) (2%)	0.44	0.04 <sup>b</sup>	0.03 <sup>bc</sup>	0.05 <sup>c</sup>	35.06
T <sub>2</sub> -T <sub>1</sub> + Seed treatment and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	0.23	0.04 <sup>b</sup>	0.01 <sup>c</sup>	0.11 <sup>a</sup>	-42.85
T <sub>3</sub> -T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb 63% (0.2%) + foliar spray with copper hydroxide (0.2%),	0.20	0.01 <sup>c</sup>	0.04 <sup>bc</sup>	0.009 <sup>d</sup>	88.3
T <sub>4</sub> - T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb 63% (0.2%) +foliar spray with mancozeb (0.2%),	0.35	0.01 <sup>c</sup>	0.07 <sup>b</sup>	0.007 <sup>d</sup>	90.90
T <sub>5</sub> -T <sub>1</sub> +Foliar spray with tebuconazole (0.1%)	0.24	0.01 <sup>c</sup>	0.12 <sup>a</sup>	0.010 <sup>d</sup>	87.01
T <sub>6</sub> -T <sub>1</sub> +Foliar spray with difenconazole (0.05%)	0.20	0.006 <sup>c</sup>	0.07 <sup>ab</sup>	0.013 <sup>d</sup>	83.11
T <sub>7</sub> -Untreated control	0.34	0.012 <sup>a</sup>	0.03 <sup>bc</sup>	0.077 <sup>b</sup>	

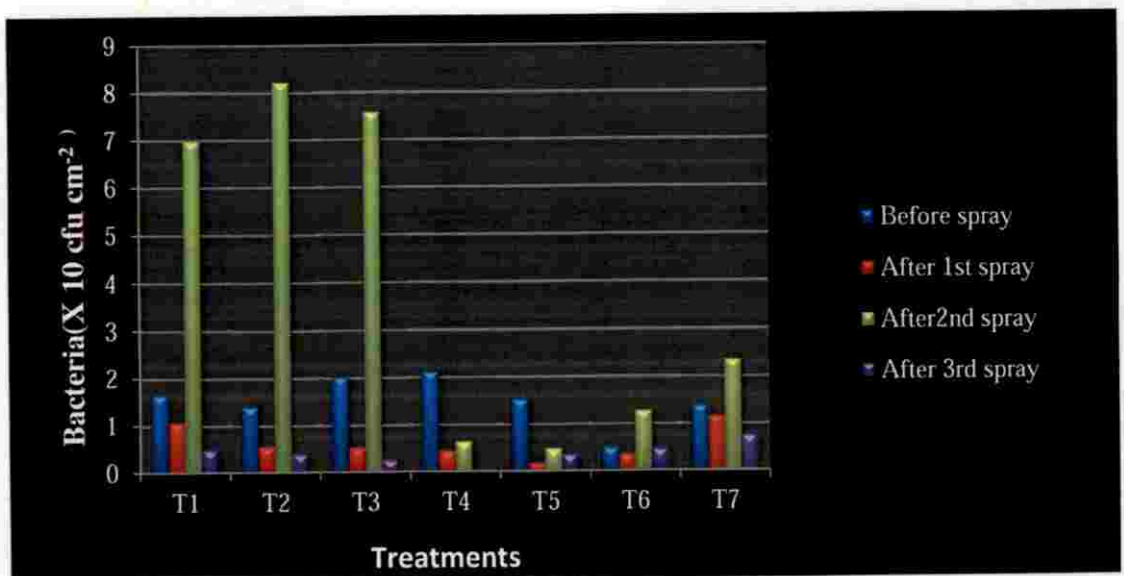
#### 4.6.4 Population of phylloplane fungi in rain shelter

Data on Population of phylloplane fungi in rain shelter presented (Table 4.19).The first spray was given at five months after planting. Before spray there was more or less uniform phylloplane fungal population on the leaves, but after the first spray there was significant difference among the treatments at 1% level of significance. It was observed that, there was a drastic increase in the population of fungi during this period in control (T7).While there was decline in the population of T3,T4,T5,T6 as plant grows, there is increase in phylloplane fungi as it evidence from

**Fig.-4.8 Effect of foliar treatments on phyloplane fungi of capsicum in polyhouse**



**Fig.-4.9 Effect of foliar treatments on phyloplane bacteria of capsicum in polyhouse**



T1-Soil solarization +Soil application of *Trichoderma viride* (*asperellum*)

T2-T1+Soil application and foliar spray of *P. fluorescens*

T3-T1+Seed treatment with carbendazim+ mancozeb (0.2%) + foliar spray with copper hydroxide (0.2%)

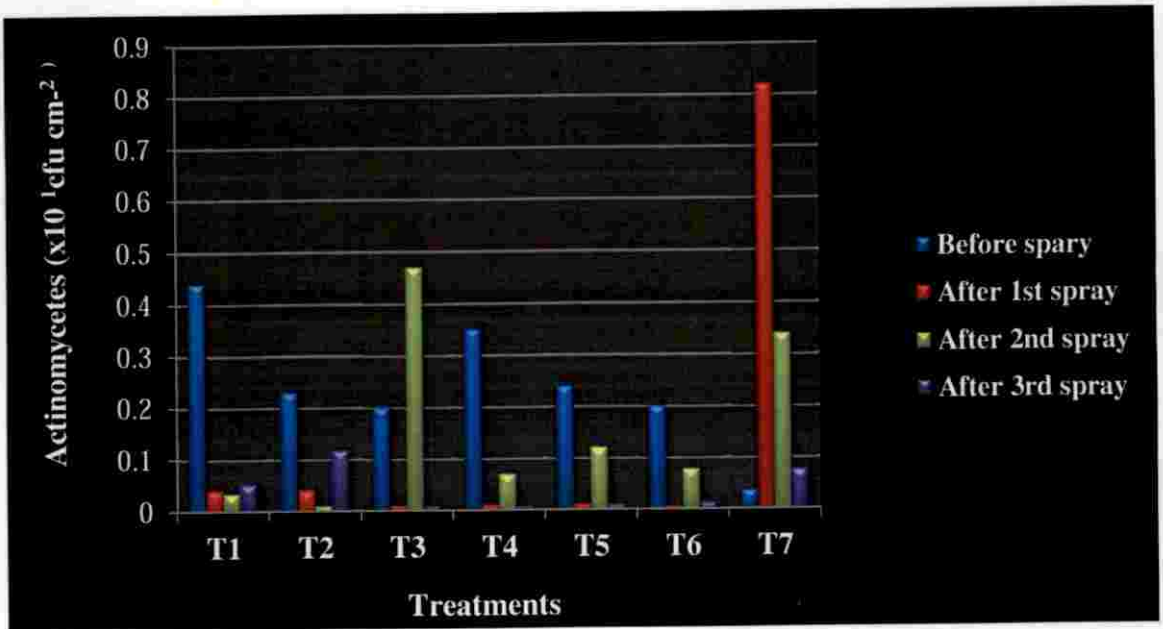
T4-T1+Seed treatment with carbendazim+ mancozeb (0.2%) + foliar spray with mancozeb (0.2%)

T5-T1+Foliar spray with tebuconazole (0.1%)

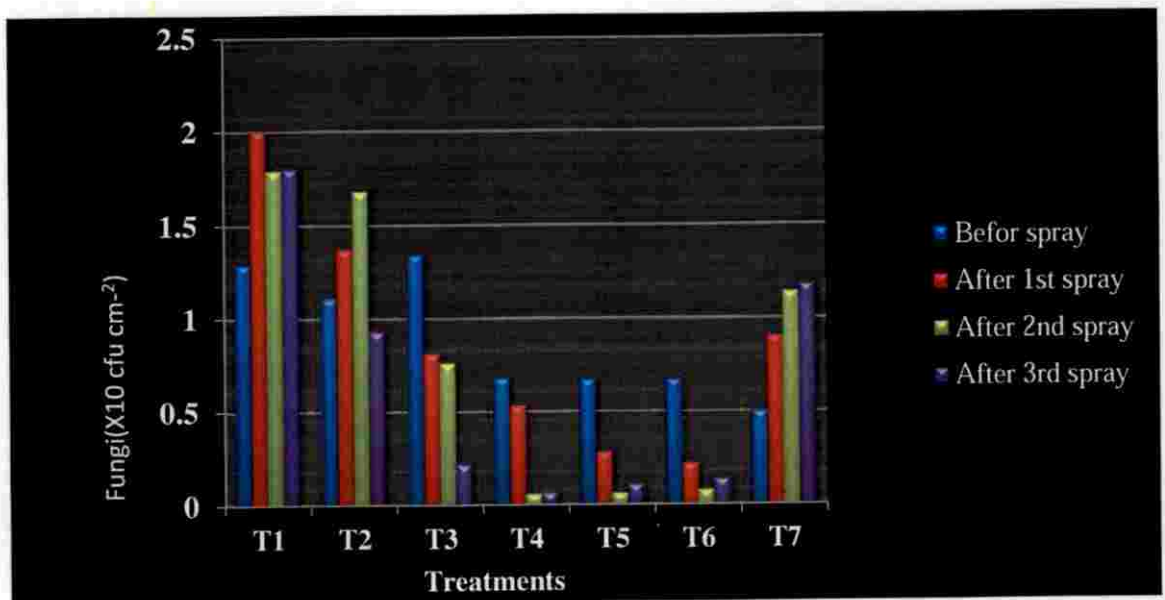
T6-T1+Foliar spray with difenoconazole (0.05%)

