MANAGEMENT OF DOWNY MILDEW (*Pseudoperonospora cubensis* (BERK. & CURT.) (ROSTOV.) OF CUCUMBER UNDER PROTECTED CULTIVATION

By

RESHMA RAJ T.

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Management of downy mildew (*Pseudoperonospora cubensis* (Berk. & Curt.) Rostov.) of cucumber under protected cultivation"** is a bonafide 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, associateship, fellowship or other similar title, of any other university or society

Vellanikkara,
Date 7/11/2016

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CERTIFICATE

Certified that the thesis entitled **"Management of downy mildew (*Pseudoperonospora cubensis* (Berk. & Curt.) Rostov.) of cucumber under protected cultivation"** is a record of research work done independently by **Ms. Reshma Raj T.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her

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Reshma Raj T

Dedicated to my beloved parents

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Introduction

1. INTRODUCTION

Protected cultivation of vegetables is a fascinating technology gaining tremendous importance in India as a part of increased commercialization of agriculture It makes use of recent advances in technology to control the environment for higher crop productivity and better quality of produce India has entered into the area of protected vegetable cultivation recently and the total area under polyhouses is not more than 10,000 hectares The government of Kerala is also promoting cultivation of vegetables under polyhouse to a great extent The precise environmental and nutritional control in the protected structures leads to new heights of growth and productivity At the same time, this can generate chronic stress conditions mainly those of biological origin

Prevalence of microclimate which is congenial for multiplication and spread of plant pathogens, high density cropping and monocropping of high yielding genotypes make the plants under polyhouse predisposed to pathogens So far, enough attention has not been paid to standardize technology for control of diseases and pests in polyhouses Though polyhouse cultivation provides opportunity to grow vegetables all through the seasons in Kerala, ram shelter, which is comparatively low cost structure, is also considered as a better alternative It provides more variability in the choice of crops and varieties Major vegetable crops cultivated under protected system are cucumber, capsicum, tomato, French beans, cauliflower, chilli, broccoli, knol khol, and coriander (Wittwer and Castilla, 1995)

Cucumber, *Cucumis sativus* (L) is one of the most preferred vegetables grown under protected conditions in the developed world In India it is traditionally grown during January-March and September-December Compared to open field, very high yield of cucumber has been reported even under naturally ventilated polyhouse

(Srivastava and Singh, 1997) But sometimes, the crop is devastated by diseases like powdery mildew, downy mildew, anthracnose, *Alternaria* blight and *Fusarium* wilt (Yucel *et al* , 2013), and among them, the major disease is downy mildew

Cucurbit downy mildew caused by the oomycete *Pseudoperonospora cubensis* (Berk and Curt)(Rostov) is the most severe disease of cucumber under polyhouse in Kerala According to Singh and Banyal (2007), it is the major disease followed by powdery mildew especially during rainy season Downy mildew is the most widely distributed disease of cucurbitaceous crops in the open field and under cover (Labeda and Cohen, 2011) Farmers use fungicides with high residual toxicity against the disease Owing to increasing awareness about the health hazards that may occur due to the consumption of vegetables with high amount of pesticide residues, organic cultivation is being promoted by policy makers Since there is provision for controlled atmosphere in a protected structure, organic farming and use of biocontrol agents will be more effective compared to open fields

Use of chemical fungicides leads to destruction of valuable beneficial microbes which in turn lead to increased vulnerability of crops and development of resistant strains of pathogens Moreover, due to high input costs, crop failure by diseases results in heavy economic losses Hence, precise disease management with emphasis on biocontrol is essential for assuring profitable farming in protected structures

In this back ground, the present study was undertaken with the following objectives,

1 Assessment of incidence and severity of downy mildew of cucumber under protected and open condition in farmers' fields in Thrissur district
2 Characterization of the pathogen
3 Management of downy mildew of cucumber under polyhouse and rain shelter condition

4 Enumeration of phylloplane microflora of cucumber under protected condition

5 Survival of the biocontrol agents on the phylloplane of cucumber under protected condition

Review of Literature

2. REVIEW OF LITERATURE

Cucumber, *Cucumis sativus* (L) is one of the most preferred vegetables grown under protected conditions in the developed world The crop is devastated by various diseases and among them, the major foliar disease is downy mildew Cucurbit downy mildew, caused by the oomycete *Pseudoperonospora cubensis*, (Berk & Curt) (Rostov), causes heavy crop loss in protected structure as well as in open field Annual loss to the cucumber crop at global level due to the pathogen has been estimated to be $ 246 2 million Since the use of chemical fungicides leaves pesticide residue in the commercial produce which is consumed as raw vegetable (salad), organic farming is being promoted in Kerala Therefore the present study aims at management of downy mildew of cucumber under polyhouse and rain shelter, with emphasis on biological control

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

2 1 Protected cultivation of vegetables
2 2 Symptomatology of cucumber downy mildew
2 3 Characterization of the pathogen
2 4 Epidemiology
2 5 Management by chemical control
2 6 Management by biological control
2 7 Management by biofungicides
2 8 Phylloplane microflora
2 9 Survival of biocontrol agents

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) In India, presently around 25,000 ha are under protected structures, out of which the area under vegetable crops is about 2,000 ha So far, 1108 polyhouses of size 40-400 m^2 are actively engaged in crop production in Kerala and the major vegetables being cultivated in polyhouses are salad cucumber, capsicum, cow pea, bhindi, tomato *etc* However, 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, chillies, cowpea, cauliflower and capsicum in rain shelter in a rotational manner gave bumper yield compared to cultivation in open field Moreover, salad cucumber gave 180 per cent more yield in rain shelter than open condition

Cucumber, popularly known in India as 'khira' and gherkins, are extensively grown in tropics, subtropics and milder temperate zones of the country In India it is traditionally grown during January-March and September-December Compared to open field, very high yield of cucumber have been reported under naturally ventilated polyhouse (Srivastava and Singh, 1997) Singh and Kumar (2006), reported that parthenocarpic cucumber cultivation in greenhouse can be a profitable venture for the vegetable growers and yearly three crops can be taken Cucumber is used as salad, pickle and also as cooked vegetable because of its low calorie content The fruits and seeds possess cooling properties on consumption, hence used as astringent and antipyretic

2.1.1 Diseases of vegetable crops under protected cultivation

Greenhouse vegetable crops are vulnerable to various pests and diseases Since polyhouses are enclosed structures, it provides favourable environment for rapid multiplication of plant pathogens Once inoculum enters in to the structures, the

spread of the disease become rapid Epidemic initiate when the available inoculum meets a susceptible host in a favourable environment Once infection is established on plants in the greenhouse, it provides inoculum for secondary spread The spores of airborne pathogens like downy mildew and gray mold are produced in large quantities, under humid conditions, but are released most readily when humidity drops The most frequently occurring airborne fungal pathogens in protected structures are species of *Alternaria Botrytis Cladosporium Didymella Erysiphe Fulvia, Leveillula Phoma Phytophthora Pseudoperonospora Puccinia Sclerotinia, Septoria and Sphaerotheca* and the common fungal diseases that thrive under the polyhouses include grey mold (*Botritis cinerea*), tomato late blight (*Phytophthora infestans*), tomato leaf mold (*Cladosporium fulvun* syn *Fulvia fulva)* and powdery mildews in cucurbits (*Sphaerotheca fusca* syn *S fuliginia*), roses (*S pannosae*) and various other crops (*Leveillula taurica*) (Yucel *et al* , 2013)

The major diseases affecting cucumber in protected structures are fusarium wilt (*Fusarium oxysporum* f sp *cucumerinum*), downy mildew (*Pseudoperonospora cubensis*), damping off (*Pythium aphanidermatum*) and powdery mildew (*Erisiphe cichoracearum*) and among them, downy mildew, caused by the oomycete *Pseudoperonospora cubensis* is favoured by high humidity (Rusell, 2004, Sabir and Singh, 2013)

2.1.2 Distribution and severity of cucumber downy mildew

Downy mildew leads to serious losses in cucumber production when the crop is grown under protected structures These structures provide ideal temperature and promote high humidity for development of the disease which becomes the main limiting factor for cucumber production (Wehner and Shetty, 1997) Abawi and Widmer (2000) reported that more consistent environment, moderate temperature, high humidity, less air movement, overcrowded conditions and lush growth in the polyhouse favours downy mildew outbreak The mild temperature and high-humidity

environment in protected structures are suitable for the development of downy mildew disease and can result in infections and epidemics (Yang *et al* , 2007)

Singh and Banyal (2007) reported that in cucumber grown under polyhouses, downy mildew causes 20-90 per cent disease severity and it was also reported that the severity of downy mildew was high during rainy season The disease is damaging in warm, humid climates where the pathogen thrives and it affects cucurbit crops in the field as well as in greenhouses Downy mildew decreases flower set and fruit development by killing the foliage (Kristkova *et al* , 2009) According to Aujla *et al* (2010), downy mildew on cucumber grown under polyhouses shifts in the time of appearance of the disease from April in open field to February Its early appearance is attributed to favourable relative humidity and temperature and early growth of the crop

2.2 Symptomatology of cucumber downy mildew

Initially the lesions of downy mildew are angular and restricted by the veins of the leaf, particularly on *Cucumis sativus* L (salad cucumber) As the disease progess, chlorotic lesions become necrotic, during hot and dry weather conditions (Oerke *et al* , 2006) Savory *et al* (2010) characterized the disease as a 'downy' or 'felt' appearance on leaves which is due to the sporangia found on the abaxial side of the leaf Primary lesions of *Pseudoperonospora cubensis* are between 3 -10 mm, as the disease progresses the lesions combine to form larger lesions that can eventually cover the entire leaf In extreme conditions, leaves turn necrotic and sometimes lead to the death of whole plant (Lebeda and Cohen, 2011)

2 3 Characterization of the pathogen

The pathogen causing downy mildew of cucumber was discovered in Cuba by Berkeley and Curtis, (1868) as '*Peronospora cubensis* It was reclassified based on zoosporic germination of sporangia to *Pseudoperonospora cubensis* in 1903 by Rostovzev (Skalicky, 1961) *Pseudoperonospora* species have true sporangia that

germinate *via*, cytoplasmic cleavage to produce zoospores whereas species of *Peronospora* have sporangia that germinate directly *via* germ tube (Palti and Cohen, 1980) The genus *Pseudoperonospora* belongs to a taxonomic group on the border between a genus that regularly produce zoospores (*Pythium* spp) and another that never produce zoospores *Peronospora* spp (Goker *et al* , 2007)

Pseudoperonospora cubensis is one of the most economically important and widespread biotrophic plant parasite belongs to kingdom Chromista, phylum Oomycota, subphylum Peronospororomycotina, class Peronosporomycetes (Oomycetes), order Peronosporales (downy mildews), family Peronosporaceae (Voglmayr, 2008, Savory *et al* , 2010)

Being a biotroph or obligate parasite, the organism requires living host tissue to grow and reproduce and so it cannot be cultured on artificial media Morphologically the first sign of disease include sporulation as the 'downy' appearance on the lower leaf surface (Rotem *et al* , 1978) Sporangiophore morphology of *P cubensis* can vary with temperature, and sporangia dimensions are influenced by the cucurbit host (Waterhouse and Brothers, 1981) Recent work with a single isolate of the pathogen inoculated onto six different cucurbit species has shown that the host cell matrix plays a role in influencing five morphological criteria, including sporangiophore length, length of ultimate branchlets, sporangial length and width, and the ratio between sporangial length and width (Runge and Thines, 2011)

Pseudoperonospora spp has dichotomously-branched sporangiophores with terminal growth and the sporangia of similar age are present at the ends of sterigma (Voglmayr, 2003, Choi *et al* , 2005) whereas, *Pseudoperonospora cubensis* forms hyaline sporangiophores (180–400 mm) bearing large (20-40 x 14-25 mm in diameter), lemon-shaped grey–purple sporangia with a conspicuous papilla The sporangia are borne singly on the pointed tips of sporangiophores that branch at acute angles The sporangiophore ranges from 180 600 mm in height, 20 mm in diameter and 5 7 mm in width Moisture prompts the sporangia to release 5 to 15 asexual,

ovoid zoospores that measure 10-13 mm in diameter The zoospores are biflagellate, with one posterior whiplash and one anterior tinsel flagellum The purpose of the flagella is to assist the zoospores as they swim through free moisture on the leaf surface to the stomata (Cohen, 1981) Once a stoma is located, the zoospore will encyst (produce a cell wall) and then form a germ tube (50-95 mm), which will enter through the stoma and infect the host plant cell

Once a host plant is infected, mycelium grows within and between host cells and serves as the body of the pathogen The intercellular mycelium is hyaline (colorless, transparent) and coenocytic (aseptate) The mycelium develops in the mesophyll, but also penetrates the palisade tissue The hyphal diameter is 5 4-7 2 mm (Colucci, 2008) Haustoria are formed within the host cells and allow for the absorption of nutrients They vary in shape and appear stunted, inflated or as branched clusters of hyphae The pathogen reproduces predominantly asexually, but has been reported to reproduce sexually *via,* the production of oospores, although oospore production is rare (Lebeda and Cohen, 2011)

2.4 Epidemiology

Plant disease epidemiology is the study of factors that affect the spread of diseases in time and space Being an oomycete, nicknamed as water moulds, downy mildew prefer high humidity and excess water Moisture on the leaf surface is necessary for infection to occur When zoospores land on a wet leaf surface, they swim in the film of water on leaves during humid or wet conditions and enter and infect through stomata Optimum temperature for infection ranges between 16°C and 22°C The period of wetness needed for infection on cucumber leaves are about 12hr at 10-15°C, 6 hr at 15- 19°C and 2hr at 20°C (Palti and Cohen,1980) The incubation period from initial infection to visible symptoms varies depending on temperature, free moisture and inoculum level, but generally ranges between three to 12 days Symptoms may vary slightly depending on the host plant

Sporangia and sporangiophores are greatly affected by changes in temperature and humidity Once sporangia land on a susceptible host, free moisture is required for each sporangium to release 5-15 zoospores Free moisture is also important for zoospore movement, germ tube development and penetration of host tissue by the germ tube However, excess moisture may reduce the duration of sporangia viability Optimal temperature for sporulation is 15 °C with 6 to 12 hours of moisture Warm and dry atmosphere at early morning hours causes twisting of the sporangiophores, which is important for the detachment of sporangia If sporangia experience dry periods even for a short interval, the spore is prevented from germination (Ledeba and Cohen, 2011)

New sporangiophores, differentiated from the mycelium, emerge singly or in groups from the epidermis, usually through the stomata The new sporangia are produced 4-12 days after initial infection, depending on temperature and humidity High humidity is required for the emergence of sporangiophores High temperatures (>35°C) are not favorable for disease development However if cooler night time temperatures occur, disease development may progress (Labeda *et al*, 2011) At normal greenhouse temperatures, if relative humidity exceeds 70-75 per cent there is chance of occurrence of downy mildew (Ferguson, 2007) Spores can quickly spread within the greenhouse *via* moist air currents, contaminated tools, equipment, fingers and clothing The spores become less infective under conditions of high temperature and low humidity in the greenhouse

Asexual spores do not survive for a long time under common environmental conditions When detached from sporangiophores, they lose viability and infection ability quickly (Cohen and Rotem, 1971) Because of the obligate biotrophic nature of *P cubensis* survival of mycelium in dead leaves is not possible (Lebeda, 1991) The main way of survival in unfavourable conditions (overwintering) is by the formation of thickwalled resting oospores (Cohen *et al*, 2003, Zhang *et al*, 2008)

2.5 Management by chemical control

Cucurbit downy mildew is one of the most economically important and prevalent diseases of cucurbitaceous crops with worldwide distribution (Lebeda and Cohen, 2011, Lebeda *et al*, 2011) The continual changes in pathogen populations worldwide make it difficult to manage cucurbit downy mildew Cucumber downy mildew is the most destructive cucumber disease, causing significant losses in yield and quality, and its control requires extensive chemical measures (Savory *et al*, 2010)

Even though chemical control by fungicides may have negative environmental effects, fungicides still constitute the predominant part of the control measures used against oomycetes (Gisi and Sierotzki, 2008) According to Gisi (2002), the sales value of fungicides against downy mildews amounted to 1 2 billion in 1996, of which 10 per cent were used to fight mainly *P cubensis* on cucurbit crops

Chemical control of *P cubensis* was done for many decades by contact fungicides and dithiocarbamates (Cohen, 1981) These fungicides prevent zoospore release and cystospore germination Their application is effective only if spraying is done before sporangial deposition (Urban and Lebeda, 2006) Spraying with fungicides like dithane M- 45 (Maneb), Dithane Z-78 (Zineb), blue copper blitox (0 4%) and Tricop-50 have been recommended for management of downy mildew of cucumber and among them spraying with blue copper blitox (0 4%) thrice at 8 days showed maximum disease reduction (Bains and Jhooty, 1976) Besides this, frequent spray of copper containing fungicides (Bordeaux mixture and copper oxy chloride) is economical but it leaves residual effect in fruits and foliage

Call *et al* (2013), tested the efficacy of contact fungicide, mancozeb against downy mildew of cucumber and the results showed that mancozeb cause a significant reduction in the germination of sporangia of the downy mildew pathogen Results also revealed that among the tested fungicides, *i e* Folu Gold, Indofil M45, Galben

Copper, Previcure-N and Redomil Gold, mancozeb (Indofil M45) caused a significant reduction to the germinated sporangia of cucumber downy mildew Because of the resistance of the *Pseudoperonospora cubensis* to systemic fungicides (metalaxyl, azoxystrobin, propamocarb, fosetyl Al and oxadixyl), these fungicides can be used alone or in combination with protective fungicides (chlorothalonil, zineb, mancozeb and copper), and proved to be successful in the control of the disease (Keinath *et al*, 2007, Call *et al*, 2013) Zhan-Bin Sun *et al* (2013) reported a 69 per cent disease reduction in cucumber downy mildew by spraying with metalaxyl-mancozeb WG in greenhouses