T7-Untreated control

**Fig.4.10 Effect of foliar treatments on phylloplane actinomycetes in poly house**



**Fig.4.11 Effect of foliar treatments on phylloplane fungi in rain shelter**



T1-Soil solarization +Soil application of *Trichoderma viride* (*asperellum*)

T2-T1+Soil application and foliar spray of *P. floescens*

T3-T1+Seed treatment with carbendazim+ mancozeb (0.2%) + foliar spray with copper hydroxide (0.2%)

T4-T1+Seed treatment with carbendazim+ mancozeb (0.2%) + foliar spray with mancozeb (0.2%)

T5-T1+Foliar spray with tebuconazole (0.1%)

T6-T1+Foliar spray with difenoconazole (0.05%)

T7-Untreated control

increase in fungi in control(T7). However population declined after 2<sup>nd</sup> spray in all the treatments including control (T7), and after 3<sup>rd</sup> spray in all the treatments there was drastic reduction in the population of phylloplane fungi. (Fig 4.11).

**Table 4.19 Effect of foliar treatments on phylloplane fungi of capsicum in rain shelter**

Treatments	X 10 cfu /cm <sup>2</sup> of leaf area				
	Before spray	After 1 <sup>st</sup> spray	After 2 <sup>nd</sup> spray	After 3 <sup>rd</sup> spray	Per cent reduction over control
T <sub>1</sub> -Soil solarisation +soil application of <i>Trichoderma viride (asperellum)</i> (2%)	1.29 <sup>a</sup>	2.00 <sup>a</sup>	1.79 <sup>a</sup>	0.80 <sup>b</sup>	32.20
T <sub>2</sub> -T <sub>1</sub> + Seed treatment and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	1.11 <sup>a</sup>	1.37 <sup>b</sup>	1.68 <sup>a</sup>	0.93 <sup>b</sup>	21.18
T <sub>3</sub> -T <sub>1</sub> +Seed treatment with carbendazim12%+mancozeb 63% (0.2%) + foliar spray with copper hydroxide (0.2%),	1.34 <sup>a</sup>	0.81 <sup>c</sup>	0.76 <sup>bc</sup>	0.22 <sup>c</sup>	81.3
T <sub>4</sub> - T <sub>1</sub> +Seed treatment with carbendazim12%+mancozeb 63% (0.2%) +foliar spray with mancozeb (0.2%),	0.68 <sup>b</sup>	0.53 <sup>cd</sup>	0.06 <sup>c</sup>	0.07 <sup>d</sup>	94.0
T <sub>5</sub> -T <sub>1</sub> +Foliar spray with tebuconazole (0.1%)	0.67 <sup>b</sup>	0.28 <sup>d</sup>	0.063 <sup>c</sup>	0.11 <sup>cd</sup>	90.6
T <sub>6</sub> -T <sub>1</sub> +Foliar spray with difenconazole (0.05%)	0.67 <sup>b</sup>	0.22 <sup>d</sup>	0.08 <sup>c</sup>	0.14 <sup>cd</sup>	88.13
T <sub>7</sub> -Untreated control	0.50 <sup>b</sup>	0.90 <sup>c</sup>	1.14 <sup>ab</sup>	1.18 <sup>a</sup>	

#### 4.6.5 Population of phylloplane bacteria in rain shelter

In Table 4.20, changes in bacterial population due to foliar treatments in rain shelter are furnished. In the case of bacteria also, before the treatment application the population was more or less uniform on the leaves, but after the 1<sup>st</sup> spray there was significant difference among the treatments. It was observed that there was decrease in the population of bacteria during this period in all the treatments whereas, the population was declined drastically in treatments T5 and T6 (tebuconazole and

difenconazole respectively). After the 2<sup>nd</sup> spray bacterial population was the highest in T2 (2.63). However, after 3<sup>rd</sup> spray reduction in the phylloplane fungi was observed in all the treatments. The data in Table 4.20 indicate differential effect of fungicide treatments on phylloplane bacteria. The systemic fungicides (T5 and T6) cause a sudden decline of bacteria but after the second spray, the effect is reversed as there is increase in population (Fig.4.12).

**Table 4.20 Effect of foliar treatments on phylloplane bacteria of capsicum in rain shelter**

Treatments	X 10 cfu /cm <sup>2</sup> of leaf area				
	Before spray	After 1 <sup>st</sup> spray	After 2 <sup>nd</sup> spray	After 3 <sup>rd</sup> spray	Per cent reducti on over control
T <sub>1</sub> -Soil solarisation +soil application of <i>Trichoderma viride (asperellum)</i> (2%)	1.28 <sup>a</sup>	1.37 <sup>b</sup>	2.04 <sup>b</sup>	0.40 <sup>b</sup>	-11.11
T <sub>2</sub> -T <sub>1</sub> + Seed treatment and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	1.34 <sup>a</sup>	2.44 <sup>a</sup>	2.63 <sup>a</sup>	0.93 <sup>a</sup>	-158.3
T <sub>3</sub> -T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb63% (0.2%) + foliar spray with copper hydroxide (0.2%),	1.38 <sup>a</sup>	0.95 <sup>cd</sup>	1.23 <sup>c</sup>	0.17 <sup>c</sup>	52.7
T <sub>4</sub> - T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb 63% (0.2%) +foliar spray with mancozeb (0.2%),	0.67 <sup>b</sup>	0.53 <sup>d</sup>	0.36 <sup>d</sup>	0.10 <sup>c</sup>	72.2
T <sub>5</sub> -T <sub>1</sub> +Foliar spray with tebuconazole (0.1%),	1.17 <sup>a</sup>	0.73 <sup>d</sup>	0.60 <sup>d</sup>	0.10 <sup>c</sup>	72.2
T <sub>6</sub> -T <sub>1</sub> +Foliar spray with difenconazole (0.05%)	1.28 <sup>a</sup>	1.25 <sup>bc</sup>	0.66 <sup>d</sup>	0.06 <sup>c</sup>	0.83
T <sub>7</sub> -Untreated control	1.55 <sup>a</sup>	0.05 <sup>e</sup>	0.57 <sup>d</sup>	0.36 <sup>b</sup>	

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#### 4.6.6 Population of phylloplane actinomycetes in rain shelter

Change in the population of phylloplane actinomycetes in rain shelter are presented in Table 4.21 before and after the treatments. There was no significant differences in population of actinomycetes. The population of actinomycetes decreased in all treatments during the experiment, and the reduction was more in fungicides (Fig.4.13).

**Table 4.21 Effect of treatments on phylloplane actinomycetes of capsicum in rain shelter**

Treatment	X 10 cfu /cm <sup>2</sup> of leaf area				
	Before spray	After 1 <sup>st</sup> spray	After 2 <sup>nd</sup> Spray	After 3 <sup>rd</sup> spray	Per cent reduction over control
T <sub>1</sub> -Soil solarisation +soil application of <i>Trichoderma viride (asperellum)</i> (2%)	0.06	0.067	0.047	0.03	0
T <sub>2</sub> -T <sub>1</sub> + Seed treatment and foliar spray with <i>Pseudomonas fluorescens</i> (2%)	0.03	0.023	0.042	0.04	-33.3
T <sub>3</sub> -T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb 63% (0.2%) + foliar spray with copper hydroxide (0.2%),	0.05	0.047	0.078	0.02	33.3
T <sub>4</sub> - T <sub>1</sub> +Seed treatment with carbendazim12%+ mancozeb 63% (0.2%) +foliar spray with mancozeb (0.2%),	0.08	0.063	0.090	0.021	30
T <sub>5</sub> -T <sub>1</sub> +Foliar spray with tebuconazole (0.1%)	0.06	0.063	0.063	0.02	33.3
T <sub>6</sub> -T <sub>1</sub> +Foliar spray with difenconazole (0.05%)	0.02	0.024	0.130	0.01	66.6
T <sub>7</sub> -Untreated control	0.08	0.055	0.063	0.03	

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#### 4.7 Survival of bio control agents in the phylloplane of capsicum in poly house

Survival of *Pseudomonas fluorescens* on phylloplane of capsicum are furnished below in Table 4.22 (Plate 19). On spraying with *Pseudomonas fluorescens* there was generally an increase in its population in poly house and rain shelter. Initially, phylloplane population of *Pseudomonas fluorescens* was 2.03cfu/cm<sup>2</sup> leaf area, which increased three fold by third spray 8.83cfu/cm<sup>2</sup> leaf area. In rain shelter also same trend was observed, where initially phylloplane population was 1.55 cfu/cm<sup>2</sup> which reached 6.92cfu/cm<sup>2</sup> leaf area after third spray. *Pseudomonas fluorescens* survived up to 15days after spraying as it is shown in Fig.4.14 and 4.15.