Cymoxanil is a cyanoacetamide oxime group fungicide which quickly penetrates into the leaf tissue and destroys the already emerging pathogens It allowed a 50 per cent reduction in the amount of mancozeb needed to control downy mildew when applied in a mixture with mancozeb, and treatment with this mixture at 10 day intervals provided satisfactory protection It was found to have a curative effect when applied during the incubation period (Klopping and Delp, 1980) When this product was applied at fixed times, excellent control was obtained with good economic savings compared to traditional chemical treatments Ziogas and Davidse (1987) reported that with the action of cymoxanil, RNA synthesis was differentially inhibited in the various developmental stages of the fungus

During the last decades, systemic fungicides have been developed and widely used for downy mildew management, e g , (cymoxanil in 1976, phosetyl-Al in 1977, phenylamides in 1977–1983, propamocarb in 1978, dimethomorph in 1988, cyazofamid in 2001, zoxamide in 2001, mandipropamid in 2005, fluopicolide in 2006) (Lebeda and Cohen, 2011) Bhat *et al* (2013) reported that out of the 11 fungicides evaluated for the management of downy mildew of cucumber, minimum disease severity (7 10 %) was recorded with Ridomil MZ application followed by Ridomil Gold (9 35 %), Amistar 25 SC (10 28 %), Curzate M-8 (17 01 %), Acrobat (19 26 %), Indofil M 45 (22 94 %), Infinito (23 11 %), Mandipropamid (24 34 %), Antracol (24 57 %), Polyram (26 27 %) and Kocide (28 45 %) as compared to 77 48 per cent in untreated check

The major site-specific fungicides that are effectively used in chemical control of *P cubensis* are from four chemical classes which include, the quinone outside inhibitors(QoI), (strobilurins, e g azoxystrobin, famoxadone, fenamidone), phenylamides (e g metalaxyl-M), carboxylic acid amides (e g dimethomorph, iprovalicarb, benthiavalicarb, mandipropamid) and cyanoacetamide oximes (e g cymoxaml) Azoxystrobin, a novel fungicide showed maximum disease control with low residues (Gisi and Sierotzki, 2008)

Under greenhouse conditions, the treatments with fungicides gave better results although it is environmentally unsafe Most systemic fungicides have a specific, single-site mode of action, which means that they are active at one point in one metabolic pathway of the pathogen (Urban and Lebeda, 2006) The introduction of these systemic fungicides significantly increased the efficiency of fungicides against downy mildews (Wang *et al*, 2009) Systemic fungicides also have some curative effects, as they can stop the development of disease for a certain time after infection (Urban and Lebeda, 2006)

But systemic fungicides can be both harmful to the environment and for human consumption with continued use Additionally, resistance can occur with use of fungicide products with similar active groups There are now a range of alternative fungicides available including biological control agents, foliar fertilizers, natural and mineral oils and hydrogen peroxide Some may be suitable to effectively control downy mildew (Jovicich, *et al* , 2010)

2 6 Management by biological control

Pseudoperonospora cubensis is categorized as the pathogen possessing a high risk of developing fungicide resistance by the Fungicide Resistance Action Committee (FRAC) and considered to be one of the 10 highest risk pathogens with high evolutionary potential (Russell, 2004 , Lebeda and Cohen, 2011) These reports indicate the need for biological management of the pathogen

The term 'biological control' literally refers to the utilization of one or more organisms in combination to eliminate detrimental microbes In recent years, public concern has increasingly focused on food safety issues, especially the effects of pesticide residues (Vukovic *et al* 2012) Since cucumbers are harvested every second or third day, the presence of fungicide residues and their degradation rates are a limiting factor in the production of high quality and residue free fruits Biological control is an alternative means of management of foliar pathogens and only a few micro-organisms have been fully commercialized for this (Fravel *et al* 1994) 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) Recently, attempts were also made to use a consortium of biocontrol agents to get persistent control of plant pathogens (Chaube and Sharma, 2002) Antagonistic fungi especially *Trichoderma* spp and the bacteria, *fluorescent pseudomonads* have been widely used against a number of phytopathogens and some strains induce plants to change their native defense mechanisms (Rini and Sulochana, 2006) It is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection and it can be introduced in organic system of food production (Harman *et al*, 2004, Kowalska, 2010)

One of the most studied commercial biocontrol agents is *Trichoderma harzianum* which can be regarded as a biocontrol model to demonstrate control under commercial conditions (Elad, 1994) The mechanisms involved in biocontrol of foliar pathogens by *Trichoderma* species are mycoparasitism, antibiosis and competition for space and nutrients, induction of systemic resistance, *etc* It is possible that more than one mechanism is involved in a host antagonist-pathogen interaction (Sawant, 2014) In addition to direct antagonism, biocontrol agents triggers/activates plants' latent defense mechanisms in response to infection by pathogen and increases the activities of various defense related enzymes and chemicals in response to infection by pathogen Inducing the plant's own defense mechanism by prior application of biological agents is a novel strategy in plant disease management

Vivekanandan *et al* (2004) reported that *P fluorescens* produce antibiotics *viz* , HCN, pyrrolnitrin, phenazine and 2,4-diacetyl phloroglucinol and lytic enzymes against fungal pathogens According to Panpatte *et al* (2014), the mechanism of biological control in *P fluorescens* against foliar pathogens include antibiotic production, competition, HCN production and elicitation of a disease-resistance response, *i e* , induced systemic resistance (ISR)

The biocontrol agent *Trichoderma* spp controls the foliar pathogens like *Pseudoperonospora cubensis* and *Sclerotinia sclerotiorum* in cucumber under commercial greenhouse *Pseudomonas fluorescens* and *Trichoderma harzianum* have markedly reduced the downy mildew pathogen in cucumber hosts to the 10 extent of 46 per cent (Elad, 2000) Machenahalli *et al* (2013) reported that among biocontrol agents *Pseudomonas fluorescens* was found to be superior in inhibiting sporangial germination in *P cubensis* at six per cent concentration

P fluorescens control downy mildew by both seed treatment and foliar application but efficacy was significantly higher when seed treatment was followed by foliar application (Umesha *et al* 1999) Some isolates of filamentous fungi, yeast-like fungi, yeasts and strains of bacteria inhibited the activities of various pathogenic foliar fungi (Yohalem, 2000) Biological control, therefore, acts as a strategy for disease management and it is environment friendly also *Pseudomonas fluorescens* and mixture of *Trichoderma* spp isolates were the most effective biocontrol agents in controlling air borne diseases (powdery and downy mildew) on varieties of cucumber and the highest yield was obtained when only *P fluorescens B subtilis* and mixture of *Trichoderma* spp isolates were used (Abd-El Moity *et al* , 2003)

2.6.1 Soil Solarization

Soil solarization is a cost saving and environmentally safe nonchemical soil disinfestation method that, under appropriate conditions, can ensure an effective control of a wide range of pathogens, weeds and arthropod pests Increase in soil

temperature by solarization must be sufficiently high and prolonged to cause irreversible damage to most soil-borne pathogens (Fenoll *et al* 2010) Therefore, this technique is particularly suitable for the Mediterranean climate, where the occurrence of high summer temperatures can ensure an effective control of fungi, nematodes and weeds (Camprubi *et al*, 2007) Since oospores of downy mildew harbour and survive in plant debris of cucumber in soil (Labeda and Cohen, 2011), solarization also helps to bring down the initial inoculum and inoculum build up in soil

Moistened soil is covered 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 wavelength on reflection and thus increasing the soil temperature (Katan, 1981) This increase in soil temperature in the presence of high moisture is the most important mechanism by which soil solarization controls diseases Greenberger *et al* (1987) reported that solarized soil is frequently more suppressive and less conducive to certain soil borne pathogens than non-solarized soils Soil solarization has also been successfully combined with the fungal biological control agents *Trichoderma hazianum* and *Talaromyces flavus*, which were added to the soil or planting material Soil solarization in greenhouses produces significantly higher soil temperatures than solarization in fields and can therefore be more effective in cooler weather (Elmore *et al* 1997)

Candido *et al* (2008) conducted soil solarization in greenhouse for a period of 79 days and reported that the difference in temperature between solarized and nonsolarized soil was on an average 7 1, 6 4 and 6 0 °C respectively, at 10, 20, 30 cm depth and the maximum temperatures were 47 3, 45 2 and 43 9 °C in solarized soil and 39 4, 37 9 and 36 9 °C in nonsolarized soil respectively, at 10, 20 and 30 cm depth Moreover, soil solarization improves soil structure and increases the availability essential plant nutrients and further increases plant growth, harvestable yield, and crop quality The increased growth response of plants in solarized soil is a well-documented phenomenon and has been verified both in green house experiments and under field conditions (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 Bagyaraj, 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 polyhouses effectively reduced the soil microflora *viz*, fungi, bacteria and actinomycetes Changes in the populations of soil-borne microorganisms 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 Escherichia coli, Pseudomonas aeruginosa Bacillus anthracis and Bacillus subtilis,* are the predominant species in the soil samples Similarly, different types of fungi dominating in soil samples include, *Aspergilllus niger Aspergillus flavus Trichoderma* sp, *Fusarium oxysporum, Rhizopus* sp

2.7 Management by biofungicides

Biofungicides such as garlic and Calphomil plays a prominent role in management of diseases in polyhouses Portz *et al* (2008), reported that allicin in garlic juice reduced the severity of cucumber downy mildew caused by *Pseudoperonospora cubensis* by approximately 50–100 per cent The results emphasis the potential for developing preparations from garlic for its use in specialised aspects of organic farming, under greenhouses Calphomil is leaf extract of many leaves, of which a major constituent is *Aegle marmalos* It has been reported that it increases nitrate reductase activity and chlorophyll content when applied on leaves and fruits It also reduces the phylloplane microflora which helps to further increase the shelf life of the produce (Azhagumurukan *et al*, 2013) Besides this, it contains different types of alkaloids (solasodine and solanine) and terpinoids which causes antifungal activity (Nigam and Nambiar, 2015)

2.8 Phylloplane microflora

Numerous micro-organisms reside on the aerial surface of plants especially on leaves In recent years, the use of these epiphytic microorganisms of either non-pathogenic or saprophytic origin, play an important role in reducing foliar diseases (Leben, 1965) , and they have attained prominent role in biological control of foliar pathogens (Gowdu and Balasubramanian, 1988) Some of these organisms such as bacteria, fungi called as residents (Andrews and Kinkel, 1986), may survive on the leaf surface for a longer period and together form the microflora of the phylloplane (Bonnen and Hopkins, 1997) Common phylloplane microflora include *Cladosporium* spp , *Phoma* spp *Epicoccum* spp , *Alternaria* spp , *Aureobasidium pullulans* and *Pseudomonas* spp

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 Among the bacteria, most predominant ones are Gram-negative, often chromogenic, and include the following genera *Erwinia Pseudomonas Xanthomonas, and Flavobacterium* Gram-positive bacteria such as *Lactobacillus Bacillus*, and *Corynebacterium* are isolated less frequently In addition to saprophytic bacteria, pathogenic bacteria, e g *Pseudomonas syringae* pv *syringae P syringae* pv *morsprunorum, P syringae* pv *glycinea Erwinia amylovora* and *E carotovora*, can live in a nonpathogenic epiphytic phase on leaf surfaces

For enumerating the phylloplane microflora, 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 cause 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 makes 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) To evaluate the potential risk associated with the use of pesticides, it is important to know the fate of a pesticide on a plant (Pucarevic *et al* 2013)

Dik and Pelt van (1992) summarised that there is seasonal succession of microorganisms that colonise living leaves Eventhough in early spring, the levels of epiphytic nutrients and airborne inoculum are low, the epiphytic bacteria predominate in the phylloplane because bacteria take up scarce nutrients more readily than fungal spores Mechanisms of mycoparasitism by *Trichoderma* sp and antibiosis and competition afforded by *P fluorescens*, have a wide application in controlling foliar plant pathogens by increasing the population of non-pathogenic phylloplane microflora (Harman *et al* , 2004)

2.9 Survival of biocontrol agents on leaf surface

The fungal biocontrol agent *T viride* survive mainly on the hyphae of the pathogen present on the leaf surface or else it may get transferred to resting spores whereas 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 the serial dilution method on a 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

declined within 30 days after inoculation on plant species such as pepper, tomato, eggplant and strawberry Freeman *et al*, (2004) who studied the viability of the *Trichoderma* strains T-39, T-105, T-161 and T-166, applied individually on strawberry leaf surfaces, reported that the population of *Trichoderma* declined rapidly to a lower level after 3 days, but less rapidly, 7-14 days after application Furthermore, survival of the strains declined to undetectable levels after 2 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 (1 g) were transferred to a test tube containing 100 ml of sterile water and after thorough shaking, the population of *P fluorescens* and *T viride* in the suspension was estimated 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 in the process it multiplied at such fast rate that it occupied the active multiplication sites on the leaf 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 downy mildew (*Pseudoperonospora cubensis* (Berk and Curt) (Rostov) of cucumber under protected cultivation" was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2014-2016 The details of the materials used and the techniques adopted for the investigation are described below

3.1 Survey for assessment of incidence and severity of downy mildew of cucumber under polyhouses in Thrissur district

A survey was conducted in Thrissur district during January-December of 2015 by selecting nine different polyhouses located at Thanniyam, Peringottukara, Manaloor, Chendrapinni, Chavakkad and College of Horticulture, Vellanikkara The incidence and severity of downy mildew in polyhouses was assessed using standard score chart and procedures

Percentage of disease incidence was calculated using the formula

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

In each polyhouse, 10 plants were selected randomly, and a total of five leaves, two each from bottom and middle and one from the top of the plant were observed Disease severity was assessed using 0-5 scale as mentioned below

Disease rating	Percentage of leaf area infected
0	No infection
1	Below 10 % infection
2	>10-25% infection
3	>25-50% infection
4	>50-75%infection
5	Above 75% infection

Based on the percentage of leaf area affected, per cent disease severity (PDS) was calculated using the following formula (Wheeler, 1969)

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

The major meteorological factors *i e* temperature and relative humidity (RH) influencing the crop and the pathogen prevailing in the structures and open condition were recorded using a whirling psychrometer

3.1.1 Correlation analysis of incidence and severity of downy mildew with meteorological factors

Data collected during the survey was subjected to correlation analysis using SPSS v16 0 data editor to assess the influence of meteorological factors on incidence and severity of downy mildew in protected structures

3.1.2 Analysis of soil nutrients and other soil parameters

Soil samples (500g) were collected from inside and outside of the polyhouses during the survey by cutting a 'V' shaped notch at a depth of 10 15 cm Soil collected using soil auger from different locations in the polyhouses in which symptoms of nutrient deficiency are noticed were analyzed for major and minor nutrients, soil reaction, and electrical conductivity Soil nutrients like organic carbon,

available P, K, Ca, Mg, S and micronutrients like Fe, Zn, Mn and B were analysed in the Radiotracer Laboratory (RTL), College of Horticulture, Vellanikkara

3.1.3 Population of soil microflora

Soil samples were collected from inside and outside of the polyhouses during the survey as per 3 1 2 Then it was thoroughly mixed, and using quadrant method, the quantity was reduced to 100g It was then air dried and filtered using a sieve of mesh size 2mm Enumeration of soil microflora *viz* , fungi, bacteria and actinomycetes was done using serial dilution and plating technique The samples (1g) were added to 99ml sterile water, shaken well for one minute and serially diluted upto 10^{6} dilution For isolation of fungi, bacteria and actinomycetes 10^{3}, 10^{6} and 10^{5} dilutions were used respectively The media used were Martin's Rose Bengal Streptomycin Agar, Nutrient Agar and Ken Knights Agar for fungi, bacteria and actinomycetes respectively

Microbial suspension (1ml) of the respective dilution was pippeted into sterile petri plate and 15 ml of molten cooled medium was added Three replications were kept for each sample The plates were then incubated at room temperature After incubation, fungal, bacterial and actinomycetes colonies were counted at 24h, 48h and on 7^{th} day respectively Population of microflora was expressed as number of cfu g^{-1} of soil

3.2 Symptomatology and characterization of the pathogen

Infected leaves with symptoms of downy mildew were collected from different locations during the survey and morphological characters of the oomycete, like sporangial length, width, length to width ratio were studied Sporangia and sporangiophores were examined using sticky tape method (Yang, 2003), where an infected leaf was taken and a piece of sticky tape was placed and pressed over the infected area on the abaxial side of the leaf The piece of sticky tape having sporangia and sporangiospores imprinted on the sticky surface was then pressed on a clean glass

slide and viewed through 100x magnification Sporangiophore length, height of first branching, ratio between length and height of first branching, width of trunk and number of branch orders were recorded The sporangial length, width *etc* were also recorded using ultrascope to know variation if any

3.3 Field experiment for management of downy mildew of cucumber under polyhouse and rain shelter condition.

Field experiments were conducted under polyhouse of size 300m^2 and rain shelter of size 200 m^2 both having gable type roof, constructed in North- South direction in the Department of Plant Pathology, College of Horticulture, Vellanikkara The experiments were carried out during November to May of 2015-2016 to evaluate different treatments for the management of downy mildew of cucumber under protected condition The details of the experiment are as follows

Design	RBD
Treatments	12
Replication	3
Plot size	3 0 X 1 0 m^2
Spacing	1 0 X 0 5 m^2
Variety	Hilton (hybrid for polyhouse condition)
	AAUC-2 (rain shelter)
Season	November to May (2015-2016)

Treatment details

Treatment	Description	Concentration (%)
T_1	Soil solarization + seed treatment and soil application of *Trichoderma viride*+ foliar spray with *Trichoderma viride*	2%
T_2	Soil solarization + seed treatment and soil application of *Pseudomonas fluorescens* + foliar spray with *Pseudomonas fluorescens*	2%
T_3	Foliar spray with *Trichoderma viride*	2%
T_4	Foliar spray with *Pseudomonas fluorescens*	2%
T_5	Foliar spray with cowdung supernatant	5%
T_6	Foliar spray with cowdung supernatant (5%) + *Pseudomonas fluorescens* (2%)	5% +2%
T_7	Foliar spray with garlic extract	2%
T_8	Foliar spray with calphomil	0 3%
T_9	Foliar spray with combined formulation of potassium phosphonate + hexaconazole (Samarth)	0 3%
T_{10}	Foliar spray with cymoxanil + mancozeb (Curzate M8)	0 2%
T_{11}	Foliar spray with mancozeb (Indofil M45)	0 2%
T_{12}	Control	

Details of the plant protection chemicals

The following fungicides were used for the experiment

Sl.no	Chemical name	Trade name of formulation	Contact/systemic	Concentration (%)
1	Potassium phosphonate + hexaconazole (5%)	Samarth SC	systemic	0 3%
2	Cymoxanil (8%) + mancozeb (64%)	Curzate M8 WP	systemic	0 2%
3	Mancozeb	Indofil M45 75 WP	contact	0 2%

Details of the biocontrol agents

The following biocontrol agents were used for the experiment

Sl.no	Common name	Concentration (%)
1	*Trichoderma viride*(KAU)	2%
2	*Pseudomonas fluorescens*(KAU)	2%
3	Cowdung(Fresh cowdung supernatant)	5%
4	Cowdung slurry + *Pseudomonas fluorescens*	5% + 2%

Details of biofungicides

The following biofungicides were used for the experiment

Sl.no	Common name	Concentration (%)
1	Garlic extract	2%
2	Calphomil	0 3%

3.3.1 Preparation of field

Field experiments were conducted in polyhouse and rain shelter simultaneously Land inside the structure was ploughed and thoroughly prepared

using power tiller and beds of size 3 0 x 1 0 m^2were taken Well decomposed farm yard manure (FYM) and poultry manure were incorporated to the soil The beds were subjected to soil solarization as per the treatment details On completion of soil solarization for 80 days, polythene sheet was removed, soil thermometers were dismantled and basal dose of fertilizers were applied Treated seeds of cucumber were sown in the beds at a spacing of 1 0 x 0 5m^2 and 3-4 cm depth and then a thin layer of soil was spread over the beds Fertigation and irrigation was carried out using drip irrigation system Relative humidity inside the structure was maintained above 80 per cent by operating fogger

3.3.1.1 Soil solarization

Beds of 3 0 x 1 0 m^2 size and 10 cm height were taken Well decomposed FYM and poultry manure were incorporated in to the soil, 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 is placed touching the surface and there are no air pockets in between The sides of the polythene sheet were sealed by putting soil Soil solarization was carried out during October – December for a period of 80 days in protected structures (Candido *et al*, 2008) Soil thermometers were installed at 10 cm depth in solarized and non- solarized beds in both polyhouse and rain shelter Soil temperature was recorded at 7 30 am and 2 30 pm daily during the period of solarization

3.3.1.2 Enumeration of soil microflora

Soil samples were collected from polyhouse and rain shelter before and after soil solarization The population of fungi, bacteria and actinomycetes were enumerated using serial dilution plating technique as described in 3 1 3 and expressed as number of cfu per gram of soil

3.3.2 Management of downy mildew of cucumber under protected condition

The effectiveness of selected plant protection chemicals, biocontrol agents and biofungicides as per 3 3 was tested against the downy mildew of cucumber under protected condition The first foliar application was given at the onset of the disease Subsequent sprays were given at 15 days interval Systemic fungicides were sprayed two times and contact fungicides, biocontrol agents and biofungicides were sprayed three times Plants in each plot were separated using plastic screen during foliar spray, in order to avoid drift Disease severity was recorded using 0-5 scale (3 1) before spraying and 15 days after each spraying From each plot, four plants were selected randomly, and a total of five leaves from each plant were observed Based on the percentage of leaf area infected, disease severity was calculated as described in 3 1

3.3.2.1 Assessment of crop loss

To estimate crop loss due to downy mildew, the yield from the plot having maximum yield T1(Soil solarization + seed treatment + soil application + foliar spray of *Trichoderma viride*) and minimum yield (control) were recorded Percentage yield loss was estimated using the formula (Simon, 1996) as shown below

$$\text{Percentage yield loss} = \frac{\text{Yield in treatment-Yield in control} \times 100}{\text{Yield in treatment}}$$

Yield in treatment=Yield of plot sprayed with T_i

Yield in control=Yield of plot without fungicide spray

3.3.2.2 Biometric observations

Biometric observations such as days to flower, days to harvest, vine length, fruit weight, fruit length and shelf life of cucumber was recorded and tabulated to know the effect of treatments on these parameters

3.3.2.3 Economic analysis

Total cost incurred and total returns were calculated separately for the treatments The benefit cost ratio was calculated at market price (Rs 30 kg^{-1}) of cucumber for all treatments as well as at 20 percent premium price for organic treatments alone

3.4 Meteorological parameters

Temperature and relative humidity inside the polyhouse and rain shelter was recorded at 7 30 am and 2 30 pm daily during the experiment using temperature and moisture meter which is 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

3.5 Incidence of other diseases and pests

Incidence of pests and diseases other than downy mildew in polyhouse and rain shelter was observed and percentage disease incidence was worked out as per (3 1)

3.6 Enumeration of phylloplane microflora of cucumber

The phylloplane microflora (fungi, bacteria and actinomycetes) of the crop was enumerated using serial dilution plating of leaf washings to know the changes due to the treatments The methodology adopted by Elad and Kirshner (1993) was used for studying the enumeration of phylloplane microflora Cucumber leaves were collected from all treatments in polyhouse and rain shelter on the next day and 15th day after each spray Area of the leaf taken 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 serially diluted upto 10^6 dilution The respective dilutions were plated as per 3 1 3 Population of the phylloplane microflora was expressed as cfu per cm^2 area of leaf

3.7 Survival of the biocontrol agents on cucumber phylloplane

The biocontrol agents sprayed on leaves were enumerated at periodical intervals of 5, 10, 15 days after spraying using serial dilution plating of leaf washings (3 6) Here, the media used were TSM (*Trichoderma* selective agar medium) and King's B agar for isolation of *Trichoderma viride* and *Pseudomonas fluorescens* respectively Leaves were collected from field before and after treatment application at an interval of 5 days For isolation of *T viride* and *P fluorescens*, 10^3 and 10^6 dilutions were used respectively The dilutions were plated as per 3 1 3 and colonies were counted at 24h and 48h of incubation for *Trichoderma viride* and *Pseudomonas fluorescens* respectively Population of biocontrol agents in the phylloplane was expressed as cfu per cm^2 area of leaf

3.8 Statistical analysis

The data collected during field experiments were analysed using web agri-stat package (WASP) 2 0

Results

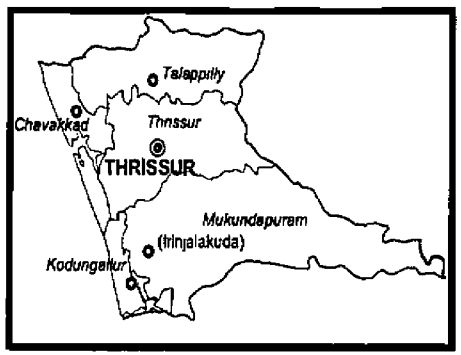
4. RESULTS

The studies on "Management of downy mildew (*Pseudoperonospora cubensis* (Berk & Curt) Rostov) of cucumber under protected cultivation" was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2014-2016 The study consisted of survey on incidence and severity of downy mildew of cucumber in farmer's fields and field experiments in polyhouse and rain shelter The survey was conducted in Thrissur district during January-December of 2015 by selecting nine different polyhouses located at Thanniyam, Peringottukara, Manaloor, Chendrapinni, Chavakkad and College of Horticulture, Vellanikkara (Plate 1) The incidence and severity of downy mildew of cucumber in protected structures and open condition were assessed using standard score chart (Plate 2) and procedures The major meteorological factors *i e* temperature and relative humidity (RH) influencing the crop and the pathogen prevailing in the structures and open condition were also recorded using whirling psychrometer

4.1 Survey for assessment of incidence and severity of downy mildew

Incidence of downy mildew was noticed in all the polyhouses where cucumber is cultivated Per cent disease incidence (PDI) varied from 4 67 to 15 45 Per cent disease severity was calculated using 0-5 scale (Plate 2) and per cent disease severity (PDS) varied from 11 33 to 35 78 in different polyhouses surveyed (Table 1) Incidence and severity of the disease were found to be high during rainy season *i e* 15 45 and 35 75 respectively as observed in the polyhouse (1) at Chentrapinni It is clear from the table that there is occurrence of cucumber downy mildew inside the polyhouse irrespective of the season

Meteorological data was recorded using whirling psychrometer and temperature and moisture meter (Plate 3), which showed a wide variation among the polyhouses It varied mainly with the season, irrigation schedule, type of the structure, location *etc* In general, temperature and RH are higher inside the



A Locations visited

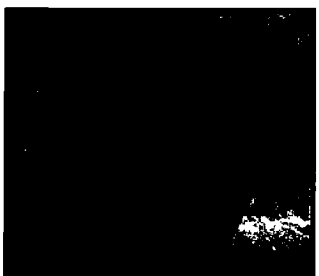
B Quonset type polyhouse

C Field visit

D Symptoms of downy mildew

F Symptoms of nutrient deficiency

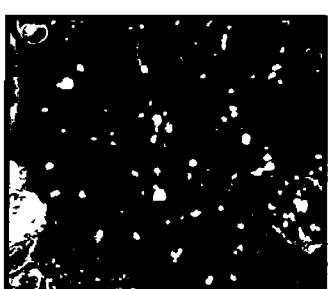
Plate 1 Field visit for survey



0 (no infection)

1(below 10% infection)

2(>10-25% infection)



3(>25-50% infection

4 (>50-75% infection)

5(above 75% infection)

Plate 2 Score chart for downy mildew

polyhouse compared to outside However, out of the nine polyhouses visited, in polyhouse (1) at Peringotukara outside temperature was higher than inside Similarly, a low RH was observed in the polyhouse at Manaloor compared to outside

Table 1. Incidence and severity of downy mildew of cucumber under polyhouses in farmers' fields in Thrissur district

	Polyhouse			Disease		Meteorological parameters			
Sl. No	Location	Area (m^2) of polyhouse	Period (2015)	PDI	PDS	Inside polyhouse		Outside polyhouse	
						Temp. (^{0}C)	RH	Temp. (^{0}C)	RH
1	Peringottukara(1)	220	January	12 70	24 56	30 5	93	31 1	93
2	Manaloor	400	March	4 67	11 33	29 4	79	28 0	81
3	Thanmyam (1)	365	May	13 34	25 00	31 6	87	30 5	86
4	Chenthrapinni(1)	420	June	15 45	35 75	24 7	97	23 3	91
5	Chenthrapinni (2)	400	July	12 67	28 90	26 4	95	24 6	93
6	Thanniyam (2)	400	August	5 33	15 67	33 8	81	32 7	80
7	Peringottukara(2)	180	September	6 98	16 17	32 0	83	28 1	81
8	Vellanikkara	200	November	11 33	14 67	32 7	83	31 2	82
9	Chavakkad	400	December	9 75	22 39	30 0	87	26 1	79

PDI Per cent disease incidence PDS Per cent disease severity, Temp Temperature, RH Relative humidity

The temperature inside the polyhouse varied from 24 7 to 33 8°C and the increase in temperature inside the polyhouse compared to outside varied from 0 6 to 3 9°C The RH inside the polyhouse varied from 79 to 97 per cent and the increase in RH inside the polyhouse varied from 0 to 8 per cent Data presented in the table shows that high humidity and low temperature influences the development of downy mildew of cucumber inside polyhouse

4.1.1 Correlation analysis of incidence and severity of downy mildew with meteorological factors

Correlation analysis of disease incidence and severity with major meteorological parameters inside the polyhouse was performed (Table 2)

Table 2. Correlation analysis of disease incidence and severity with major meteorological parameters.

Correlation coefficients	PDS	PDI	RH
PDI	0 859**		
RH	0 956**	0 845**	
Temperature	-0 757*	0 546*	-0 768*

*Correlation is significant at 0 05 level ** Correlation is significant at 0 01 level

It is observed that there is a significant positive correlation between PDI/PDS with RH inside the polyhouse whereas they are negatively correlated with temperature There is a significant positive correlation between PDI and PDS also

4.1.2 Analysis of soil nutrients and other soil parameters

During the survey, deficiency of potassium, calcium and boron was noticed cucumber grown in the polyhouses (Plate 4) Soil nutrient analysis was carried out for soil samples collected from polyhouses where severe deficiency symptoms were observed Soil samples collected from inside and outside of such polyhouses were analyzed for soil reaction, electrical conductivity and major and minor nutrients (Table 3)

Soil pH was found to be in the range of 6 7 to 7 6 inside the polyhouse whereas pH varied from 6 2 to 6 7 in the open condition The pH of soil from inside is more by 0 5 to 1 9 compared to soil from outside the polyhouse Electrical conductivity was found to be high inside the polyhouse (2) at Thannium, whereas in all other polyhouse and in the open condition EC of soil was found to be normal in

the places surveyed However, EC was found to be higher inside the polyhouses than outside

Table 3. Soil nutrient status inside and outside the polyhouse

Soil nutrient/ parameter	Soil nutrients quantity and status					
	Thanniyum- 1		Thannium- 2		Peringottukara- 1	
Parameter	Outside	Inside	Outside	Inside	Outside	Inside
pH	6 2	7 6	6 7	7 6	6 6	6 7
Electrical conductivity (dS/m)	0 03 (N)	0 839 (N)	0 03 (N)	2 23 (H)	0 04 (N)	0 55 (N)
Organic Carbon (%)	0 47 (L)	1 38 (M)	0 29 (L)	0 52 (L)	0 85 (M)	0 97 (M)
Available Phosphorous (kg ha $^{-1}$)	44 34 (H)	187 06 (H)	37 41 (H)	51 27 (H)	77 59 (H)	471 11 (H)
Available Potassium (kg ha $^{-1}$)	43 70 (L)	862 40 (H)	34 70 (L)	1359 7 (H)	42 60 (L)	357 30 (H)
Available Calcium (mg kg $^{-1}$)	192 00 (D)	1686 5 (S)	465 75 (S)	1024 0 (S)	666 75 (S)	1004 75 (S)
Available Magnesium (mg kg $^{-1}$)	37 50 (D)	149 50 (S)	374 25 (S)	34 75 (D)	144 25 (D)	152 50 (S)
Available Sulphur (mg kg $^{-1}$)	5 11 (S)	78 90 (S)	9 38 (S)	96 60 (S)	24 7 (S)	80 40 (S)
Micronutrients						
Copper(mg kg $^{-1}$)	0 55 (S)	0 95 (S)	0 84 (S)	0 62 (S)	1 50 (S)	5 90 (S)
Iron(mg kg $^{-1}$)	133 50 (S)	11 69 (S)	19 30 (S)	18 37 (S)	17 94 (S)	18 87 (S)
Zinc (mg kg $^{-1}$)	0 80 (S)	1 70 (S)	0 53 (D)	1 00 (S)	22 68 (S)	18 41 (S)
Manganese (mg kg $^{-1}$)	4 84 (S)	7 47 (S)	2 09 (S)	40 98 (S)	4 64 (S)	18 09 (S)
Boron (mg kg $^{-1}$)	0 12 (D)	0 86 (S)	0 18 (D)	2 56 (S)	0 14 (D)	0 15 (D)

N-Normal M Medium, L Low, H High D Deficient S Sufficient

Organic carbon was found to be medium inside the soil of all the polyhouses surveyed except for polyhouse (2) at Thannium In the case of potassium (K), soil inside the polyhouses contain sufficient amount of K except in polyhouse (2) of Thannium Phosphorous was found to be high in all the samples whereas K was high in inside soil and low in outside Though Ca deficiency was expressed by the plants it was found to be present in sufficient quantities in the soil collected from inside the polyhouses Symptoms of boron deficiency were also observed in plants but in soil it was available in sufficient quantities except in polyhouse (1) of Peringottukara

In general the soil micronutrients especially copper, iron, zinc and manganese are present in sufficient quantities inside and outside the polyhouses and compared to outside soil, micro nutrients are found to be more inside the polyhouse

4.1.3 Population of soil microflora

Soil samples collected from inside and outside of the polyhouses visited during the survey were subjected to enumeration of population of soil miroflora and the data are furnished in Table 4