**Table 4.22 Survival of *Pseudomonas fluorescens* in the phylloplane of capsicum in poly house and rain shelter**

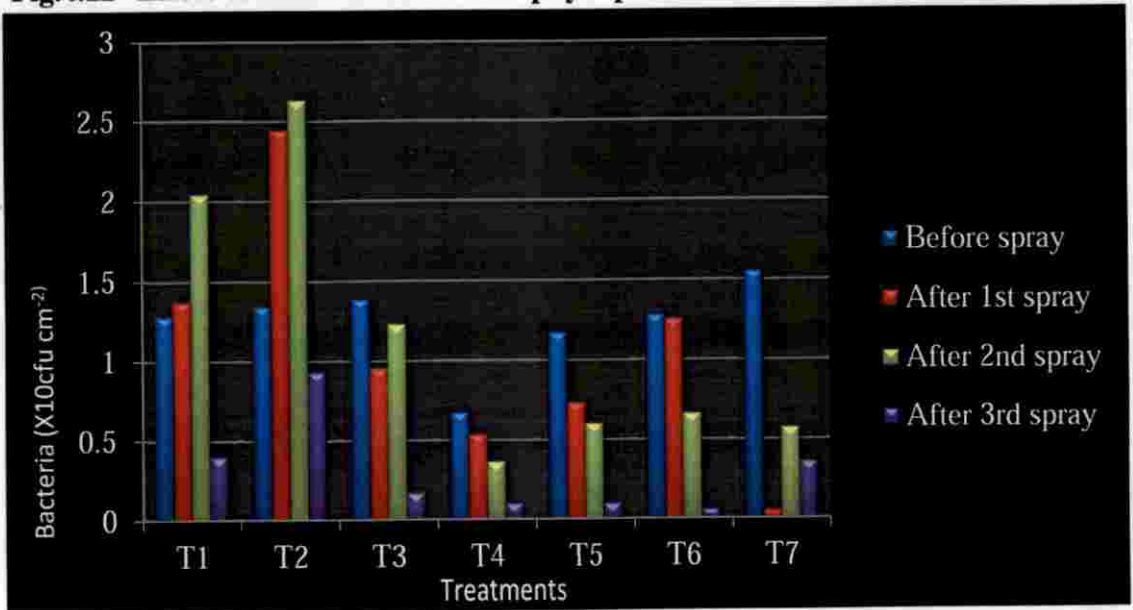
Treatment	<i>Pseudomonas fluorescens</i> (X 10cfu cm <sup>-2</sup> )							
T2-T1+ ST with foliar spray <i>Pseudomonas fluorescens</i> (2%)	Poly house				Rain shelter			
	Before spray	After 1 <sup>st</sup> spray	After 2 <sup>nd</sup> spray	After 3 <sup>rd</sup> spray	Before spray	After 1 <sup>st</sup> spray	After 2 <sup>nd</sup> spray	After 3 <sup>rd</sup> spray
	2.03	2.63	5.097	8.83	1.55	3.22	5.21	6.92

T1-T1-Soil solarisation +soil application of *Trichoderma viride* (*asperellum*) (2%)

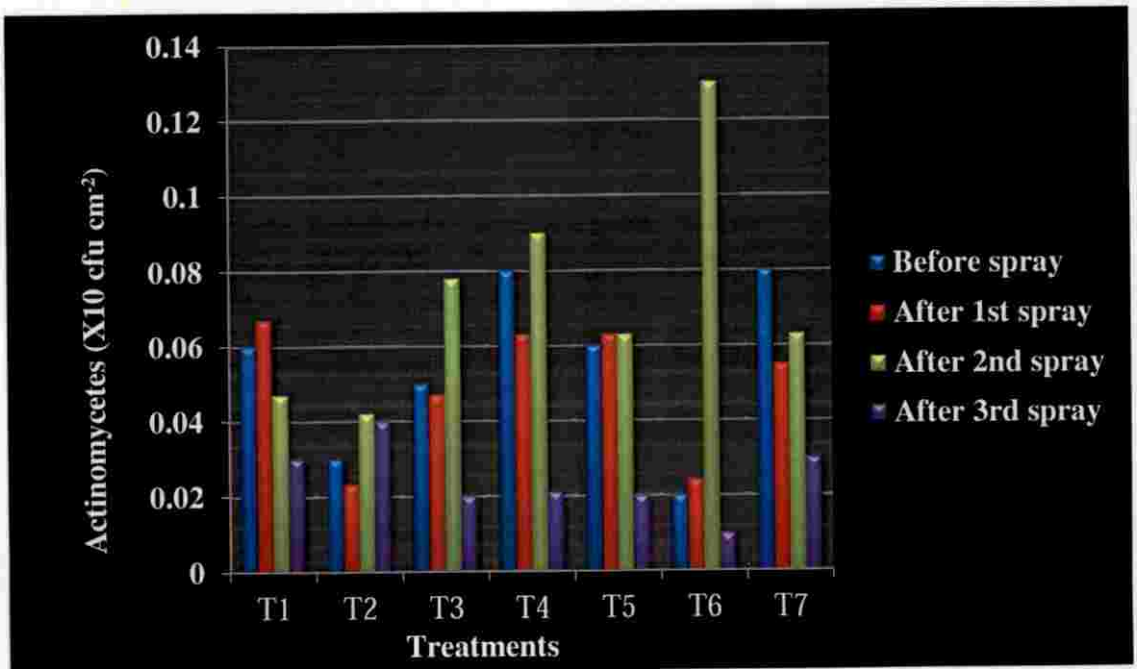
ST-Seed Treatment



**Fig.4.12 Effect of foliar treatments on phyloplane bacteria in rain shelter**



**Fig.4.13 Effect of foliar treatments on phyloplane actinomycetes in rain shelter**



T1-Soil solarization +Soil application of *Trichoderma viride* (*asperellum*)

T2-T1+Soil application and foliar spray of *P. florescens*

T3-T1+Seed treatment with carbendazim+ mancozeb (0.2%) + foliar spray with copper hydroxide (0.2%)

T4-T1+Seed treatment with carbendazim+ mancozeb (0.2%) + foliar spray with mancozeb (0.2%)

T5-T1+Foliar spray with tebuconazole (0.1%)

T6-T1+Foliar spray with difenoconazole (0.05%)

T7-Untreated control

Plate 19 *Pseudomonas fluorescens* in phylloplane of capsicum



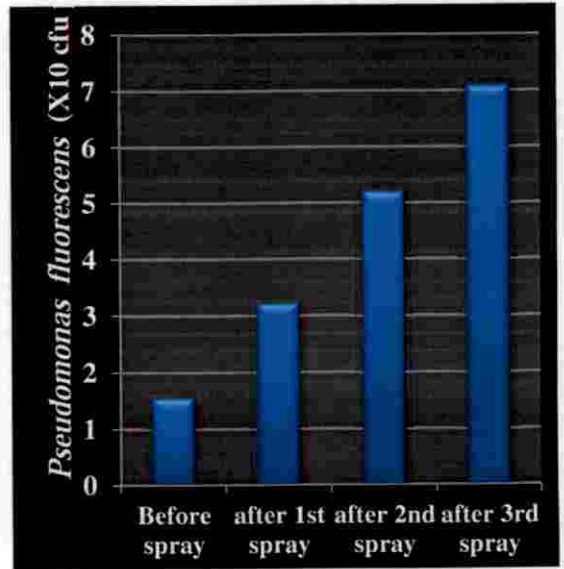
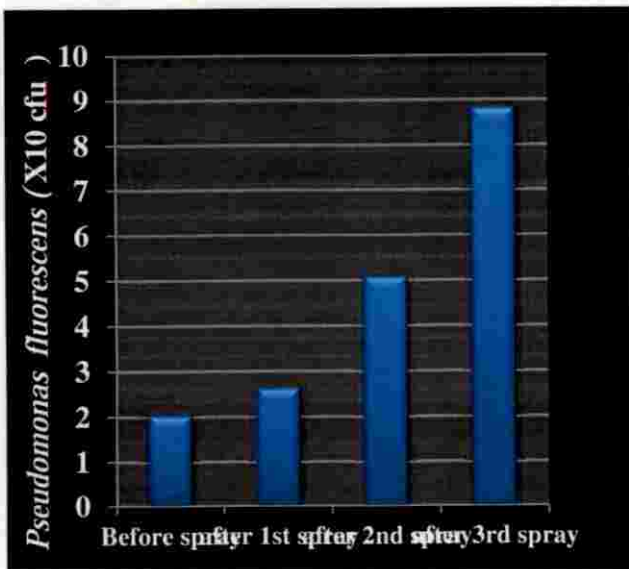
Special media used King's B Agar



*Pseudomonas fluorescens*

Fig.4.14 Survival of *P. fluorescens* in Phylloplane of capsicum in polyhouse

Fig.4.15 Survival of *P. fluorescens* in phylloplane of capsicum in rain shelter



T2- Foliar spray of *Pseudomonas fluorescens* (20g.L<sup>-1</sup>)

# *Discussion*

## 5. DISCUSSION

Protected cultivation of vegetables is a fascinating technology gaining tremendous importance in India in the recent years. Capsicum, (*Capsicum annuum* L.) is one of the most preferred vegetables grown under protected condition in the developed world. In India also, protected cultivation is being adopted on a large scale. Government of Kerala is also encouraging polyhouse and rain shelter cultivation of vegetable crops. Capsicum is a cool season crop, but it can be grown round the year in protected structures where temperature and relative humidity (RH) can be manipulated. However, in protected structures, prevalence of microclimate which is congenial for multiplication and spread of plant pathogens predispose the plants to diseases. Among the various diseases, fungal diseases are causing major loss in tropical and subtropical condition, resulting in qualitative and quantitative losses. Farmers have for many years relied up on chemical control to manage the diseases which resulted in many undesirable effects like environmental pollution, accumulation of toxic substances and development of resistance in pathogen. However, biological control alone may not be effective for economic management of diseases. Hence there is an urgent need to develop bio intensive methods for disease management. Accordingly the present study was to undertaken assess the incidence and severity of major fungal diseases of capsicum under protected cultivation and to formulate an eco-friendly management practices against the diseases.