Table 4. Population of soil microflora inside and outside the polyhouses

Location	Population of soil microflora*					
	Inside polyhouse			Open condition		
	Fungi ($x10^3$ cfu g^{-1})	Bacteria ($x10^6$ cfu g^{-1})	Actmomycetes ($x10^5$ cfu g^{-1})	Fungi ($x10^3$ cfu g^{-1})	Bacteria ($x10^6$ cfu g^{-1})	Actmomycetes ($x10^5$ cfu g^{-1})
Peringottukara(1)	25 67	13 27	3 24	48 67	2 00	5 33
Manaloor	52 28	9 67	3 75	71 32	20 68	3 00
Thanniyam (1)	54 00	3 67	2 24	57 00	12 67	3 28
Chenthrapinni(1)	52 00	25 33	3 26	59 67	1 34	3 37
Chenthrapinni (2)	98 00	16 57	2 32	81 33	6 64	3 34
Thanniyam (2)	70 33	22 81	8 67	93 65	2 24	2 40
Peringottukara(2)	73 32	10 00	5 89	53 54	3 24	2 55
Vellanikkara	18 89	44 35	8 75	65 23	31 67	1 42
Chavakkad	62 67	15 24	5 24	51 67	34 67	1 74

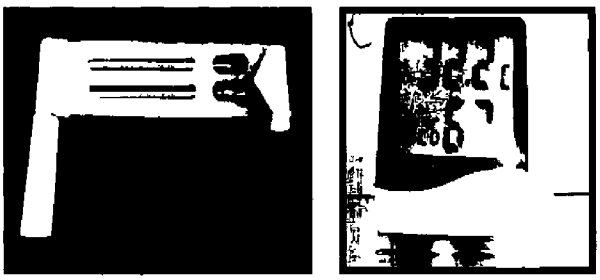
*Mean of three replication

Population of soil fungi inside the polyhouse varied from 18 89 to 98 00 $x10^{3}$ cfu g^{-1} of soil and in general soil fungi are more in outside soil than inside the polyhouse The population of soil bacteria inside the polyhouse varied from 3 67 to 44 35 $x10^{6}$ cfu g^{-1} of soil and in the case of actinomycetes the population in polyhouse varied from 2 24 to 8 75 $x10^{5}$ cfu g^{-1} and they are more in inside soil than in open However, in three polyhouses *i e*, polyhouse(2) at Peringottukara and Chentrapinni and polyhouse at Chavakkad, the fungal population is found to be higher inside the polyhouse Similarly out of the nine polyhouses visited, in three, bacterial population was found to be more inside compared to outside

4.2 Symptomatology and characterization of the pathogen

Leaves showing symptoms of downy mildew were collected from different locations during the survey The initial symptoms of the disease on the cucumber leaves were observed as pale yellow lesions on the upper side of the leaves, sometimes restricted by leaf veins The size of lesions varied from 3-9mm As the disease developed further, lesions enlarged, coalesced and eventually covered the entire leaf area Under extreme conditions leaves turned necrotic and lead to death of whole plant (Plate 5)

Morphological characterization of cucumber downy mildew pathogen was carried out by microscopic examination of the infected area on the leaves (Table 5) The leaves with symptoms of downy mildew were collected from inside the polyhouse during the survey Sporangia and sporangiophores were examined using sticky tape method, where an infected leaf was taken and a piece of sticky tape was placed and pressed over the infected area on the abaxial side of the leaf The slide was viewed through 100X magnification



A Whirling psychrometer B Temperature and moisture meter

Plate 3 Instruments for recording temperature and relative humidity

Potassium deficiency Calcium deficiency Boron deficiency

Plate 4 Symptoms of nutrient deficiency

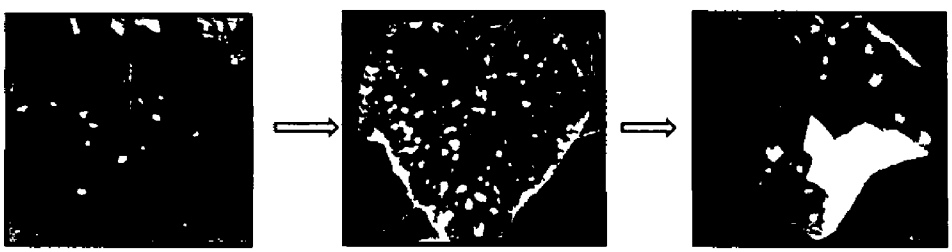


Plate 5 Symptomatology of downy mildew

Table 5. Characters of the pathogen observed under microscope (100 X)

Sporangiophore	
Length(µm)	69 23-87 91
Height of first branching(µm)	37 67-55 34
Ratio length / height of first branching	1 69 1
Width of trunk(µm)	3 01-3 44
Number of branch orders	2-3
Sporangia	
Length(µm)	52 35-62 77
Width(µm)	18 53-34 26
Average(µm)	57 08 x 26 39
Ratio length/ width	2 16 1

It was observed that the pathogen has dichotomously-branched sporangiophores with terminal growth (Plate 6) Each mature sporangiophore bears sporangia at the end The pathogen forms hyaline sporangiophores of length 69 23-87 91µm The sporangia are borne singly on the pointed tips of sporangiophores that branch at acute angles and these are large lemon-shaped and brownish-yellow in colour Ratio of length/ width of sporangia is 2 16 1 0 Sporangia lie on sterigmata which emerge in groups of one to four from stomata on the abaxial surface of infected leaves The morphological characters of the pathogen observed under microscope from different locations during the survey were same as that of the *Pseudoperonospora cubensis* (Berk & Curt) (Rostov) Hence it is confirmed that the symptoms observed on cucumber leaves collected from polyhouses during the survey are those of downy mildew caused by *P cubensis*

4.3 Management of downy mildew of cucumber under protected condition.

Field experiments were conducted simultaneously in newly constructed polyhouse (300m^2) and rain shelter (200m^2) during November to May of 2015-2016 to evaluate different treatments for the management of downy mildew of cucumber under protected condition (Plate 7) The structures are facing North South direction and with gable type roof

4.3.1 Soil solarization

In treatments T_1 and T_2, soil solarization was carried out before sowing for a period of 80 days in polyhouse and rain shelter Soil thermometers were installed at 10 cm depth in solarized and nonsolarized plots and temperature was recorded at 7 30 am and 2 30 pm daily during the period of solarization (Plate 8)

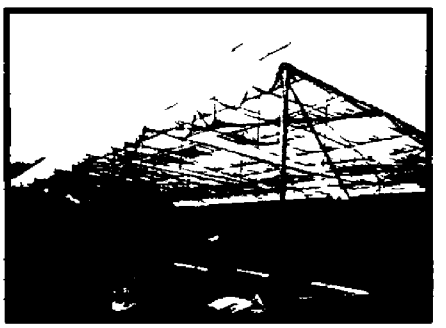
Table 6. Soil temperature of solarized and nonsolarized beds in polyhouse and rain shelter

Std. week	Soil temperature at 10 cm depth (°C)							
	Polyhouse				Rain shelter			
	7.30 am		2.30 pm		7.30 am		2.30 pm	
	S	NS	S	NS	S	NS	S	NS
41	30 5*	30 0	37 0	33 5	29 5	28 5	36 0	32 5
42	32 0	30 5	35 0	32 0	31 0	29 0	36 0	33 0
43	32 0	30 5	35 5	33 0	31 0	29 0	34 0	31 5
44	32 5	31 0	37 5	33 5	31 5	30 0	36 0	33 0
45	32 5	30 5	37 0	34 0	30 0	28 5	32 0	30 0
46	31 0	30 0	35 5	33 0	31 5	30 0	34 0	31 0
47	32 0	30 5	36 5	34 0	31 5	29 0	36 5	33 5
48	31 0	30 0	37 5	34 0	31 0	29 0	35 5	32 5
49	31 0	30 0	37 0	33 5	31 5	30 0	35 5	33 0
50	32 5	31 0	37 0	33 5	31 0	29 0	35 0	32 0
51	30 0	28 5	37 5	34 0	31 0	29 0	34 5	33 5
52	30 0	28 5	37 0	34 0	31 0	30 0	36 0	33 5

*average temperature of seven days S solarized soil NS-nonsolarized soil

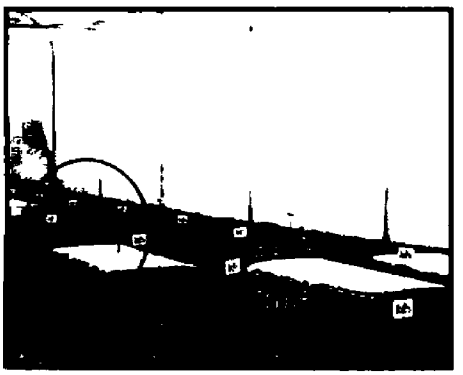
Plate 6 Sporangia and sporangiophores of
Pseudoperonospora cubensis (100X magnification)

Polyhouse



Rain shelter

Plate 7 Protected structures for field experiments



A Polyhouse

B Rain shelter

Plate 8 Soil solarization and installation of soil thermometer in protected structures

Data on soil temperature of both solarized and nonsolarized beds before and after solarization are presented in Table 6 In general, soil temperature is found to be high in polyhouse compared to rain shelter, and in both the temperature in solarized beds was higher than nonsolarized beds In polyhouse there is an increase in soil temperature upto of 4^0C in solarized beds over nonsolarized beds at 2 30 pm During this period temperature in solarized beds ranged from 33 5-37 5^0C in solarized beds, whereas in nonsolarized beds from 32-34^0C (Fig 1) At 7 30 am, soil temperature in solarized beds inside the polyhouse varied from 30-32 5^0C, whereas in nonsolarized beds it ranged from 28 5-31^0C

In rain shelter, there is an increase in soil temperature upto of 3 5^0C in solarized beds over nonsolarized beds at 2 30 pm During this period temperature varied from 34 to 36 5 ^{0}C, whereas in nonsolarized beds it ranged from 30-33 5^0C (Fig 2) At 7 30 am, soil temperature in rain shelter varied from 29 5-31 5 ^{0}C, whereas in nonsolarized beds from 28 5-30^0C

4.3.1.1 Enumeration of soil microflora in polyhouse and rain shelter

The population of fungi, bacteria and actinomycetes in solarized and nonsolarised soil was enumerated in polyhouse and rain shelter (Plate 9) The results are presented in Table 7 During the period of solarization, there was reduction in the population of soil microflora in all the plots, but it was highly prominent in solarized beds The percentage reduction in the fungal flora in polyhouse was 79 34, whereas in nonsolarized beds it was only 28 18 per cent Similarly the bacterial population was reduced by 77 50 per cent in solarized beds, while in nonsolarized beds the reduction was only 31 33 per cent The population of actinomycetes was also reduced by 67 85 per cent in solarized beds, compared to 25 92 per cent in nonsolarised soil

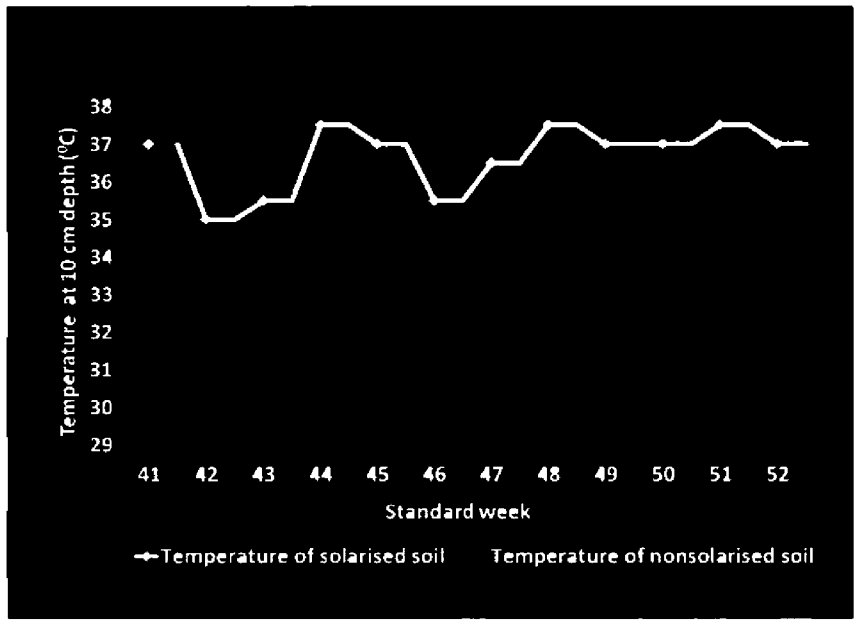


Fig 1 Soil temperature in solarized and nonsolarized beds in polyhouse

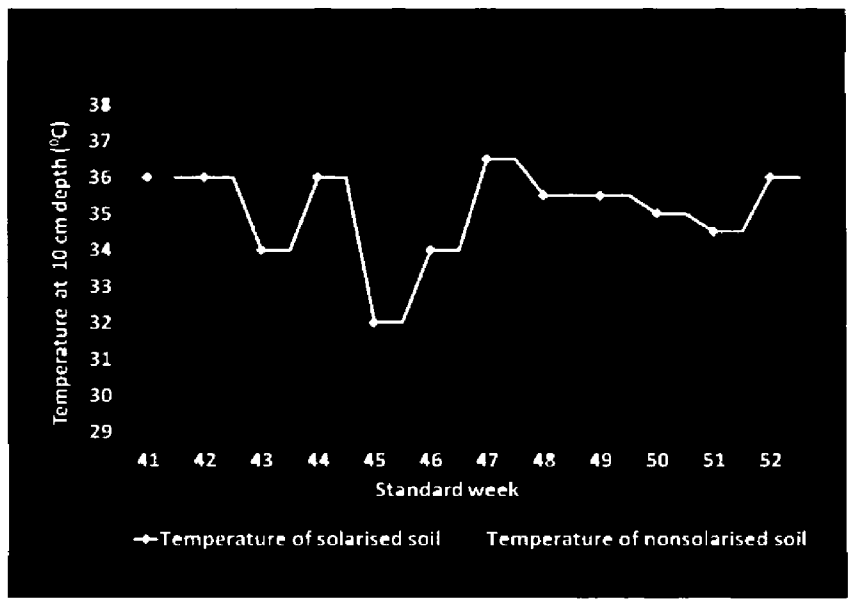


Fig 2 Soil temperature in solarized and nonsolarized beds in rain shelter

Table 7. Population of soil microflora in polyhouse and rain shelter

Soil microflora	Population of soil microflora*											
	Polyhouse						Rain shelter					
	Solarized soil			Nonsolarized soil			Solarized soil			Nonsolarized soil		
	Before	After	Per cent reduction	Before	After	Per cent reduction	Before	After	Per cent reduction	Before	After	Per cent reduction
Fungi($x10^3$ cfu g^{-1})	151 67	31 33	79 34	145 89	104 78	28 18	133 45	35 66	73 28	128 89	89 00	30 95
Bacteria($x10^6$ cfu g^{-1})	56 30	12 67	77 50	52 45	31 33	40 27	34 89	9 34	73 23	32 45	18 33	43 51
Actinomycetes ($x10^5$ cfu g^{-1})	9 33	3 00	67 85	10 34	7 66	25 92	12 34	4 30	65 15	11 66	8 56	26 59

*Mean of three replication

In rain shelter, the same trend was observed and the per cent reduction of fungal flora in solarized beds was found to be 73 28, whereas in nonsolarized beds the reduction was only 30 95 per cent Similarly the bacterial population was reduced by 73 23 per cent in solarized beds, while in nonsolarized beds the reduction was only 43 51per cent In the case of actmomycetes the reduction was 65 15 and 26 59 per cent, in solarized and nonsolarized beds respectively

4.3.2 Management of downy mildew of cucumber under polyhouse and rain shelter

The effectiveness of selected plant protection chemicals, biofungicides and biocontrol agents were tested against the downy mildew of cucumber under polyhouse and ram shelter The crop was raised in polyhouse and rain shelter during January to May of 2016 Relative humidity inside the structures was maintained >80 per cent by operating fogger Incidence of downy mildew was noticed at 49 days after sowing (DAS) in polyhouse and at 54 DAS in rain shelter The first foliar application was given at the onset of disease Subsequent sprays were given at 15 days interval Disease severity was recorded using 0-5 scale before spraying and 15 days after each praying From each plot, four plants were selected randomly, and a total of five leaves from each plant were observed Based on the per cent of leaf area infected, disease severity was calculated (Wheeler, 1969)

Table 8. Effect of different treatments on downy mildew and yield of cucumber under polyhouse

Treatment	Per cent disease severity*							Mean yield (kg plot $^{-1}$)**	Per cent increase over control
	Before treatment	After first treatment	Per cent reduction over control	After second treatment	Per cent reduction over control	After third treatment	Per cent reduction over control		
T_1 -S + ST + SA + foliar spray - *T viride*	4 33	12 67^{abc}	43 28	16 67^{ab}	54 13	23 67^{abc}	60 99	23 40^{a}	71 01
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	5 00	13 67^{abcd}	38 80	17 00^{abc}	53 21	24 67$^{a1 cd}$	59 34	22 60^{ab}	65 16
T_3 -*T viride*	6 67	15b^{cde}	32 84	18 33^{abcd}	49 54	26 67^{bcd}	56 15	20 63^{abc}	50.79
T_4 -*P fluorescens*	6 33	15 67^{cde}	29 85	20 33^{bcd}	44 04	26 00^{abcd}	57 15	19 14^{c}	39 85
T_5 cowdung supernatant	7 00	17 33^{e}	22 39	22 33^{d}	38 53	28 33^{d}	53 30	20 93^{abc}	52 98
T_6 -cowdung supernatant + *P fluorescens*	6 00	17 00^{e}	23 88	20 33^{bcd}	44 04	28 00^{d}	53 85	20 32^{bc}	48 47
T_7 -garlic extract	6 33	16.00d^{e}	28 36	21 00^{cd}	42 20	27 33^{cd}	54 95	19 87^{bc}	45 19
T_8 –Calphomil	7.00	16 00d^{e}	28 36	20 33^{bcd}	44 04	27 00^{bcd}	55 50	20 47^{bc}	49 57
T_9 -potassium phosphonate + hexaconazole	5 67	16 33d^{e}	26 87	20 00$^{a1 c d}$	44 95	27 00^{bcd}	55 50	20 48^{bc}	49 69
T_{10} cymoxanil + mancozeb	6 00	11 67^{a}	47 76	16 00^{a}	55 96	22.67^{a}	62 63	22 53^{ab}	64 68
T_{11} -mancozeb	7 00	12 33^{ab}	44 78	16 33^{ab}	55 05	22 33^{ab}	61 55	21 75^{abc}	58 95
T_{12} -control	6 67	22 33^{f}	-	36 33^{e}	-	60 67^{e}	-	13 68^{d}	-
CV	15 96	12 25		12 45		8 15		8 16	
CD	NS	3 22		4 31		3 97		2 83	

*Mean of three replications, values followed by same superscript are not significantly different by DMRT (P=0 05) **plot size 3m^2

S-Soil solarization ST- Seed treatment, SA-Soil application

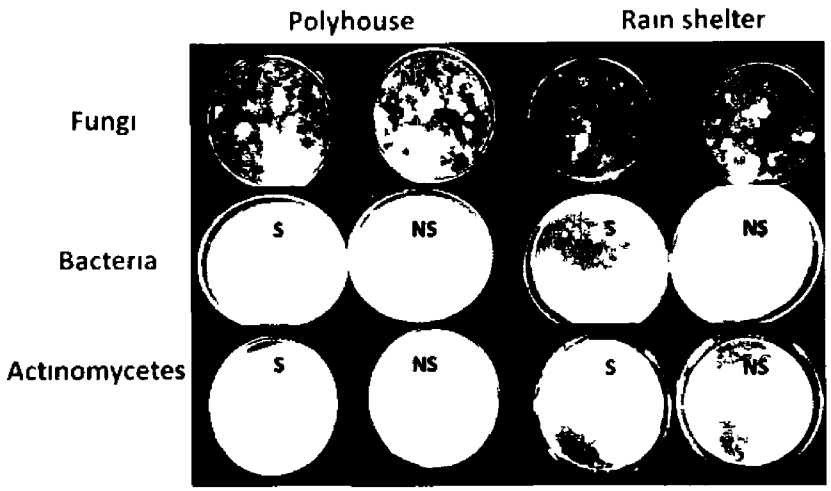
4.3.2.1 Effect of different treatments on downy mildew of cucumber under polyhouse

Results of the field experiment for management of downy mildew of cucumber in polyhouse are presented in Table 8 Before treatment application there was no significant difference in disease severity among the treatments There was a gradual increase in the disease throughout the experiment However, all 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 T_{10} (cymoxanil + mancozeb) at all stages of observation and the per cent reduction over control is 62 63 per cent after the third spraying followed by T_{11} (mancozeb) which shows 61 55 per cent reduction in disease severity after last spray However, treatments T_{10}, T_{11} were found to be on par after each spraying

Among biocontrol agents, *Trichoderma viride* along with soil solarization (T_1) gave the best result in the reduction of downy mildew which accounted for 60 99 per cent over control (Plate 10) T_1 is followed by T_2 (solarization + foliar spray of *P fluoi escens*) and T_4 (foliar spray with *Pseudomonas fluoi escens*) where the reduction is 59 34 per cent and 57 15 per cent respectively over control Treatments T_1, T_2 and T_4 were found to be on par with chemical treatments T_{10} and T_{11}

Between the two biofungicides, T_8 (Calphomil) gave reduction in downy mildew upto 55 50 per cent followed by T_7 (garlic) where the per cent reduction over control is 54 95

Effect of treatments was reflected on yield of cucumber also and all the treatments recorded higher yield than control Eventhough the lowest PDS was recorded in T_{10}(cymoxanil + mancozeb), mean yield was found to be more in the treatment T_1 and T_2 with an increase of 71 01 per cent and 65 16 per cent respectively over control However mean yield was found to be on par for treatments T_1 (23 4 kg plot $^{-1}$), T_2 (22 6 kg plot $^{-1}$), T_{10} (22 53 kg plot $^{-1}$), T_{11} (21 75kg plot $^{-1}$), T_3 (20 63 kg plot $^{-1}$), T_5 (20 93 kg plot $^{-1}$)



S solarized soil NS nonsolarized soil

Plate 9 Population of soil microflora in solarized and nonsolarised soil

T_1- solarisation + seed treatment + soil application of *Trichoderma viride* + foliar spray with *T viride* (2%)

T_{12} Control

Plate 10 Effect of treatments on downy mildew in polyhouse

Table 9 Effect of different treatments on downy mildew of cucumber under rain shelter

Treatment	Per cent disease severity*							Mean yield (kg plot $^{-1}$)**	Per cent increase over control
	Before treatment	After first treatment	Per cent reduction over control	After second treatment	Per cent reduction over control	After third treatment	Per cent reduction over control		
T_1 -S + ST + SA + foliar spray - *T viride*	4 33	7 67^{abc}	41 03	10 67^{abc}	59 57	11 67^{abc}	52 70	16 78^{a}	54 92
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	4 00	8 00^{abc}	38 46	11 00^{abc}	57 45	12 00^{abc}	51 35	16 03^{ab}	48 00
T_3 -*T viride*	5 00	8 00^{abc}	38 46	11 67^{bcd}	53 19	12 33^{abc}	50 00	15 15^{abc}	39 85
T_4 -*P fluorescens*	6 00	8 67^{bc}	33 33	11 67^{bcd}	53.19	12 67^{abc}	48 65	14 88^{abc}	37 38
T_5 -cowdung supernatant	6 00	9 00^{c}	30 77	13 33de	42 55	13 67^{c}	44 60	13 93^{bc}	28 62
T_6 -cowdung supernatant + *P fluorescens*	5 33	8 67^{bc}	33 33	12 67^{cde}	46 81	13 00^{bc}	47 30	14 02^{bc}	29 38
T_7 -garlic extract	6 00	9 00^{c}	30 77	14 33^{e}	36 17	13 67^{c}	44 60	13 77^{c}	27 08
T_8 –Calphomil	5 67	8 67^{bc}	33 33	12.67^{cde}	46 81	13 33^{bc}	45 95	13 97^{bc}	28 92
T_9 -potassium phosphonate + hexaconazole	5 67	8 67^{bc}	33 33	11 67^{bcd}	53 19	13 00^{bc}	47 30	14 20^{bc}	31 08
T_{10}-cymoxanil + mancozeb	6 67	7 00^{a}	46 15	9 33^{a}	68 06	11 00^{a}	55 41	15 38^{abc}	41 97
T_{11} -mancozeb	5 67	7 33^{ab}	43 59	10 33^{ab}	61 70	11 33^{ab}	54 05	15 18^{abc}	40 09
T_{12} –control	6 33	13 00^{d}	-	20 00^{f}	-	24 67^{d}	-	10 83^{d}	-
CV	16 85	9 75		9 78		9 86		8 55	
CD	NS	1 43		2 06		2 26		2 10	

*Mean of three replications, values followed by same superscript are not significantly different by DMRT (P=0 05) **plot size 3m^2
S-Soil solarization, ST- Seed treatment, SA-Soil application

4.3.2 2 Effect of different treatments on downy mildew of cucumber under rain shelter

Results of the field experiment in rain shelter are presented in Table 9 Incidence of downy mildew was noticed after 54 days of sowing (DAS) in rain shelter Spraying as well as recording of observation were done as in polyhouse

Before treatment application there was no significant difference in disease severity among the treatments As in polyhouse there was a gradual increase in the severity of downy mildew throughout the experiment However, all treatments at all the stages of observation were found to be superior over the control in reducing he rate of increase of the disease Throughout the experiment, T_{10} (cymoxanil + mancozeb) was the best treatment with lowest PDS and this treatment gave disease reduction of 55 41 per cent over control It is followed by T_{11} (mancozeb) which gave 54 05 per cent disease reduction over control However, these two treatments were on par after each spraying

Among the biocontrol treatments, T_1(*Trichoderma viride* along with soil solarization) gave the best result in the reduction of downy mildew, which accounted for 52 70 per cent disease reduction over control and this is followed by T_2 *i e* solarization + foliar spray of *Pseudomonas fluorescens*(51 35per cent reduction) After the third spraying, the PDS in T_1(11 67%), T_2(12%), T_3(foliar spray of *Trichoderma viride*-12 33%), T_4 (foliar spray of *Pseudomonas fluorescens*-12 67%), T_6 (foliar spray with cowdung + *Pseudomonas fluorescens* 13%) and T_9(foliar spray with potassium phosphonate + hexaconazole-13%) were found to be on par with T_{10}(11%) and T_{11}(11 33%)

The biofungicides tested *viz* Calphomil(T_8) and garlic(T_7) also gave reduction in downy mildew upto 45 95 per cent and 44 60 per cent respectively

Effect of treatments was reflected on yield of cucumber also Eventhough the lowest PDS was recorded in T_{10} (cymoxanil + mancozeb), mean yield was found to be the highest in T_1(16 78 kg plot ^{1}) followed by T_2(16 03 kg plot ^{1}) which accounts for an yield increase of 54 92 per cent and 48 per cent respectively over control

4.3.2.3 Assessment of crop loss

Yield from the plot having maximum yield T_1(Soil solarization + seed treatment + soil application + foliar spray of *Trichoderma viride*) and minimum yield T_{12}(control) were recorded and percentage yield loss in polyhouse and rain shelter was worked out and presented in Table 10

Table 10. Per cent yield loss in cucumber due to downy mildew disease

Protected structure	Yield(kg plot $^{-1}$)*		
	T_1(S + ST + SA + foliar spray of *Trichoderma viride*)	T_{12}(control)	Per cent yield loss
Polyhouse	70 20	41 05	41 52
Rain shelter	50 34	32 49	35 46

S Soil solarization, ST- Seed treatment, SA Soil application * plot size $3m^2$

In polyhouse, yield loss was found to be 41 52 per cent, whereas in rain shelter, it was 35 46 per cent

4.3.2 4 Effect of treatments on the biometric characters

Biometric characters of cucumber such as days to flower, days to harvest, vine length, fruit weight, fruit length and shelf life of cucumber was recorded during the field experiments (Table 11)

Table 11. Effect of treatments on biometric characters of cucumber in polyhouse

Treatment	Biometric characters*					
	Days to flowering	Days to harvest	Vine length(m)	Fruit weight(kg)	Fruit length(cm)	Shelf life (days)
T_1 -S + ST + SA + foliar spray - *T. viride*	27 33	37 33	5 00	0 15	17 17	$2\ 33^{dc}$
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	27 33	37 33	4 88	0 14	17 83	$2\ 67^{cde}$
T_3 -*T viride*	27 33	38 00	4 75	0 15	17 00	$2\ 67^{cde}$
T_4 -*P fluorescens*	27 33	37 67	4 88	0 14	17 67	$3\ 00^{bcd}$
T_5 -cowdung supernatant	28 00	37 67	4 87	0 14	16 50	$2\ 33^{de}$
T_6 -cowdung supernatant + *P fluorescens*	27 67	38 00	4 75	0 14	17 67	$2\ 67^{cde}$
T_7 garlic extract	27 67	37 67	4 61	0 12	17 50	$2\ 00^{e}$
T_8 Calphomil	27 67	38 00	4 63	0 13	17 50	$4\ 33^{a}$
T_9 -potassium phosphonate + hexaconazole	27 67	37 67	4 90	0 14	17 67	$3\ 33^{bc}$
T_{10}-cymoxanil + mancozeb	27 67	37 67	4 81	0 14	17 67	$3\ 67^{ab}$
T_{11} -mancozeb	28 00	37 67	4 76	0 13	16 83	$2\ 67^{cde}$
T_{12} -control	28 00	37 67	4 69	0 12	17 00	$2\ 00^{e}$
CV	2 80	1 92	4 52	9 22	3 69	18 53
CD	NS	NS	NS	NS	NS	0 88

*Mean of three replications, values followed by same superscript are not significantly different by DMRT (P=0 05)

S Soil solarization, ST Seed treatment, SA Soil application

In polyhouse experiment, there was no significant difference among the treatments in any of the biometric parameters except shelf life (Plate 11) However, there is a slight earliness in the case of flowering and harvest in the treatments T_1 and T_2 and also the fruit weight, vine length and fruit length found to be more in treatments T_1 and T_2 Vine length of cucumber in polyhouse ranges from 4 61 to 5m and fruit weight ranges from 0 12 to 0 15kg Fruit length showed a variation from 16 50 to 17 83cm The longest shelf life of fruits was in T_8(Calphomil) followed by T_{10}(cymoxanil + mancozeb) when stored at room temperature

Plate 11 Different stages of the crop in polyhouse (cucumber variety Hilton)

Table 12. Effect of treatments on biometric characters of cucumber in rain shelter

Treatment	Biometric characters*					
	Days to flowering	Days to harvest	Vine length(m)	Fruit weight(kg)	Fruit length(cm)	Shelf life (days)
T_1 -S + ST + SA + foliar spray - *T. viride*	33 67	42 33	4 90	0 24	18 17	2 00
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	32 67	42 67	4 73	0 25	18 00	2 00
T_3 -*T viride*	32 67	42 67	4 46	0 22	18 00	1 67
T_4 -*P fluorescens*	32 33	42 67	4 03	0 23	17 50	2 33
T_5 -cowdung supernatant	33 00	43 33	4 55	0 23	18 00	2 00
T_6 cowdung supernatant + *P fluorescens*	32 67	42 67	4 00	0 23	17 83	1 67
T_7 garlic extract	33 00	43 33	4 51	0 22	18 00	1 33
T_8 Calphomil	33 33	43 00	4 71	0 23	17 83	2 67
T_9 -potassium phosphonate + hexaconazole	33 00	43 00	4 24	0 23	17 83	2 33
T_{10} cymoxanil + mancozeb	33 00	43 00	4 63	0 23	17 67	3 33
T_{11} mancozeb	33 67	43 33	4 49	0 23	17 50	2 33
T_{12} -control	33 67	43 33	4 53	0 22	17 67	2 00
CV	2 43	1 92	11 14	3 45	3 66	38 10
CD	NS	NS	NS	NS	NS	NS

*Mean of three replications, values followed by same superscript are not significantly different by DMRT (P=0 0:
S-Soil solarization, ST- Seed treatment, SA-Soil application

Biometric observations of cucumber in rain shelter are presented in Table 12 (Plate 12) There was no significant difference among the treatments in any of the biometric parameters Days to flowering and days to harvest were lesser for cucumber in treatments T_1 and T_2 Fruit weight, fruit length and vine length also more in treatments T_1 and T_2 as in polyhouse Vine length ranges from 4 00 to 4 90m and fruit weight varied from 0 22 to 0 14 kg Fruit length ranges from 17 50 to 18 17cm Shelf life of cucumber was observed to be higher for treatment T_{10} (cymoxanil + mancozeb) followed by treatment T_8 (Calphomil) in rain shelter However shelf life was not significantly different among the treatments in rain shelter

Plate 12 Different stages of the crop in rain shelter (cucumber variety AAUC-2)

4.3.2.5 Economic analysis

Benefit cost ratio was calculated at the market price *i e* Rs 30 kg ^{1}for all the treatments and also with 20 per cent premium price for nonchemical treatments

Table 13. Economic analysis of treatments in polyhouse

Treatment	Benefit : Cost ratio					
	Total cost (Rs.)	Yield (kg)	@ Rs.30 kg ^{1}		@ 20% premium price for nonchemical produce	
			Total returns (Rs)	B C ratio	Total returns (Rs)	B C ratio
T_1 -S + ST + SA + foliar spray - *T. viride*	1342	70 2	2106	1 57 1	2527 2	1 88 1
T_2 -S + ST + SA + foliar spray - *P. fluorescens)*	1271 5	67 8	2034	1 60 1	2440 8	1 92 1
T_3 -*T viride*	1417	61 9	1857	1 31 1	2228 4	1 57 1
T_4 -*P fluorescens*	1471 5	57 41	1722 3	1 17 1	2066 76	1 40 1
T_5 -cowdung supernatant	1539	62 8	1884	1 22 1	2260 8	1 47 1
T_6 -cowdung supernatant + *P fluorescens*	1571 5	60 95	1828 5	1 16 1	2194 2	1 40 1
T_7 -garlic extract	1567	59 6	1788	1 14 1	2145 6	1 37 1
T_8 -Calphomil	1617	61 4	1842	1 14 1	2210 4	1 37 1
T_9 -potassium phosphonate + hexaconazole	1527	61 45	1843 5	1 21 1	1843 5	1 21 1
T_{10}-cymoxanil + mancozeb	1149	67 60	2028	1 77 1	2028	1 77 1
T_{11} -mancozeb	1290	65 25	1957 5	1 52 1	1957 5	1 52 1
T_{12} -control	1159	41 05	1231 5	1 06 1	1477 8	1 28 1

S Soil solarization, ST- Seed treatment, SA Soil application

Benefit cost ratio of different treatments in polyhouse was calculated and presented in Table 13 At the market price for all treatments of Rs 30 kg^{-1}, the highest B C ratio was observed in treatment T_{10}(cymoxanil + mancozeb) *i e* 1 77 1 However when calculated with 20 per cent premium price for nonchemical treatments, T_2 (solarization+ *Pseudomonas fluorescens*) is found to be highly economic with B C ratio (1 92 1) However, all the treatments recorded a B C ratio, higher than control in both the structures