### 5.1 Survey for assessment of incidence and severity of fungal diseases of capsicum

Survey was conducted in three districts of Kerala by selecting eight poly houses where capsicum was being cultivated during August-February 2016-17. Incidence and severity of fungal disease of capsicum were assessed using standard score charts. Powdery mildew was noticed in all the poly houses except Vellanikkara,

irrespective of the season. However *Cercospora* leaf spot was noticed only during rainy season (July-August). During the survey it was found that, temperature and RH are higher inside the poly house compared to outside. The increase in temperature inside polyhouse is the result of greenhouse effect brought about by the polythene roof, *i.e* retention of major parts of reflected radiation from the earth's surface. Thus most of the energy which is transmitted into the poly house is retained inside and causes increase in atmospheric temperature (Board.2004). Use of fogger and increased plant transpiration due to high atmospheric temperature are major reasons for high RH in poly house. Studies have revealed that closed environments enable the spores of the pathogens to stay inside the poly house for longer period so as to cause more infection. This coupled with higher RH leads to higher rate of diseases in poly houses (Reshma, 2016)

## 5.2 Symptomatology and characterization of pathogens

Plant samples with symptoms of fungal diseases such as powdery mildew, *Cercospora* leaf spot, anthracnose, and stem and fruit rot were collected from various poly houses. Powdery mildew was found to be the most devastating disease of capsicum in polyhouse. Symptoms on leaves such as white-grey to purple growth of the causal organism, powdery growth on lower side of the leaf were observed initially, later infected area of leaves became dried up and turned brown. There was severe defoliation also. But symptoms was not observed on stem and fruits. The observation confirmed earlier reports of (Palti 2013).

*Cercospora* leaf spot disease appeared on leaves as small brown spots, which enlarged later. When numerous spots occurred, the leaves turned yellow and dropped off. Concentric zonations were seen on the spots. Incidence of anthracnose on fruits occurred either from field or after harvest. Infection began with appearance of small circular, depressed lesions that rapidly expanded and hence no defined diameter. According to Lopes and Avia (2005), when relative humidity is high, the formation

of pink or orange conidial mass may be observed. However, in the present study, conidial mass observed was white. Stem and fruit rot of sweet pepper first appeared as slight vein clearing on the outer portion of young leaves followed by epinasty of older leaves. Infection at the seedling stage may cause wilt and drying of seedlings soon after symptom appearance. But incidence of the disease was not observed during seedling stage in the present study. Pathogenicity of the fungi associated with the diseases was proved by artificial inoculation on healthy plant parts. The cultural and morphological characters of the pathogens were studied and the pathogens were identified. The identity of the organism was further confirmed by molecular characterization. It was confirmed that the leaf spot, fruit rot and stem and fruit rot of capsicum are caused by *Cercospora capsici*, *Colletotrichum capsici*, and *Fusarium* sp. respectively. Major meteorological parameters such as temperature and RH were recorded during survey. However, there was no significant correlation observed between these factors and the severity of diseases in farmer's poly house. Later in the study it was found that meteorological factors 15 days prior to the disease incidence are influential on the severity of diseases.

### **5.3 *In vitro* evaluation of fungicides and biocontrol agents on powdery mildew of capsicum**

Prior to the incidence of diseases, the fungicides and biocontrol agents were evaluated *in vitro* to study their efficiency against powdery mildew of capsicum. Data presented in Table-4.2 indicates that T5-tebuconazole (0.1per cent) is the most effective fungicide to control powdery mildew followed by T6-difenoconazole (0.05per cent) these two fungicides belonging to the triazole group are dimethylation inhibitors(DMI) which have preventative, curative and antispore action, against powdery mildew. These, fungicide also cause abnormal growth of the fungi and eventually death (Muller, 2007). The results of the present study is in confirmation with the findings of Manojkumar *et al.*,(2008) who reported that among the systemic fungicides, tebuconazole was best chemical for control of powdery mildew of bell

pepper, among the bio control agents *Pseudomonas fluorescens* also recorded better results comparative to other treatments the results of the present study is in confirmation with the findings of Raghavendra (2005) reported that among several biocontrol tested *Pseudomonas fluorescens* was superior over the other treatments for control of powdery mildew in chilli.

#### **5.4 Field experiments for management of fungal diseases of capsicum under protected condition**

Field experiments were conducted under polyhouse and rain shelter at the Department of Plant Pathology, College of Horticulture, Vellanikkara during August to February 2016-17, to evaluate different treatments for the management of fungal diseases of capsicum under protected condition.

##### **5.4.1 Soil solarisation inside poly house and rain shelter**

Solarisation has been commercially exploited in sick soils under open condition, where direct sunlight is available. However, it has been found to be effective under protected condition also, when the period of solarization is extended for growing high value crops Elmore *et al.*,(1997), Reshma (2016) reported that solarisation in green house produces higher soil temperature than solarization in open fields during cooler weather. Therefore it will be more effective in cooler weather. Hence, in the present study solarisation in polyhouse and rain shelter was carried out for a period of 90 days before transplanting capsicum seedlings. Soil temperature at 10cm was recorded during the period of solarisation. . It was found that in both poly house and rain shelter the temperature in solarized beds was higher than non-solarized beds. In polyhouse, there was an increase in soil temperature by 6.35<sup>0</sup>C at 2:30pm, and in rain shelter soil temperature increased up to 4.5<sup>0</sup>C over non-solarized beds. It was also observed that there was lesser diseases in solarized plots compared to control. Since fungal pathogens were killed by the increased temperature in presence of moisture. When moistened soil is covered with transparent polythene

which allows the sun's radiant energy to be trapped in soil and most of this energy transmitted to the soil is not reflected back and thus increasing the soil temperature (Katan, 1981). Increase in soil temperature is the most important mechanism by which soil solarisation brings down the population of pathogenic organisms, weeds and nematodes (Camprubi *et al.*, 2007).

#### **5.4.2 Enumeration of soil microflora**

There was a reduction in the population of fungi, bacteria, and actinomycetes in solarized soil. This is because of higher temperature prevailing under the polythene sheet, the results confirms the findings of (Candido *et al.*, 2008) who reported that soil solarisation under greenhouse condition also increases soil temperature to level lethal to many soil borne plant pathogens and weeds.

#### **5.5 Management of fungal diseases of capsicum under protected condition**

The main objective of the study was to assess the incidence and severity of fungal diseases of capsicum under protected cultivation and to formulate an effective and eco-friendly management strategy against the diseases. Therefore it was essential to test the efficacy of selected plant protection chemicals and biocontrol agents against the major diseases of capsicum under poly house and rain shelter. Initially an *in vitro* evaluation was conducted to compare the efficacy of different treatments against powdery mildew of capsicum. It was found that, tebuconazole (0.1%) was the best effective fungicide against powdery mildew which gave 100 per cent control of the disease.

Two experiments were conducted simultaneously during June-February 2016-2017 in polyhouse and rain shelter in the Department of Plant Pathology College of Horticulture Vellanikkara, the treatments included systemic and contact fungicides, and biocontrol agents.



### 5.5.1 Effect of different treatments on *Cercospora* leaf spot of capsicum under poly house and rain shelter

In polyhouse, incidence of *Cercospora* leaf spot was noticed 77 days after transplanting and there was a gradual increase in the severity of the disease throughout the period of experiment. However all the treatments recorded lower disease severity compared to control. The lowest disease severity was recorded in the contact fungicide, T4 (mancozeb foliar spray) and the per cent reduction was found to be 52.51 per cent after the second spray. Prashant (2004) has reported the efficacy of mancozeb against *Cercospora capsici* who recorded 100 per cent inhibition of mycelia. This results was further confirmed in field condition by Khali and Jalaluddin (2004). Mancozeb is an ethylene bisdithiocarbamate which inhibits spore germination of fungi. It has multisite action against a wide range of fungi including ascomycetes, basidiomycetes and imperfect fungi and also oomycetes (Gullino *et al.*, 2010). Between the two systemic fungicides T5 (tebuconazole) was more effective than difenconazole. These two fungicides belonging to the triazole group are dimethylation inhibitors (DMI) which have preventative, curative and antispore germination action against powdery mildew, these fungicides also cause abnormal growth of fungi and the eventually death (Muller, 2007). The results of the present study, confirms the findings of Suresh (2013), who could observe that among the non-systemic fungicides mancozeb was the best compound against the *Cercospora* leaf spot in chilli.