Table 14. Economic analysis of treatments in rain shelter

Treatment	Benefit . Cost ratio					
	Total cost (Rs.)	Yield (kg)	@ Rs.30 kg^{-1}		@ 20% premium price nonchemical produce	
			Total returns (Rs)	B·C ratio	Total returns (Rs.)	B:C ratio
T_1 -S + ST + SA + foliar spray - *T. viride*	1227	50 34	1510 2	1 23 1	1812 24	1 48 1
T_2 -S + ST + SA + foliar spray - *P. fluorescens)*	1112 55	48 09	1442 7	1 30 1	1731 24	1 56 1
T_3 -*T viride*	1198	45 45	1363 5	1 14 1	1636 2	1 37 1
T_4 -*P. fluorescens*	1153	44 64	1339 2	1 16 1	1607 04	1 39 1
T_5 -cowdung supernatant	1089	41 79	1253 7	1 15 1	1504 44	1 38 1
T_6 -cowdung supernatant + *P fluorescens*	1136 5	42 06	1261 8	1 11 1	1514 16	1 33 1
T_7 -garlic extract	1122	41 31	1239 3	1 11 1	1487 16	1 33 1
T_8 -Calphomil	1102	41 91	1257 3	1 14 1	1508 76	1 39 1
T_9 -potassium phosphonate + hexaconazole	1012	42 60	1278	1 26 1	1278 00	1 26 1
T_{10}-cymoxanil + mancozeb	956	46 14	1384 2	1 45 1	1384 2	1 45 1
T_{11} -mancozeb	1034	45 53	1365 93	1 32 1	1365 93	1 32 1
T_{12} -control	944	32 49	974 7	1 03 1	1169 64	1 23 1

S Soil solarization, ST Seed treatment, SA Soil application

Benefit cost ratio of different treatments in rain shelter was calculated and presented in Table 14 At Rs 30 kg ^{1}, the highest B C ratio was observed in treatment T_{10} (cymoxanil + mancozeb) *ie* 1 45 1 However, when calculated with 20 per cent premium price for nonchemical treatments, T_2 (solarization+ *Pseudomonas fluorescens*) is found to be highly economic as in polyhouse with 1 56 1 B C ratio Here also, all the treatments, gave a B C ratio higher than control

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 At the same price for all treatments, highest B C ratio was observed in treatment T_{10}(cymoxanil + mancozeb) in both polyhouse and rain shelter However, when calculated at 20 per cent premium price, treatment T_2 (solarization+ *Pseudomonas fluorescens*) is found to be highly economic in both the structures

4.4 Meteorological parameters

Temperature and relative humidity inside the polyhouse and rain shelter was recorded at 7 30 am and 2 30 pm daily during the experiment (Table 15) Temperature was found to be higher in rain shelter compared to polyhouse

Inside the polyhouse the temperature varied from 22 6 to 29 6°C at 7 30 am and during this period, in rain shelter it varied from 23 2 to 30 4°C At 2 30 pm, the temperature inside the polyhouse and rain shelter varied from 36 6 to 42 5°C and 40 6 to 44 1°C respectively (Fig 3, 4)

Table 15. Meteorological data during the period of experiments in polyhouse and rain shelter

Std. week*	Meteorological parameters											
	Polyhouse				Rain shelter				Open			
	7.30 am		2.30 pm		7.30 am		2.30 pm		7.30 am		2.30 pm	
	Temp. (^{0}C)	RH	Temp. (^{0}C)	RH	Temp. (^{0}C)	RH	Temp. (^{0}C)	RH	Temp. (^{0}C)	RH	Temp. (^{0}C)	RH
1	27.0	71	42.5	42	27.8	61	42.1	37	22.5	72	32.5	41
2	25.1	74	39.9	42	25.8	66	40.9	32	22.7	75	32.7	46
3	26.6	76	36.6	40	27.2	70	41.2	32	24.2	72	34.1	42
4	24.9	65	40.5	33	25.4	64	40.9	25	22.1	66	34.5	26
5	22.6	80	40.1	41	23.2	80	42.1	37	22.2	82	34.9	39
6	25.8	80	40.3	37	26.3	78	40.6	32	24.1	79	35.1	36
7	29.6	81	40.1	39	30.4	80	42.1	32	24.6	82	36.2	44
8	27.5	84	41.2	46	28.1	83	42.6	41	24.8	84	36.0	34
9	24.7	81	40.0	67	25.2	80	40.6	46	25.0	81	36.7	42
10	29.1	80	39.8	76	28.7	80	40.7	54	25.0	81	36.7	42
11	27.5	89	40.1	64	27.9	87	40.7	50	25.0	90	36.1	51
12	29.3	91	40.1	60	29.8	90	42.7	46	27.3	92	35.8	56
13	28.3	89	41.1	61	28.6	88	44.1	45	25.5	90	36.8	51
14	29.6	87	41.2	62	30.4	86	43.1	46	25.8	87	36.4	52
15	30.7	85	41.6	52	31.2	83	44.1	46	26.3	86	35.0	59

*average of seven days

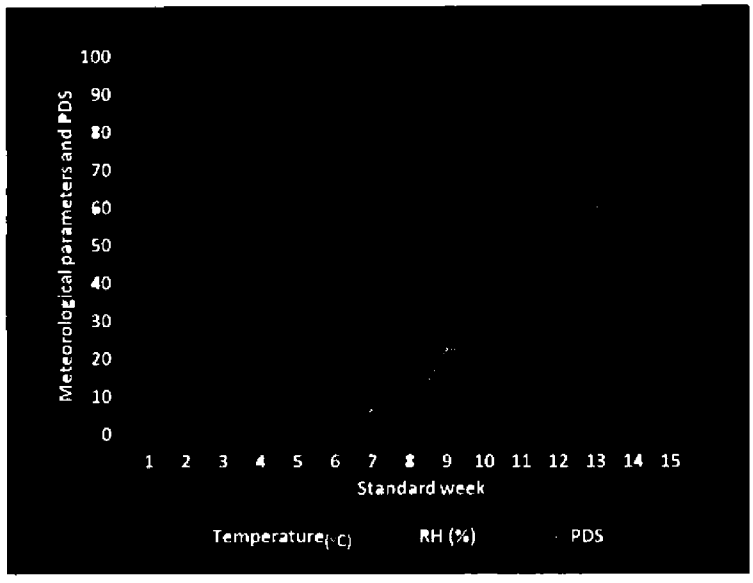


Fig 3 Influence of meteorological parameters on disease severity in polyhouse

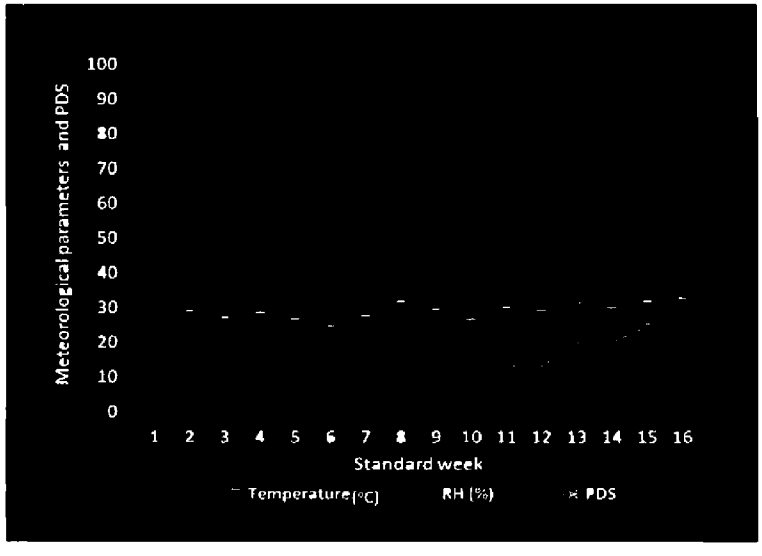
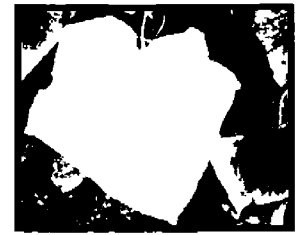


Fig 4 Influence of meteorological parameters on disease severity in rain shelter

RH Relative humidity PDS Per cent disease severity

Damping off
Pythium aphanidermatum

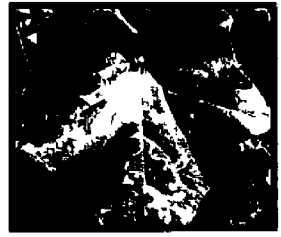
Fruit rot
Pythium aphanidermatum



Red spider mite
Tetranychus sp

Plate 13 Incidence of pests and diseases other than downy mildew in polyhouse

Powdery mildew
Erisiphe cichoracearum



Serpentine leaf minor
Liriomyza trifolii

Cucumber caterpillar
Diaphania indica

Red spider mite
Tetranychus sp

Plate 14 Incidence of pests and diseases other than downy mildew in rain shelter

Inside the polyhouse, relative humidity varied from 65 to 91 per cent at 7 30 am and during this period, in ram shelter it varied from 64 to 90 per cent At 2 30 pm, RH inside the polyhouse and ram shelter varied from 33 to 76 per cent and 32 to 54 per cent respectively

Table 16. Correlation analysis of disease severity with major meteorological parameters.

Per cent disease severity / Meteorological parameters	Correlation coefficients			
	Polyhouse		Ram shelter	
	PDS	RH	PDS	RH
RH	0 933**		0 803**	
Temperature	-0 283*	0 082	-0 427*	0 196

PDS Per cent disease incidence RH- Relative humidity

*Correlation is significant at 0 05 level ** Correlation is significant at 0 01 level

The correlation analysis was performed utilizing the data collected during the field experiment, and here also it was observed that there is a significant positive correlation between PDS and RH inside the polyhouse and rain shelter and it is negatively correlated with temperature

4 5 Incidence of other diseases and pests

Incidence of other diseases and pests were recorded during the experiment (Table 17) Minor incidence of damping off and powdery mildew was recorded in polyhouse (Plate 13) and rain shelter (Plate 14) respectively Infestation of pests such as cucumber caterpillar and serpentine leaf minor were also observed in rain shelter whereas in polyhouse the infestation of red spider mite was observed

Table 17. Per cent incidence of other diseases and pests in polyhouse and rain shelter

Pests/diseases/ physiological disorders	Per cent disease incidence(PDI)	
	Polyhouse	Rain shelter
Pests		
Cucumber caterpillar		11 57
Serpentine leaf minor		6 94
Red spider mite	5 23	
Diseases		
Damping off	4 17	
Powdery mildew		13 89
Physiological disorders		
Calcium deficiency	*	*
Boron deficiency	*	

*Presence of deficiency symptos

Symptoms of calcium deficiency were observed both in polyhouse and rain shelter towards the last stage of harvest (Plate 15) The fruits became C' shaped or dump-bell shaped when there is deficiency of calcium Yellowing and drying of immature fruits due to boron deficiency was observed in polyhouse However, these deficiencies were observed towards the last stage of the crop and hence did not affect the yield

4 6 Enumeration of phylloplane microflora under protected condition

The phylloplane microflora (fungi and bacteria) of the crop was enumerated before and after each spray using serial dilution plating in both polyhouse and rain shelter (Plate 16) Actinomycetes could not be isolated from any of the samples

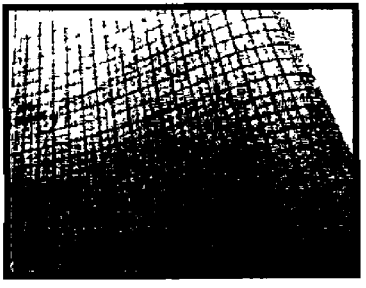
4.6.1 Population of phylloplane fungi

There was more or less uniform population of phylloplane fungi on the crop inside the polyhouse before spraying (Table 18) which ranged from 62 01 x 10 cfu cm^2 to 69 61 x10 cfu cm^2 But immediately after spraying, there is a drastic

A Calcium deficiency

B. Boron deficiency

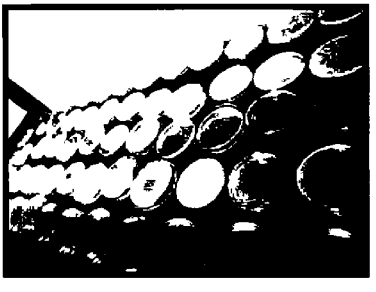
Plate 15 Nutrient deficiency symptoms in cucumber in protected structures



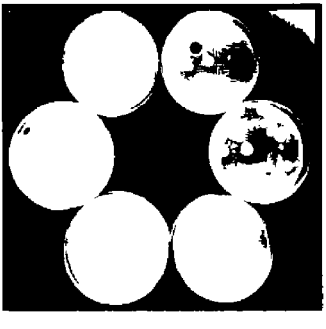
A Assessment of leaf area

B Extraction of phylloplane microflora in sterile water

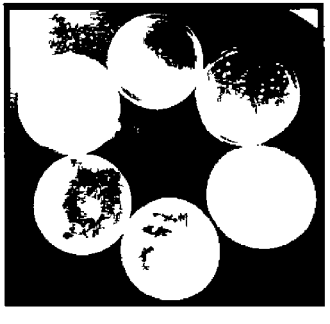
C. Media used
Fungi- Martin's Rose Bengal Agar
Actinomycetes- Ken Knights Agar
Bacteria- Nutrient Agar



D Incubation of plates



E Phylloplane fungi



F Phylloplane bacteria

Plate 16 Enumeration of phylloplane microflora of cucumber

reduction in the population in T_{10}(cymoxanil + mancozeb-17 14 x 10 cfu cm^2), T_{11}(mancozeb-23 81 x 10 cfu cm^2) and T_9(potassium phosphonate + hexaconazole-25 52 x 10 cfu cm^2) However, the population slightly increased to 21 43 x 10 cfu cm^2, 28 10 x 10 cfu cm^2, 29 05 x 10 cfu cm^2 respectively at 15 days after spraying Again after second spray, it came down further to 11 57 x 10 cfu cm^2, 22 24 x 10 cfu cm^2, 20 95 x 10 cfu cm^2 respectively Then in the case of T_{10} and T_9 since there was no third spray, the population continuously increased to 28 64 x 10 cfu cm^2, 39 56 x 10 cfu $cm,^2$ respectively at 45 DAS In the case of T_{11}, the same trend is observed after third spray also (Fig 5)

Table 18. Effect of different treatments on phylloplane fungi of cucumber in polyhouse

Treatment	Fungi (x 10 cfu cm^2)*						
	Pre treatment	First spraying		Second spraying		Third spraying	
		1DAS	15DAS	1DAS	15DAS	1DAS	15DAS
T_1 -S + ST + SA + foliar spray - *T viride*	68 63	108 84	66 34	117 68	67 45	121 67	64 96
T_2 -S + ST + SA + foliar spray - *P. fluorescens)*	66 67	38 57	62 86	34 32	65 87	33 42	64 81
T_3 -*T viride*	64 81	105 65	65 76	113 65	69 66	123 65	68 52
T_4 -*P fluorescens*	68 57	34 24	63 52	37 43	65 67	36 54	63 89
T_5 -cowdung supernatant	67 59	89 86	67 14	87 56	68 18	86 83	63 52
T_6 -cowdung supernatant + *P fluorescens*	69 52	78 61	63 33	76 53	65 34	76 23	67 34
T_7 -garlic extract	62 86	46 19	68 48	46 19	70 48	46 19	69 78
T_8 -Calphomil	64 76	37 14	61 43	37 14	71 43	37 14	68 74
T_9 -potassium phosphonate + hexaconazole	67 62	25 52	29 05	20 95	27 62	34 52	39 56
T_{10}-cymoxanil + mancozeb	69 61	17 14	21 43	11 57	14 29	21 19	28 64
T_{11} -mancozeb	62 04	23 81	28 10	22 24	29 52	21 90	31 11
T_{12} -control	63 81	65 71	67 89	68 76	66 19	65 10	69 11

*Mean of three replications DAS Days after spraying

S Soil solarization, ST Seed treatment, SA Soil application

In treatments T_1 and T_3, where *Trichoderma* was sprayed on leaves, there is a drastic increase in the population of phylloplane fungalflora and the population is