Between the two biocontrol agents, treatment T2 (*Pseudomonas fluorescens*) recorded better reduction in severity (40.51 per cent). The results agree with findings of Raguchander *et al.*, (2005) who found that talc formulation of *Pseudomonas fluorescens* is highly effective as seed and foliar treatment against foliar diseases of urd bean (*Vigna munga*). Mechanisms of biocontrol by *P. fluorescens* are well documented which include antibiotic production, competition, HCN production and elicitation of disease resistance (ISR) reported by (Vidyasekaran *et al.*,

1997. Nandakumar *et al.*, 2001. Panpatte *et al.*, 2014). Treatment T1 (soil solarisation+ soil application of *Trichoderma viride* (*asperellum*)) gave only 39.98 per cent reduction in disease, however these were on par after second spray. Elmore *et al.*, (1997) has reported that soil solarisation is successful in combination with fungal biocontrol agent *Trichoderma* for disease management in green house. Reshma, (2016) has also reported the efficacy of soil solarization and bio control agents for disease management in protected cultivation.

In rain shelter, Incidence of *Cercospora* leaf spot was observed 82 days after transplanting and there was a gradual increase in the disease severity in all treatments. However the severity was comparatively higher in rain shelter than in polyhouse, here also the best treatment was mancozeb (T4) as in poly house. But here the biocontrol agents recorded better efficiency than systemic fungicide. The reason for better efficacy may be that the bio control agents are less exposed to sunlight and desiccation inside a protected structure compared to outside. Moreover, the disease severity was less at the time of spray (3.73 per cent) at which, the biocontrol agents are more effective. The result suggests that, biocontrol agents is effective when it is done at very early stage of disease.

### **5.5.2 Effect of different treatments on powdery mildew of capsicum under poly house and rain shelter**

In poly house, incidence of powdery mildew was observed 180 days after transplanting by which the first spray of fungicides and bio control agents was over. It was noticed that all treatments recorded lower disease severity compared to control, and the lowest was in T5-tebuconazole(0.1%) followed by T6-difenoconazole(0.05%) the per cent reduction was found to be 100 and 83.23 per cent respectively after third spray. The results confirmed the findings of Hiremath (1996). In the *in vitro* evaluation conducted earlier in this study also, tebuconazole was the best treatment with 100 per cent control of the pathogen, whereas difenoconazole at 0.1 per cent

concentration gave strongest inhibitory action against conidial germination of powdery mildew among six fungicides when tested *in vitro*. Higher efficacy of difenoconazole and tebuconazole in controlling powdery mildew in comparison with other fungicides has been reported earlier by Nagaraja and Naik (1998) in pea and Smith *et al.*, (1999) in pepper. These two fungicides belonging to the triazole group are dimethylation inhibitors (DMI) which have preventative, curative and antispore action, against powdery mildew. These, also cause abnormal growth of the fungi and eventually death (Muller, 2007). The results of the present study is in confirmation with the findings of Manojkumar *et al.*, (2008) who reported that among the systemic fungicides, tebuconazole was best chemical for control of powdery mildew of bell pepper which gave the highest fruit yield. Recently Saxena *et al.*, (2016) and Ruth (2016) were also reported tebuconazole as the best chemical fungicide against powdery mildew of chillies.

Among the biocontrol agents, foliar spray with *Pseudomonas fluorescens* recorded 68.90 per cent reduction in disease severity over control. Reduction in disease severity by *Pseudomonas fluorescens* may be attributed to production of various antagonistic secondary metabolites it produce, siderophores and other antibiotics (Muthuswamy *et al.*, 1999). Raghavendra (2005) also reported that among several bioagents tested, *P. fluorescens* was superior over the other treatments for control of powdery mildew in chilli.

In rain shelter, incidence of powdery mildew was observed 116 days after transplanting the severity of the disease was found to be lesser compared to that in poly house. Here also, the results of the experiment was similar to those in poly house, and the systemic fungicides ranked first in controlling powdery mildew. However in the case of bio control agents, efficacy was better in rain shelter compared to poly house, as in the case Cercospora leaf spot.

### 5.5.3 Effect of treatments on the biometric parameters of capsicum in polyhouse and rain shelter

In poly house experiment there was significant difference among the treatments in all the biometric characters except number of branches per plant. The plant height was the highest in T2 (*Pseudomonas fluorescens*) followed by T8. Days to flowering and fruiting was earliest in T1 (soil solarisation and soil application of *Trichoderma*). Growth promoting effect of soil solarisation and biocontrol agents is well documented. *P. fluorescens* is the major plant growth promoting rhizobacterium. Hol *et al.*, (2013) reported when *P. fluorescens* enhance plant growth, interact with plants to enhance the growth and defense, mechanism of *Pseudomonas* sp. has the greatest bio control and growth promoting activity (Elad and Freeman 2002). Sarhan and Snehata, (2014) also suggested that *Pseudomonas* sp. is a potential plant growth promoter and antifungal property.

However in rain shelter, there was no significant difference among the treatments in any of the biometric characters. This confirms the better efficacy of bio control agents in growth promotion in poly house compared to rain shelter.

### 5.5.4 Economic analysis of capsicum in poly house and rain shelter

The benefit cost (B: C ratio) was calculated at same price of Rs.50/- kg<sup>-1</sup> for capsicum for all the treatments. In poly house B: C ratio was found to be highest in T5 (tebuconazole) followed by T6 (difenconazole). Among the various treatments, same trend was observed in rain shelter also. However capsicum cultivation in polyhouse is more remunerative compared to rain shelter. This is brought about by various factors like higher growth rate and early germination. The capsicum hybrid Indra was used in present study. Naryanaswamy (2013) has also reported the highest yield produced by the same hybrid in polyhouse. Many other reports also show that

greenhouse cultivation of capsicum is highly remunerative and economical. (Grijalva-Conterras *et al.*, 2006; IHR 2012).

#### **5.5.5 Meteorological factors influencing the crop and the pathogen during experiment**

On analyzing the major meteorological parameters during the field experiment, it was observed that temperature and relative humidity are more in poly house compared to rain shelter. Severity of *Cercospora* leaf spot was more or less similar in both poly house and rain shelter but severity, of powdery mildew was higher in poly house. As closed structure causes increase in RH which is favored by the disease spread (Labeda and Cohen 2011).

#### **5.5.6 Correlation analysis of incidence and severity of fungal disease of capsicum with meteorological factors**

Correlation analysis of incidence and severity of fungal diseases with meteorological factors, was performed utilizing the data collected during the field experiments. It was found that, severity of *Cercospora* leaf spot was positively correlated with temperature and RH, which is in accordance with earlier reports (Suresh, 2013).

In the case of powdery mildew, negative correlation was observed between disease severity and temperature which is quiet normal for any powdery mildew. However, the negative correlation of the disease with RH is due to the fact that at the time of disease incidence, the RH was more than 70 per cent. It was already reported by Raghavendra (2005) that when RH increase, above 70 per cent, severity of powdery mildew decrease with increase in RH.

## 5.6 Enumeration of phylloplane micro flora of capsicum under protected condition

Numerous micro-organisms reside on the aerial surface of plants. In recent years, the use of these epiphytic micro-organisms of either saprophytic or non-pathogenic origin, such as bacteria, fungi has attained prominent role in biological control of foliar pathogens (Gowdu and Balasubramanian.1988). Population of phylloplane microflora of rice, sunflower, okra have been studied by several researchers (Gokulapalan1998, Mandhare and Suryawanshi 2009, Ogwu and Oswaru 2014). The importance of beneficial microflora on the phylloplane providing resistance to foliar pathogens has been highlighted in almost all the reports, some of these organisms called as residents (Andrew and Kinkle, 1986), may survive on the leaf surface for a longer period and together form the micro flora of the phylloplane. The effect of treatments on the phylloplane micro flora *i e.* fungi, bacteria and actinomycetes of capsicum in poly house and rain shelter were studied as part of the investigation in order to select the treatment with least impact on beneficial microflora a phylloplane.

In the present study in polyhouse it was found that application of contact and systemic compounds on leaves drastically reduced the population of phylloplane micro flora. Where, the initial population of fungi was 0.28 cfu/cm<sup>2</sup> leaf area, which was reduced to 0.25cfu/cm<sup>2</sup> leaf area, after third spray. The percent reduction of microflora over control was 81. Which it indicated that fungicides affect the non-target microbes. This is in confirmation with findings of Gokulapalan (1989) who reported the harmful effect of plant protection chemicals on epiphytic micro flora of rice. In case of rain shelter also the same trend was observed. But the reduction in population of fungi was more in the contact fungicide T4 (mancozeb) compared to other fungicidal treatments. Initially the population of fungi in T4 was 0.68 cfu/cm<sup>2</sup> leaf area. After third spray, population of fungi was 0.007 cfu/cm<sup>2</sup> leaf area. Per cent reduction over control was 94 percent, after third spray. Reports also showed that

foliar pesticide to control the diseases can cause major disruption of phylloplane populations often reducing number and diversity of organisms. This causes a negative effect on naturally occurring biological control, which in some occasions makes the plants more susceptible to other disorders (Bosshard *et al.*, 1987).