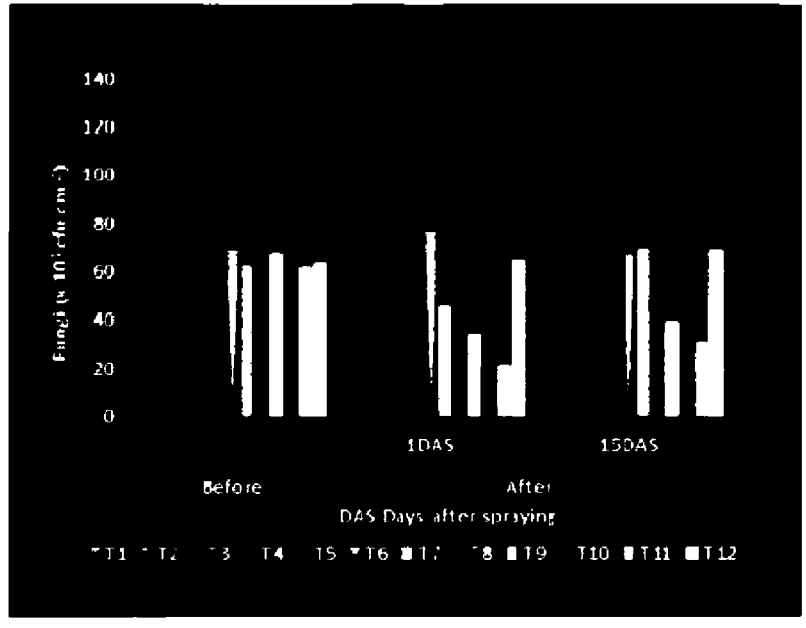


Fig 5 Effect of treatments on phylloplane fungi in polyhouse

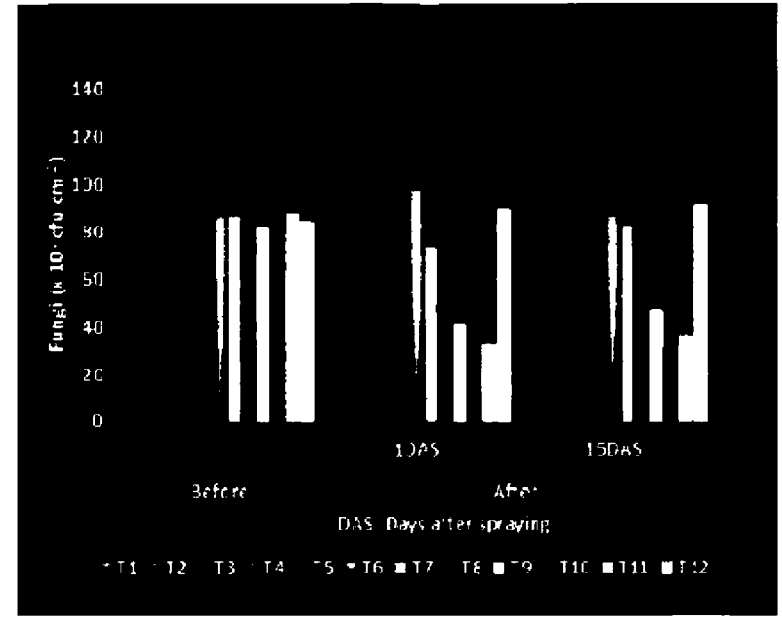


Fig 6 Effect of treatments on phylloplane fungi in rain shelter

T_1 -S + ST + SA + foliar spray - *T viride*
T_2 -S + ST + SA + foliar spray - *P fluorescens*
T_3 -*T viride*
T_4 -*P fluorescens*
T_5 -cowdung supernatant
T_6 -cowdung supernatant + *P fluorescens*
T_7 -garlic extract
T_8 -Calphomil
T_9 -potassium phosphonate + hexaconazole
T_{10} -mancozeb
T_{11} -cymoxanil + mancozeb
T_{12} -control

S- Soil solarization ST Seed treatment SA Soil Application

108 84 x 10 cfu $cm^2$and 105 65 x 10 cfu cm^2 respectively immediately after spraying In T_5(cowdung) and T_6 (cowdung +*Pseudomonas*)also there was increase in phylloplane fungal population *i e*, 89 86 x 10 cfu $cm^2$and 78 61 x 10 cfu cm^2 respectively However in all these treatments, the population came back to initial level after 15 days of spraying In T_2 and T_4 where *Pseudomonas* was sprayed there was reduction in the population of fungalflora, but the reduction was lesser compared to chemical treatments and the population increased and reached the initial level by 15 days of spraying

In T_7 (garlic) and T_8 (Calphomil) there was reduction in the population immediately after spraying, but not upto the level of chemical treatments and after 15 days, the population gradually got build upto original level The same trend is observed after second and third spray also However, the population in control did not pseudoperonospora change during the experiment

It was noticed that the trend in the variation of population of phylloplane fungalflora in rain shelter is same as that of polyhouse But in rain shelter the initial population was slightly higher (Table 19) than polyhouse and it varied from 81 61 x 10 cfu cm^2 to 89 29 x 10 cfu cm^2 But immediately after spraying, there is a drastic reduction in the population in T_{10} (cymoxanil + mancozeb-23 08x 10 cfu cm^2), T_{11}(mancozeb-32 05x 10 cfu cm^2) and T_9(potassium phosphonate + hexaconazole-36 15x 10 cfu cm^2) However, the population slightly increased to 25 23 x 10 cfu cm^2,35 21 x 10 cfu cm^2, 38 56x 10 cfu cm^2 respectively at 15 days after spraying Again after second spray it came down further to 23 46 x 10 cfu cm^2, 33 44 x 10 cfu cm^2, 37 54 x 10 cfu cm^2 respectively Then in the case of T_9 and T_{10} since there was no third spray, the population continuously increased to 46 56 x 10 cfu cm^2, 31 78 x 10 cfu cm, ^{2}respectively at 45DAS In the case of T_{11}, the same trend is observed after third spray also (Fig 6)

Table 19. Effect of different treatments on phylloplane fungi of cucumber in rain shelter

Treatment	Fungi (x 10 cfu cm ^{2})*						
	Pre treatment	First spraying		Second spraying		Third spraying	
		1DAS	15DAS	1DAS	15DAS	1DAS	15DAS
T_1 -S + ST + SA + foliar spray - *T viride*	85 71	133 67	80 77	138 34	83 33	136 26	84 94
T_2 -S + ST + SA + foliar spray - *P fluorescens*	86 90	48 46	84 62	49 85	86 67	45 38	86 36
T_3 -*T viride*	86 67	138 46	86 42	134 80	85 56	139 23	87 92
T_4 -*P fluorescens*	84 52	47 44	83 59	48 97	84 44	49 23	86 07
T_5 -cowdung supernatant	89 29	102 82	88 97	105 64	87 78	104 36	87 78
T_6 -cowdung supernatant + *P fluorescens*	85 71	92 56	87 72	97 95	88 89	97 03	85 61
T_7 -garlic extract	85 92	68 97	85 13	69 49	84 44	73 08	82 22
T_8 -Calphomil	83 33	50 70	86 15	51 38	86 67	49 87	87 78
T_9 -potassium phosphonate + hexaconazole	81 61	36 15	38 56	37 54	38 33	40 77	46 56
T_{10}-cymoxanil + mancozeb	84 52	23 08	25 23	23 46	26 17	27 72	31 78
T_{11} -mancozeb	87 18	32 05	35 21	33 44	36 11	32 41	36 00
T_{12} -control	83 33	88 46	87 23	89 65	84 44	89 49	90 60

*Mean of three replications DAS-Days after spraying

S-Soil solarization, ST- Seed treatment, SA Soil application

In treatments T_1 and T_3, where *Trichoderma* was sprayed on leaves, there is a drastic increase in the population of phylloplane fungalflora and the population is 133 67 x 10 cfu cm ^{2} and 138 46 x 10 cfu cm ^{2} respectively In T_5(cowdung) and T_6 (cowdung +*Pseudomonas*) also there was increase in phylloplane fungal population *i e*, 102 82 x 10 cfu cm ^{2}and 92 56 x 10 cfu cm ^{2} respectively immediately after first spray However in all these treatments, the population came back to initial level after 15 days of spraying In T_2 and T_4 where *Pseudomonas* was sprayed there was reduction in the population of fungalflora, but the reduction was lesser compared to chemical treatments and the population increased and reached the initial level by 15 days of spraying

In T_7 (garlic) and T_8 (Calphomil) there was reduction in the population immediately after spraying, but not upto the level of chemical treatments and after 15 days, the population gradually got built upto original level The same trend is observed after second and third spray also However, the population in control did not change during the experiment

4.6.2 Population of phylloplane bacteria

It was observed that the population of phylloplane bacteria is always higher compared to fungi (Fig 7) Inside the polyhouse there was more or less uniform population of phylloplane bacteria on the crop before spraying (Table 20) which ranged from 17 14 x 10^3 cfu cm^2 to 19 19 x 10^3 cfu cm^2 But immediately after spraying, there is a drastic reduction in the population in T_{10}(zero), T_{11}(mancozeb-0 27 x 10^3 cfu cm^2) and T_9(potassium phosphonate + hexaconazole-0 87 x 10^3 cfu cm^2) However, the population slightly increased to 2 14 x 10^3 cfu cm^2, 5 81 x 10^3 cfu cm^2, 6 85x 10^3 cfu cm^2 respectively at 15 days after spraying Again after second spray it came down further to 0 03x 10^3 cfu cm^2, 0 45 x 10^3cfu cm^2, 0 98 x 10^3cfu cm^2 respectively Then in the case of T_{10} and T_9, since there was no third spray, the population continuously increased to 4 94 x 10^3 cfu cm^2, 8 63 x 10^3cfu cm^2, respectively at 45 DAS In the case of T_{11}, the same trend is observed after third spray also As in the case of phylloplane fungal population, bacteria also did not change in control

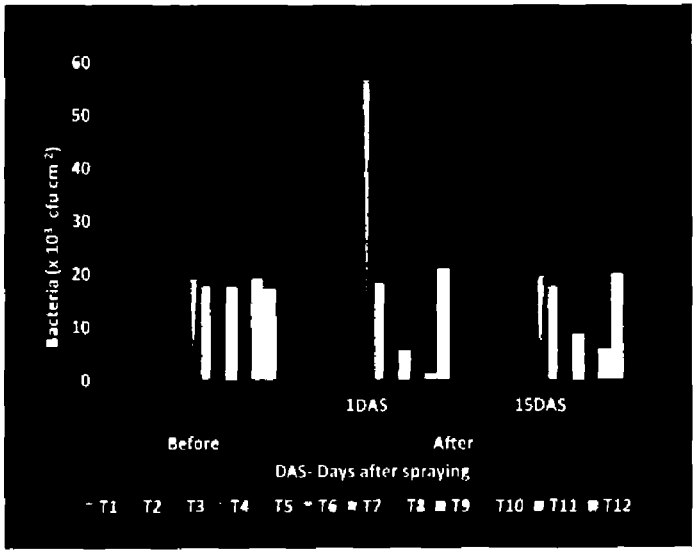


Fig 7 Effect of treatments on phylloplane bacteria in polyhouse

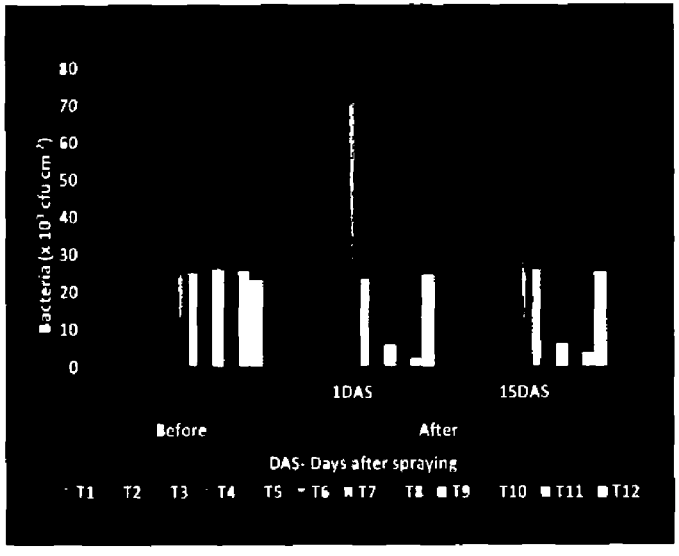


Fig 8 Effect of treatments on phylloplane bacteria in rain shelter

T_1 -S + ST + SA + foliar spray - *T viride*
T_2 -S + ST + SA + foliar spray - *P fluorescens*
T_3 -*T viride*
T_4 -*P fluorescens*
T_5 -cowdung supernatant
T_6 -cowdung supernatant + *P fluorescens*
T_7 -garlic extract
T_8 -Calphomil
T_9 -potassium phosphonate + hexaconazole
T_{11} -mancozeb
T_{10}-cymoxanil + mancozeb
T_{12} -control

S Soil solarization ST Seed treatment SA Soil Application

Table 20. Effect of different treatments on phylloplane bacteria of cucumber in polyhouse

Treatment	Bacteria (x 10^3cfu cm^{-2})*						
	Pre treatment	First spraying		Second spraying		Third spraying	
		1DAS	15DAS	1DAS	15DAS	1DAS	15DAS
T_1 -S + ST + SA + foliar spray - *T. viride*	19 05	11 90	18 24	14 29	19 07	13 57	18 82
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	19 19	42 02	19 39	46 16	19 00	49 31	18 73
T_3 -*T viride*	17 32	12 80	19 24	13 72	17 22	11 63	19 78
T_4 -*P. fluorescens*	18 18	42 86	18 10	45 10	18 85	47 48	19 69
T_5 -cowdung supernatant	16 19	34 76	17 38	39 05	18 74	38 10	18 39
T_6 -cowdung supernatant + *P. fluorescens*	19 05	55 56	19 05	56 71	18 41	56 76	19 57
T_7 -garlic extract	17 65	19 90	18 14	19 38	17 48	18 33	17 59
T_8 -Calphomil	18 44	14 85	20 76	15 22	19 78	16 52	19 67
T_9 -potassium phosphonate + hexaconazole	17 50	0 87	6 95	0 98	4 63	5 67	8 63
T_{10}-cymoxanil + mancozeb	18 10	0 00	2 14	0 03	1 70	3 52	4 94
T_{11} -mancozeb	19 05	0 27	5 81	0 45	4 67	1 28	5 90
T_{12} -control	17 14	18 10	18 38	17 14	19 44	20 95	19 84

*Mean of three replications DAS Days after spraying

S-Soil solarization, ST Seed treatment, SA-Soil application

In treatment T_6 (cowdung +*Pseudomonas*), there was a tremendous increase in the population of phylloplane bacteria (55 56 x 10^3cfu cm^{-2}) Similarly in T_2 and T_4 also, where *Pseudomonas* was sprayed on leaves, there was a drastic increase in the population of phylloplane bacteria and the population is 42 02 x 10^3cfu $cm^{-2}$and 42 86 x 10^3cfu cm^{-2} respectively In T_5(cowdung) also there was increase in phylloplane bacterial population *i e* 34 76 x 10^3cfu cm^{-2} However in all these treatments, the population came back to initial level after 15 days of spraying In T_1 and T_3 where *Trichoderma* was sprayed there was reduction in the population of bacteria, but the reduction was lesser compared to chemical treatments and the population increased and reached the initial level by 15 days of spraying

In T_7 (garlic), immediately after spraying, there was an increase in the phylloplane bacterial population, but not upto the level of T_4 (*Pseudomonas)* and in

T_8 (Calphomil) there was reduction in the population immediately after spraying, but not upto the level of chemical treatments and after 15 days, the population in both T_7 and T_8 gradually got build upto original level Same trend is observed after second and third spray also However, the population in control did not change during the experiment

It was noticed that the trend in the variation of population of phylloplane bacteria also was the same in polyhouse and rain shelter (Fig 8) The initial population was slightly higher in rain shelter (Table 21) than in polyhouse and it ranged from 23 08 x 10^3 cfu cm^2 to 26 92 x 10^3 cfu cm^2 But immediately after spraying, there is a drastic reduction in the population in T_{10}(1 33 x 10^3 cfu cm^2), T_{11}(mancozeb-2 67 x 10^3 cfu cm^2) and T_9(potassium phosphonate + hexaconazole-3 67 x 10^3 cfu cm^2) However, the population slightly increased to 2 70 x 10^3cfu cm^2,3 38 x 10^3cfu cm^2, 6 33x 10^3cfu cm^2 respectively at 15 days after spraying Again after second spray it came down further to 1 02 x 10^3cfu cm^{-2}, 2 23 x 10^3cfu cm^2, 6 77x 10^3cfu cm^2 respectively Then in the case of T_{10} and T_9, since there was no third spray, the population continuously increased to 3 03 x 10^3cfu cm^2, 6 22 x 10^3cfu cm^2, respectively at 45 DAS Again in T_{11}, the same trend is observed after third spray also

In treatment T_6 (cowdung +*Pseudomonas*), there was a tremendous increase in phylloplane bacterial population upto 67 33 x 10^3cfu cm^2immediately after first spraying In T_2 and T_4, where *Pseudomonas* was sprayed on leaves, there is a drastic increase in the population of phylloplane bacteria and the population is 64 00 x 10^3cfu $cm^2$and 64 27 x 10^3cfu cm^2 respectively In T_5(cowdung) also there was increase in phylloplane bacterial population *i e* 43 33 x 10^3cfu cm^2 immediately after first spraying However in all these treatments, the population came back to initial level after 15 days of spraying In T_1 and T_3 where *Trichoderma* was sprayed there was reduction in the population of bacteria, but the reduction was lesser

compared to chemical treatments and the population increased and reached the initial level by 15 days of spraying

Table 21. Effect of different treatments on phylloplane bacteria of cucumber in rain shelter

Treatment	Bacteria (x 10^3cfu cm^2)*						
	Pre treatment	First spraying		Second spraying		Third spraying	
		1DAS	15DAS	1DAS	15DAS	1DAS	15DAS
T_1 -S + ST + SA + foliar spray - *T viride*	25 64	14 56	25 78	15 65	24 45	16 34	25 78
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	24 36	64 00	25 24	67 23	26 07	66 39	28 97
T_3 -*T viride*	23 61	15 87	27 96	15 89	27 03	17 45	26 67
T_4 -*P fluorescens*	23 08	64 27	25 24	65 36	27 22	65 33	26 41
T_5 -cowdung supernatant	25 64	43 33	23 33	45 62	25 74	46 67	24 25
T_6 -cowdung supernatant + *P fluorescens*	24 69	67 33	25 56	69 62	27 41	70 33	27 91
T_7 -garlic extract	25 08	21 33	24 00	22 46	25 33	23 67	26 00
T_8 -Calphomil	26 92	17 67	23 98	18 67	26 78	17 89	24 78
T_9 -potassium phosphonate + hexaconazole	25 93	3 67	6 33	3 77	5 67	5 89	6 22
T_{10}-cymoxanil + mancozeb	24 36	1 33	2 70	1 02	1 94	2 87	3 03
T_{11} -mancozeb	25 64	2 67	3 38	2 23	3 44	2 33	3 83
T_{12} -control	23 08	25 33	24 33	24 49	26 00	24 48	25 20