However, with regard to bacterial population, of phylloplane there was drastic reduction after application of contact and systemic fungicides. In T4 (mancozeb) was the Per cent reduction over control after third spray was 93.33 per cent in poly house. This shows that fungicides are highly toxic to non-target bacteria also and the same trend was observed in rain shelter also.

In the case of actinomycetes, the population decreased in all the treatments during the experiment. The reduction was more in fungicides. This suggests that fungicides have a toxic effect on actinomycetes population when applied repeatedly for two or three times.

### **5.7 Survival of biocontrol agents on the phylloplane of capsicum in protected condition**

On spraying biocontrol agent like *Pseudomonas fluorescens* also there is a general increase in population of bacteria in poly house and rain shelter. In polyhouse initially the phylloplane population of *Pseudomonas fluorescens* was 2.03 cfu/cm<sup>2</sup> leaf area which increased three fold by third spray (8.83 cfu/cm<sup>2</sup> leaf area). In rain shelter also same trend was observed. Initially 1.55cfu/cm<sup>2</sup> which reached 6.92cfu/cm<sup>2</sup> leaf area after third spray. Survival of *Pseudomonas florescens* on phylloplane has been earlier reported by Cirviller *et al.*, (1999) which is in confirmation with study on survival of biocontrol agent in pepper, tomato eggplant and strawberry. Reshma (2016) has also noticed the increase in population of *Pseudomonas fluorescens* on phylloplane of cucumber when applied as foliar treatment.

# *Summary*



## 6. SUMMERY

Protected cultivation is the most contemporary approach to raise horticultural crops and it is being adopted on a large scale. It is a fascinating technology gaining tremendous importance in India. Government of Kerala is also encouraging polyhouse and rain shelter cultivation of vegetable crops. Capsicum (*Capsicum annuum* L.) is one of the most preferred vegetables grown under protected condition in the developed world. It is rich in vit-A, vit-C and minerals like calcium, magnesium, phosphorus and potassium. Crop suffer from various diseases and among which fungal diseases such as powdery mildew, *Cercospora* leaf spot, fruit rot and stem and fruit rot cause major loss. Farmers have for many years relied up on chemical control to manage the diseases, which results in many undesirable effects like environmental pollution, accumulation of toxic substances and development of resistance in pathogen. However, biological control alone may not be effective for economic management of diseases, and there is an urgent need to develop integrated management of fungal diseases. Hence the present study was undertaken with main objective of to assess the incidence and severity of major fungal diseases of capsicum under protected cultivation and formulating an eco-friendly management practice against the diseases. The experiment entitled "Management of fungal diseases of capsicum (*Capsicum annuum* L.) under protected cultivation" was conducted in department of Plant Pathology, college of Horticulture Vellanikkara, during the period from August- February 2016-17.

1. Survey was conducted in poly houses where capsicum was being cultivated. Incidence and severity of fungal diseases of capsicum was assessed eight poly houses at Vellanikkara, Manalur, Thanniyam, Elanad and Puranattukara in Thrissur, Chitali in Palakkad and Neyyatinkara, Plamootikada in Thiruvananthapuram.

2. During the survey major fungal diseases observed are powdery mildew, *Cercospora* leaf spot, fruit rot and stem and fruit rot.
3. The severity of powdery mildew varied from 5.3 to 90.2 per cent, which was present in all the poly house except Vellanikkara irrespective of the season. *Cercospora* leaf spot was observed only in rainy season and disease severity varied from 2.9 to 5.4 per cent. Severity of stem and fruit rot was very less i.e. 0.5per cent and that of fruit rot 1 per cent.
4. The pathogen causing powdery mildew was identified as *Leveillula taurica* based on conidia and conidiophore characters. The fungi associated with the diseases other than powdery mildew were isolated, pathogenicity was proved and identified based on cultural and morphological characters.
5. The purified cultures were subjected to molecular characterization and it was confirmed that the leaf spot, fruit rot and stem and fruit rot of capsicum are caused by *Cercospora capsici*, *Colletotrichum capsici* and *Fusarium* sp. respectively.
6. Field experiments were conducted simultaneously inside the poly house and rain shelter for management of fungal diseases of capsicum with seven treatments and three replications. The treatments included two biocontrol agents (*Trichoderma viride(asperellum)* and *Pseudomonas fluorescens*) two systemic fungicides (tebuconazole and difenoconazole) and two contact fungicides (mancozeb and copper hydroxide)
7. Among the treatments, T<sub>4</sub> (soil solarisation + soil application of *Trichoderma* +seed treatment with carbendazim+ mancozeb (2g.kg<sup>-1</sup>) + foliar spray with mancozeb (0.2 %) was the most effective for management of *Cercospora* leaf spot in both poly house and rain shelter, followed by T<sub>2</sub> (soil solarisation+ seed treatment and foliar spray with *Pseudomonas fluorescens* (20g.L<sup>-1</sup>) and T<sub>1</sub> (soil solarisation+ soil application of *Trichoderma*) and these were statistically on par.

8. Among the treatments, T<sub>5</sub> (soil solarisation +soil application of *Trichoderma*+ foliar spray with tebuconazole (0.1 %) was the most effective for management of powdery mildew in rain shelter and poly house, followed by T<sub>6</sub> (soil solarisation +soil application of *Trichoderma viride* (*asperellum*) + foliar spray with difenoconazole (0.05%) and these were on par.
9. Correlation analysis was performed with the meteorological data recorded during the experiment and per cent disease severity (PDS) at periodic intervals and it was found that there is significant positive correlation with RH in rain shelter and temperature in poly house in case of *Cercospora* leaf spot.
10. In the case of powdery mildew, there was a significant negative correlation with PDS and temperature inside the protected structures.
11. Economic analysis of the field experiments suggested that the soil solarisation with soil application of *Trichoderma viride* (*asperellum*) in combination of tebuconazole gives the highest B:C ratio in protected structures.
12. It was found that soil temperature at 10cm depth was higher in solarized soil when compared to non-solarized soil by 6.35<sup>0</sup>C and 4.5<sup>0</sup>C inside poly house and rain shelter respectively. It was also recorded that the population of soil microflora was reduced due to solarisation in protected structures.
13. Enumeration of fungi, bacteria and actinomycetes on the leaf surface of capsicum using serial dilution planting of leaf washings, proved that there was a drastic reduction in phylloplane fungi, bacteria and actinomycetes after spraying with chemical fungicides.
14. Survival of biocontrol agents on the phylloplane of capsicum was also studied and it was found that *Pseudomonas fluorescens* survived on leaf surface up to 15 days after foliar application. It was also found that repeated sprays increases the population of *P. fluorescens* on the leaf surface.

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# *Appendices*

## APPENDIX I

### 1. Potato Dextrose Agar (PDA)

Potato	: 200.0g
Dextrose	: 20.0g
Distilled water	: 1000ml

### 2. Nutrient Agar (NA)

Peptone	: 5.0g
Beef extract	: 1.0g
Sodium Chloride	: 5.0g
Agar	: 20.0g
Distilled water	: 1000ml
PH	: 6.5 to 7

### 2. Martin's Rose Bengal Agar

Dextrose	: 10.0g
Peptone	: 5.0g
KH <sub>2</sub> PO <sub>4</sub>	: 1.0g
Mgso <sub>4</sub>	: 0.5g
Agar	: 20.0g
Rose Bengal	: 0.03g
Streptomycin	: 30.0mg (added aseptically)
Distilled water	: 1000ml

**3. Ken knight's Agar (PH 7.0)**

Dextrose	: 1.0g
KH <sub>2</sub> PO <sub>4</sub>	: 0.1g
Kcl	: 0.1g
MgSo <sub>4</sub> .7H <sub>2</sub> o	: 0.1g
Agar	: 20.0g
Distilled water	: 1000ml

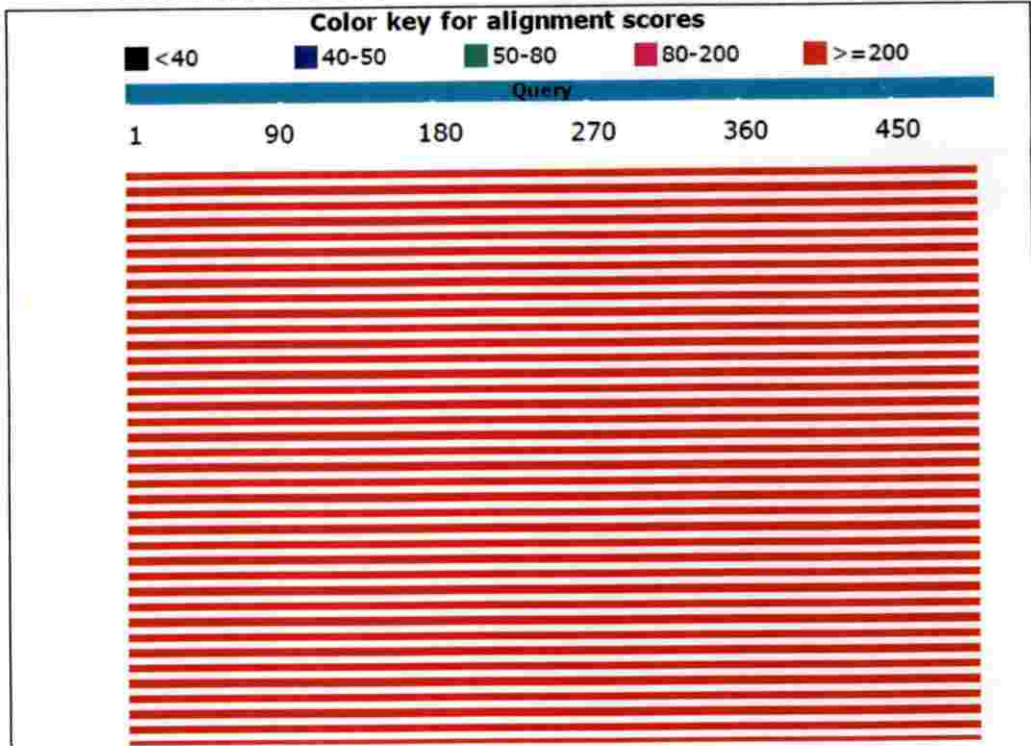
**4. King's B Agar**

Peptone	: 20.0g
Glycerol	: 10.0g
K <sub>2</sub> HPO <sub>4</sub>	: 10.0g
MgSo <sub>4</sub> .7H <sub>2</sub> o	: 1.5g
Agar	: 20.0g
Distilled water	: 1000ml
PH	: 7.2-7.4

## APPENDIX II

### Sequence analysis of *Colletotrichum capsici*

**Distribution of the top 100 Blast Hits on 100 subject sequences**



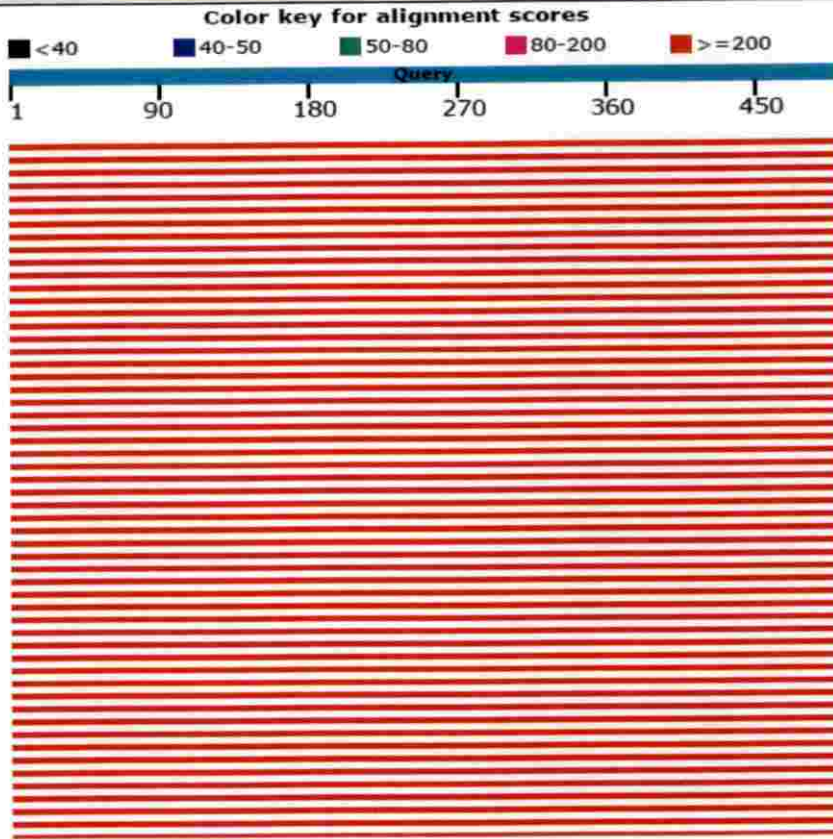
**Sequences producing significant alignments:**

Select: [All Items](#) Selected: 0

Alignments	View/Hide	Send/Save	Graphics	Statistics	View of Results		Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>						Description	1000	1000	100%	0.0	100%	LC192672.1
<input type="checkbox"/>						<i>Colletotrichum capsici</i> genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: PFR261	1000	1000	100%	0.0	100%	LC192649.1
<input type="checkbox"/>						<i>Colletotrichum capsici</i> genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: NAN2101_1	1000	1000	100%	0.0	100%	LC192642.1
<input type="checkbox"/>						<i>Colletotrichum capsici</i> genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: NAN2101_1	1000	1000	100%	0.0	100%	LC192628.1
<input type="checkbox"/>						<i>Colletotrichum capsici</i> genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: CHZ	1000	1000	100%	0.0	100%	LC192633.1
<input type="checkbox"/>						<i>Colletotrichum capsici</i> genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: FB245	1000	1000	100%	0.0	100%	LC192722.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> isolate CCC36 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, isolate: CCC36	1000	1000	100%	0.0	100%	KX649285.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> isolate CCC43 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, isolate: CCC43	1000	1000	100%	0.0	100%	KX649285.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> isolate CCJ11 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, isolate: CCJ11	1000	1000	100%	0.0	100%	KX649379.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> isolate CCP47 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, isolate: CCP47	1000	1000	100%	0.0	100%	KX649374.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> isolate HB09 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, isolate: HB09	1000	1000	100%	0.0	100%	KX294959.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> isolate HB09 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, isolate: HB09	1000	1000	100%	0.0	100%	KX364957.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> isolate H25 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, isolate: H25	1000	1000	100%	0.0	100%	KX789429.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, isolate: H25	1000	1000	100%	0.0	100%	KX885449.1
<input type="checkbox"/>						<i>Colletotrichum capsici</i> strain J105 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, isolate: J105	1000	1000	100%	0.0	100%	KX124844.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> strain F213202 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, isolate: F213202	1000	1000	100%	0.0	100%	KX197464.1
<input type="checkbox"/>						<i>Colletotrichum truncatum</i> strain F213132 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, isolate: F213132	1000	1000	100%	0.0	100%	KX197463.1

# Sequence analysis of *Cercospora capsici*

Distribution of the top 100 Blast Hits on 100 subject sequences   
 Mouse over to see the title, click to show alignments



Descriptions

Sequences producing significant alignments:

Select: All None Selected: 0

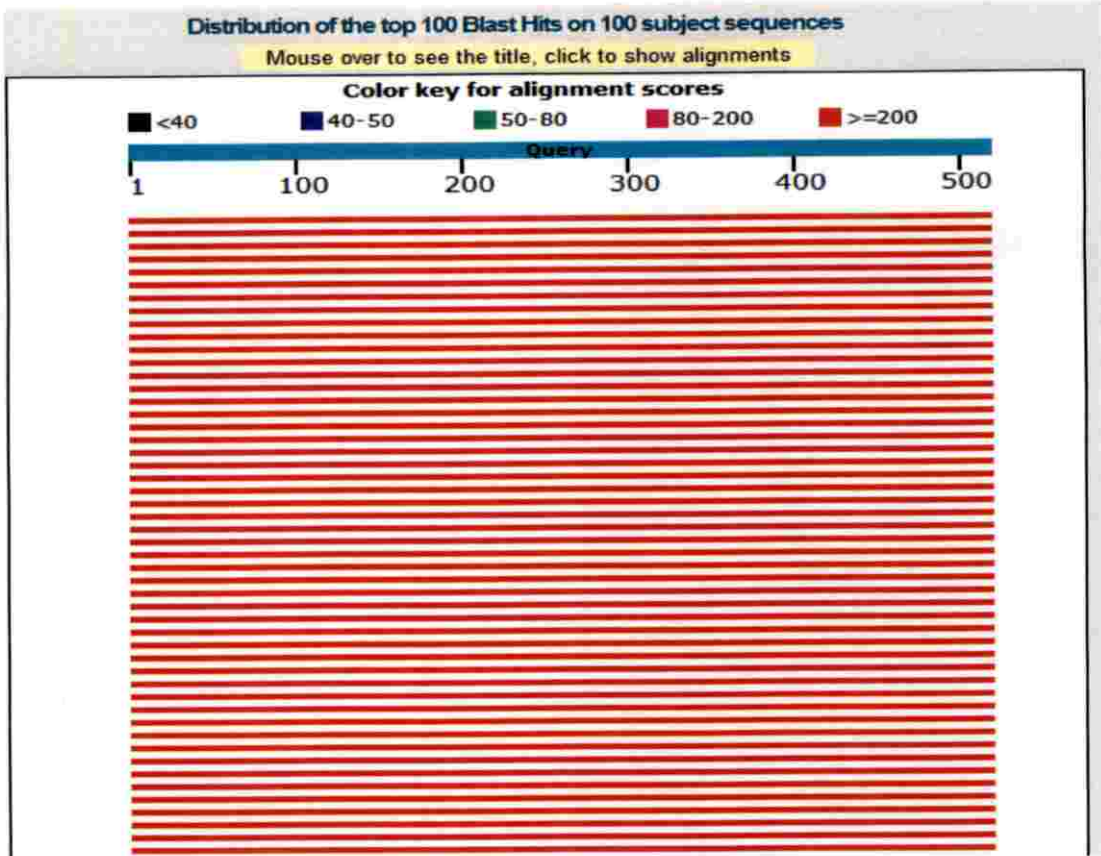
Alignments: 100 (100%)   20 (20%)   100 (100%)   100 (100%)   100 (100%)   100 (100%)

	Description	Max score	Total score	Query cover	E value	Ident	Accession
1	<i>Cercospora lyelliana</i> strain 98F-2128 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	AF232983.3
2	<i>Cercospora albivoluta</i> CPC 23832 (H) (spec. from TYPE material)	910	910	100%	0.0	100%	AF114729.3
3	<i>Cercospora</i> sp. CBS 270.21.180 (Holzner) RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	AF228723.1
4	<i>Cercospora</i> sp. strain CPC 15400 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K628727.1
5	<i>Cercospora</i> sp. Hc2013a strain CPC 13800 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
6	<i>Cercospora</i> sp. Hc2013a strain CPC 23805 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
7	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
8	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
9	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
10	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
11	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
12	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
13	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
14	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
15	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
16	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
17	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
18	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
19	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1
20	<i>Cercospora</i> cf. <i>trichomanes</i> CPC 22019 (H) ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	910	910	100%	0.0	100%	K116270.1

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## Sequence analysis of *Fusarium* sp.



Sequences producing significant alignments:

Select All None Selected 0

Alignments Download GetFacts Showtax Showtax tree of results

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	<i>Fusarium proliferatum</i> partial 18S rRNA gene for 18S ribosomal RNA, strain ITEM2409	959	959	100%	0.0	100%	U0841264.1
<input type="checkbox"/>	<i>Fusarium proliferatum</i> partial 18S rRNA gene for 18S ribosomal RNA, strain ITEM2081	959	959	100%	0.0	100%	U3841250.1
<input type="checkbox"/>	<i>Fusarium</i> sp. strain FF1474 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	KY3363354.1
<input type="checkbox"/>	Uncultured endophytic fungus genomic DNA sequence contains ITS1, ITS2, isolate H10	959	959	100%	0.0	100%	U7580084.1
<input type="checkbox"/>	Uncultured endophytic fungus genomic DNA sequence contains ITS1, ITS2, isolate H9	959	959	100%	0.0	100%	U3502278.1
<input type="checkbox"/>	<i>Fusarium</i> sp. isolate Anhe-RN412 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	K0267848.1
<input type="checkbox"/>	<i>Fusarium</i> sp. isolate Anhe-RN410 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	K0267846.1
<input type="checkbox"/>	<i>Fusarium</i> sp. isolate Anhe-RN409 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	K0267844.1
<input type="checkbox"/>	<i>Fusarium</i> sp. isolate Anhe-RN403 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	K0267840.1
<input type="checkbox"/>	<i>Fusarium</i> sp. isolate Anhe-RN401 internal transcribed spacer 1, partial sequence, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	K0267838.1
<input type="checkbox"/>	<i>Fusarium proliferatum</i> isolate FV1 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	KX891493.1
<input type="checkbox"/>	<i>Fusarium proliferatum</i> strain CCTU1232 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	KY020298.1
<input type="checkbox"/>	<i>Fusarium proliferatum</i> strain CCTU1051 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	KY020297.1
<input type="checkbox"/>	<i>Fusarium proliferatum</i> strain CCTU1216 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	KY020296.1
<input type="checkbox"/>	<i>Fusarium proliferatum</i> strain CCTU1090 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	KY020295.1
<input type="checkbox"/>	<i>Fusarium proliferatum</i> strain CCTU1091 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	KY020294.1
<input type="checkbox"/>	<i>Fusarium</i> heterotolide strain R1K17 small subunit ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	KY605583.1
<input type="checkbox"/>	<i>Fusarium verticillioides</i> isolate Lic31 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	959	959	100%	0.0	100%	K013201.1

**MANAGEMENT OF FUNGAL DISEASES OF CAPSICUM  
(*Capsicum annuum* L.) UNDER PROTECTED CULTIVATION**

By  
**DEEPA PAWAR**  
(2015-11-107)

**ABSTRACT OF THE THESIS**

Submitted in partial fulfilment of the requirement  
for the degree of

**Master of Science in Agriculture**

(PLANT PATHOLOGY)

Faculty of Agriculture  
Kerala Agricultural University



**DEPARTMENT OF PLANT PATHOLOGY  
COLLEGE OF HORTICULTURE  
VELLANIKKARA, THRISSUR 68656  
KERALA, INDIA**

2017

## ABSTRACT

The present study entitled "Management of fungal diseases of capsicum (*Capsicum annuum* L.) under protected cultivation" was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara during June to February 2016-17. The major objective was to assess the incidence and severity of fungal diseases of capsicum under protected cultivation and to formulate an eco-friendly management practice.

A survey was conducted in three districts of Kerala, Thrissur, Palakkad, and Thiruvananthapuram by selecting nine poly houses where capsicum is being cultivated during August to February 2016-17. During the survey, incidence of fungal diseases like powdery mildew, leaf spot, fruit rot, stem and fruit rot were noticed on capsicum under protected structures at various locations. Incidence of powdery mildew was observed in all the polyhouses except Vellanikkara, and the disease severity varied from 5.3 to 90.23 per cent. Leaf spot was noticed in three poly houses, during rainy season, the per cent disease severity varied from 2.97 to 5.36 per cent. Symptomatology of fungal diseases of capsicum observed during the survey was studied. The fungi associated with the diseases were isolated and the pathogenicity was proved. Cultural and morphological characterization of the pathogens was carried out and the fungi were tentatively identified as *Leviellula taurica*, *Cercospora capsici*, *Colletotrichum capsici* and *Fusarium* sp. The isolates were sent to Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram for DNA sequencing and based on molecular characterization, it was confirmed that the leaf spot, fruit rot and stem and fruit rot of capsicum are caused by *Cercospora capsici*, *Colletotrichum capsici*, and *Fusarium* sp. respectively.

Field experiments were conducted simultaneously inside the poly house and rain shelter for management of fungal diseases of capsicum with seven treatments and three replications. The treatments included two biocontrol agents (*Trichoderma viride* (*asperellum*) and *Pseudomonas fluorescens*) two systemic fungicides (tebuconazole

and difenoconazole) and two contact fungicides (mancozeb and copper hydroxide). Among the treatments, T<sub>4</sub> (soil solarisation + soil application of *Trichoderma asperellum* + seed treatment with carbendazim + mancozeb (2g.kg<sup>-1</sup>) + foliar spray with mancozeb (0.2 %) was the most effective for management of *Cercospora* leaf spot in both poly house and rain shelter, followed by T<sub>2</sub> (soil solarisation + seed treatment and foliar spray with *Pseudomonas fluorescens* (20g.L<sup>-1</sup>) and T<sub>1</sub> (soil solarisation + soil application of *Trichoderma viride* (*asperellum*)) and these were statistically on par. Among the treatments, T<sub>5</sub> (soil solarisation + soil application of *Trichoderma asperellum* + foliar spray with tebuconazole (0.1 %) was the most effective for management of powdery mildew in rain shelter and poly house, followed by T<sub>6</sub> (soil solarisation + soil application of *Trichoderma* + foliar spray with difenoconazole (0.05%) and these were on par. Correlation analysis was performed with the meteorological data recorded during the experiment and per cent disease severity (PDS) at periodic intervals and it was found that there is significant positive correlation with RH in rain shelter and temperature in poly house in case of *Cercospora* leaf spot. In powdery mildew, there was a significant negative correlation with PDS and temperature inside the protected structures. Economic analysis of the field experiments suggested that the soil solarisation with combination of biocontrol agents and tebuconazole recorded the highest B:C ratio.

It was found that soil temperature at 10cm depth was higher in solarized soil when compared to non-solarized soil by 6.35<sup>0</sup>C and 4.5<sup>0</sup>C inside poly house and rain shelter respectively. It was also recorded that the population of soil microflora was reduced due to solarisation in protected structures.

Analysis of population of phylloplane microflora proved that there was drastic reduction in the population of phylloplane fungi, bacteria and actinomycetes after spraying with chemical fungicides whereas the population increased after spraying with biocontrol agents. Survival of biocontrol agents on the phylloplane of capsicum

was also studied and it was found that *Pseudomonas florescens*, survived on the leaf surface up to 15 days after foliar application.

Thus, the study has confirmed the identity of causal agents of major fungal diseases of capsicum. *Cercospora capsici* reported for the first time on bell pepper from the country. It also suggested economical and effective management practices against the diseases. Further, the results of the study prove that, timely application of biocontrol agents gives satisfactory control of diseases without affecting beneficial microbes

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