*Mean of three replications DAS Days after spraying

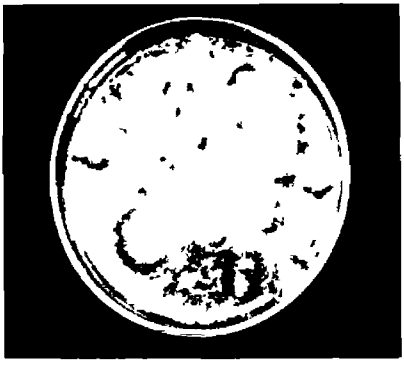
S Soil solarization, ST Seed treatment, SA-Soil application

In T_7 (garlic) and T_8 (Calphomil) there was reduction in the population immediately after spraying, but not upto the level of chemical treatments and after 15 days, the population gradually got built upto original level Similarly, same trend is observed after second and third spray also However, the population in control did not change during the experiment

4 7 Survival of the biocontrol agents on the phylloplane of cucumber under protected condition

The survival of the biocontrol agents sprayed on leaves in treatments T_1, T_2, T_3, T_4 and T_6 was assessed by re isolation and enumeration using serial dilution plating on suitable selective media at periodical intervals of 5, 10, 15 days (Plate 17)

Special media for biocontrol agents
T viride- *Trichoderma* selective Agar medium (TSM)
P fluorescens- King's B Agar medium



Trichoderma viride

Pseudomonas fluorescens

Plate 17 Biocontrol agents on cucumber phylloplane

Table 22. Survival of *Trichoderma viride* on the phylloplane of cucumber in polyhouse

Treatment	*Trichoderma viride* (x 10 cfu cm^{-2})*								
	Pre treatment	First spraying			Second spraying			Third spraying		
		5DAS	10DAS	15DAS	5DAS	10DAS	15DAS	5DAS	10DAS	15DAS
T_1 -S + ST + SA + foliar spray - *T viride*	2 75	41 24	21 51	17 68	52 63	26 04	19 61	54 60	13 64	8 33
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	2 73	2 86	3 15	2 11	2 90	2 58	2 36	2 95	2 17	3 30
T_3 -*T viride*	2 86	38 69	28 14	11 24	47 14	20 47	12 82	59 75	17 70	6 94
T_4 -*P fluorescens*	2 72	3 30	2 12	2 41	2 48	2 85	2 76	2 87	2 30	2 98
T_6 -cowdung supernatant + *P fluorescens*	2 60	11 05	7 30	8 19	19 80	10 84	2 76	28 67	8 69	3 03
T_{12} -control	2 55	2 62	2 76	2 49	2 18	2 87	1 80	2 11	2 57	2 69

*Mean of three replications DAS Days after spraying
S-Soil solarization, ST- Seed treatment SA-Soil application

4.7.1 Survival of *Trichoderma viride* on the phylloplane of cucumber under protected cultivation

Inside the polyhouse, before treatment application, the natural population of *Trichoderma* was almost the same in all the treatments (Table 22) which ranged from 2 55 x 10 cfu cm^2 to 2 86x 10 cfu cm^2 whereas after spraying, there was a 13 fold increase in the population in treatment T_1 and T_3 (Fig 9) Thereafter it gradually decreased from 41 24 and 38 69 x 10 cfu cm^2 at 5 DAS to 17 68 and 11 24 x 10 cfu cm^2 at 15 DAS respectively in T_1 and T_3 Consequent to second and third spraying the treatments showed same trend as in first spraying

In treatment T_6(cowdung + *P fluorescens)* immediately after the first spray, there was an increase in the population of *T viride* But it gradually decreased from 11 05 x 10 cfu cm^2 at 5 DAS to 8 19 x 10 cfu cm^2 at 15 DAS In T_2, T_4 and T_{12}(control), the population of *T viride* remained more or less uniform throughout the experiment The results proved that *T viride* survived on the phylloplane of cucumber upto 15 days after spraying

In rain shelter also, the population of *T viride* followed the same pattern as in polyhouse and before treatment application, the natural population of *Trichoderma* inside the rain shelter was almost the same in all the treatments (Table 23) which ranged from 2 39 x 10 cfu cm^2 to 2 56 x 10 cfu cm^2 whereas after spraying, there was a 24 fold and 11 fold increase in the population in treatment T_1 and T_3 respectively In T_1 the population of *T viride* gradually decreased from 48 35 x 10 cfu cm^2 at 5 DAS to 13 61 x 10 cfu cm^2 at 15 DAS (Fig 10)

Table 23 Survival of *Trichoderma viride* on the phylloplane of cucumber in rain shelter

Treatment	*Trichoderma viride* (x 10 cfu cm^{-2})*								
	Pre treatment	First spraying			Second spraying			Third spraying		
		5DAS	10DAS	15DAS	5DAS	10DAS	15DAS	5DAS	10DAS	15DAS
T_1 -S + ST + SA + foliar spray - *T viride*	2 53	48 35	25 95	13 61	53 33	34 95	22 99	52 34	39 86	14 49
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	2 50	2 90	2 81	1 66	1 25	1 90	2 06	2 17	2 63	2 63
T_3 -*T viride*	2 64	33 67	21 51	15 33	52 63	15 87	15 43	56 74	24 02	15 02
T_4 -*P fluorescens*	2 49	2 71	2 88	3 12	2 70	2 87	1 54	2 23	2 27	2 16
T_6 -cowdung supernatant + *P fluorescens*	2 39	5 70	3 66	3 06	6 26	4 10	3 48	8 58	5 90	4 60
T_{12} -control	2 56	2 28	2 30	2 14	2 03	2 55	2 77	2 25	2 23	2 89

*Mean of three replications DAS-Days after spraying
S-Soil solarization, ST- Seed treatment SA-Soil application

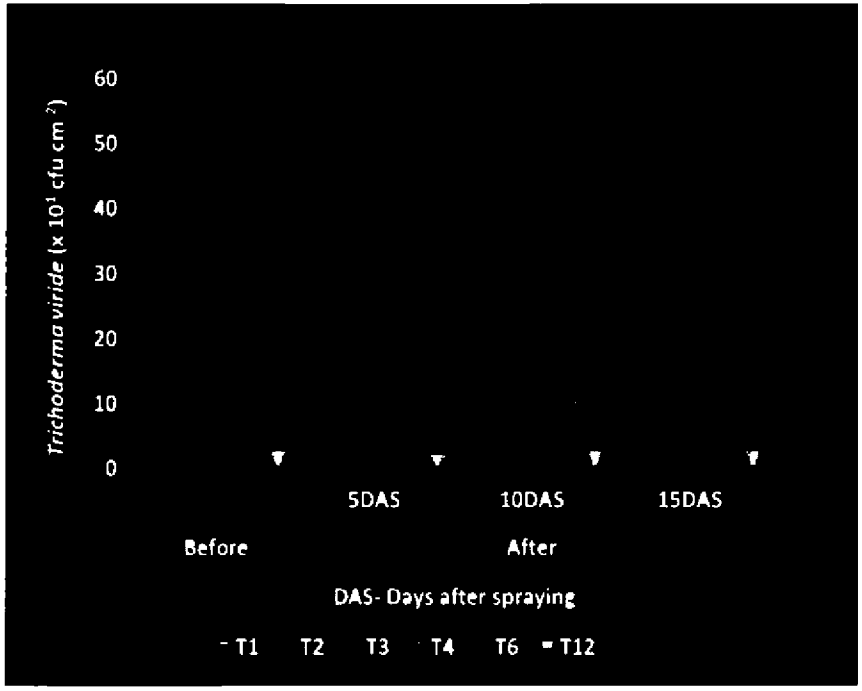


Fig 9 Survival of *Trichoderma viride* on phylloplane in polyhouse

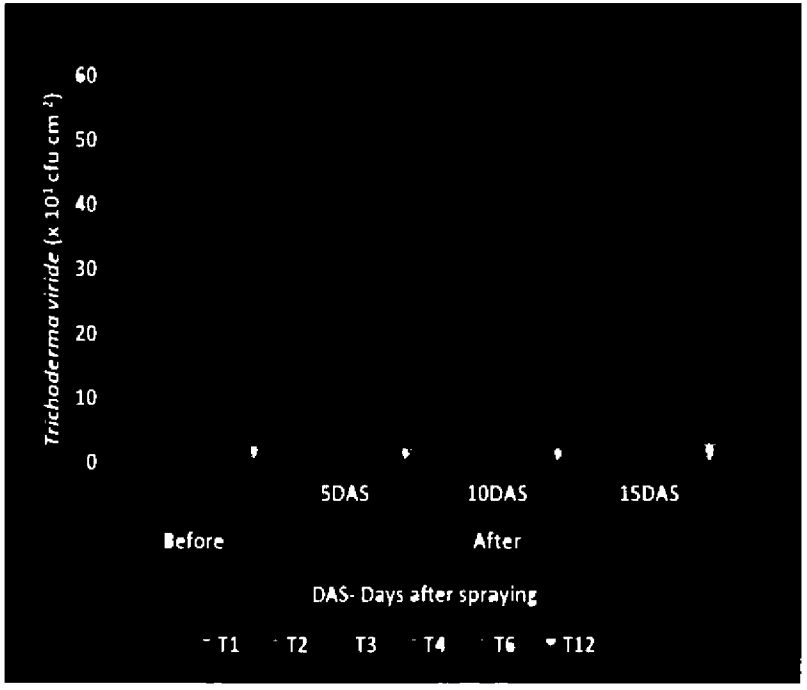


Fig 10 Survival of *Trichoderma viride* on phylloplane in rain shelter

T_1 -S + ST + SA + foliar spray - *T viride*
T_2 -S + ST + SA + foliar spray - *P fluorescens*
T_3 -*T viride*
T_4 -*P fluorescens*
T_6 -cowdung supernatant + *P fluorescens*
T_{12} -control

S- Soil solarization ST- Seed treatment SA- Soil Application

The same trend is observed in treatment T_3 also where population gradually decreased from 33 67 x 10 cfu cm^{2} at 5 DAS to 15 33 x 10 cfu cm^{2} at 15 DAS The same trend was noticed after second and third spraying also

In treatment, T_2 and T_4 where *Pseudomonas* was sprayed, the population of *T viride* was found to be more or less same as that of the pre-treatment population But in treatment T_6(cowdung + *P fluorescens),* there was an increase in the population of *T viride* at 5 DAS which gradually decreased from 5 70 x 10 cfu cm^{2} to 3 06 x 10 cfu cm^{2} at 15 DAS The same trend was observed after second and third spray However, in T_{12} (control), the population of *T viride* in the phylloplane did not change throughout the experiment This result confirms the result obtained in polyhouse

4 7.2 Survival of *Pseudomonas fluorescens* on the phylloplane of cucumber

Inside the polyhouse, before treatment application, the natural population of *P fluorescens* was same in all the treatments (Table 24) However, after spraying, it was observed that the population of *P fluorescens* increased in T_2 at 5 DAS But it gradually decreased from 49 46 x 10^3 cfu cm^{-2} at 5 DAS to 11 17 x 10^3 cfu cm^{2} at 15 DAS The same trend was observed before spraying in treatment T_4 also, where the population was more than that (44 65 x 10^3 cfu cm^{2}) at 5 DAS and then decreased to 13 86 x 10^3 cfu cm^{2} at 15 DAS (Fig 11)

Table 24.Survival of *Pseudomonas fluorescens* on the phylloplane of cucumber in polyhouse

Treatment	*Pseudomonas fluorescens* (x10^{3}cfu cm^{-2})*								
	Pre treatment	First spraying			Second spraying			Third spraying	
		5DAS	10DAS	15DAS	5DAS	10DAS	15DAS	5DAS	10DAS	15DAS
T_1 -S + ST + SA + foliar spray - *T viride*	6 30	0 18	0 22	0 16	0 28	0 52	0 35	0 56	0 15	0 16
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	6 28	49 46	29 00	11 17	43 50	32 07	17 70	60 73	26 13	17 86
T_3 -*T viride*	6 41	0 19	0 22	0 24	0 26	0 39	0 34	0 35	0 29	0 17
T_4 -*P fluorescens*	6 27	44 65	31 63	13 86	40 22	28 71	10 45	43 45	24 57	10 63
T_6 -cowdung supernatant + *P fluorescens*	6 15	25 62	10 83	4 42	25 62	15 85	5 62	28 18	16 67	7 45
T_{12} -control	6 10	6 22	5 95	4 76	4 50	6 08	8 09	6 72	8 25	6 94

*Mean of three replications DAS Days after spraying
S Soil solarization ST Seed treatment SA-Soil application

Fig 11 Survival of *Pseudomonas fluorescens* on phylloplane in polyhouse

Fig 12 Survival of *Pseudomonas fluorescens* on phylloplane in rain shelter

T_1 -S + ST + SA + foliar spray - *T viride*
T_2 -S + ST + SA + foliar spray - *P fluorescens*
T_3 -*T viride*
T_4 -*P fluorescens*
T_6 -cowdung supernatant + *P fluorescens*
T_{12} -control

S- Soil solarization ST- Seed treatment SA Soil Application

In treatment T_6(cowdung + *P fluorescens),* there was an increase in the population of *P fluorescens* after spraying at 5DAS but it gradually decreased from 25 62 x 10^3 cfu cm^2 to 4 42 x 10^3 cfu cm^2 at 15 DAS Similar trend was observed after second and third spray In treatment, T_1 and T_3 where *T viride* is sprayed, the population of *P fluorescens* declined from the pre-treatment population at 5, 10 and 15 DAS The same trend was observed after second and third spray in treatments T_1 and T_3 In general, the survival of *P fluorescens* was observed upto 15 days after treatment application In general, the survival of *P fluorescens* on the phylloplane of cucumber was observed upto 15 days after spraying

It was noticed that the trend in the variation of population of *Pseudomonas fluorescens* in rain shelter was same as that in polyhouse But in rain shelter the initial population was slightly lower (Table 25) than polyhouse and it ranged from 2 23 x 10^3 cfu cm^2 to 2 48 x 10^3 cfu cm^2 It was also observed that the population of *P fluorescens* is the highest in T_4 followed by T_2 In T_4 the population gradually decreased from 47 98 x 10^3 cfu cm^2 at 5 DAS to 12 91 x 10^3 cfu cm^2 at 15DAS Similarly in T_2 the population gradually decreased from 41 23 x 10^3 cfu cm^2 at 5 DAS to 14 78 x 10^3 cfu cm^2 at 15 DAS (Fig 12) Even after second and third spray, the population of *P fluorescens* in T_2 and T_4 showed the same trend In treatment T_6 (cowdung + *P fluorescens),* there is an increase in the population of *P fluorescens* at 5 DAS But it gradually decreased from 25 62 x 10^3 cfu cm^2 at 5 DAS to 4 42 x 10^3 cfu cm^2 at 15 DAS The same trend is observed after second and third spray

Table 25. Survival of *Pseudomonas fluorescens* on the phylloplane of cucumber in rain shelter

Treatments	*Pseudomonas fluorescens*(x10^3cfu cm^{-2})*								
	Pre treatment	First spraying			Second spraying			Third spraying		
		5DAS	10DAS	15DAS	5DAS	10DAS	15DAS	5DAS	10DAS	15DAS
T_1 -S + ST + SA + foliar spray - *T viride*	2 37	0 68	0 20	0 25	0 46	0 27	0 27	0 65	0 36	0 41
T_2 -S + ST + SA + foliar spray - *P fluorescens)*	2 34	41 23	26 99	14 75	43 93	23 95	13 04	50 38	22 88	17 90
T_3 -*T viride*	2 48	0 57	0 27	0 17	0 93	0 20	0 29	0 62	0 30	0 47
T_4 -*P fluorescens*	2 33	47 98	27 65	12 91	50 33	25 95	12 25	48 43	46 08	8 85
T_6 -cowdung supernatant + *P fluorescens*	2 23	35 76	35 06	9 57	33 33	17 48	8 94	22 74	15 31	7 69
T_{12} -control	2 40	2 86	2 30	2 07	3 03	3 55	3 02	3 22	3 12	1 81

*Mean of three replications DAS-Days after spraying
S-Soil solarization ST- Seed treatment, SA-Soil application

In treatment, T_1 and T_3 where *T viride* is sprayed, the population of *P fluorescens* was found to be reduced from the pre-treatment population at 5 days after treatment The same trend is observed after second and third spray in treatments T_1 and T_3 as in polyhouse At 45 DAS also, treatments were found to be significant In general, the survival of *P fluorescens* was observed upto 15 days after spraying As in polyhouse, the survival of *P fluorescens* on the phylloplane of cucumber was observed upto 15 DAS

Discussion

6. DISCUSSION

Protected cultivation of vegetables is a fascinating technology gaining tremendous importance in India in the recent years Cucumber, *Cucumis sativus* (L) is one of the most preferred vegetables grown under protected conditions 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 Eventhough, it was expected that polyhouses provide complete protection from insect pests and hence the produce from the structures would be free of pesticide residues, it is not true in reality Sucking pests and diseases are causing severe crop losses and farmers are forced to use chemical pesticides in polyhouse crops Recently Kerala Agricultural University has developed a high yielding F_1 hybrid, KPCH-1 suitable for polyhouse cultivation and it exhibited fair degree of tolerance against this dreaded disease (Pradeepkumar *et al* , 2015) Due to high relative humidity and temperature, crops in protected structures are prone to diseases irrespective of the season Downy mildew (*Pseudoperonospora cubensis*) is the most severe disease of cucumber under protected structures in Kerala Under highly conducive conditions for the disease, prevailing in Kerala, there are chances of heavy infection by downy mildew especially inside the polyhouse Farmers are using fungicides having high residual action against the disease Hence the main objective of the present study is to assess the incidence and severity of downy mildew of cucumber under protected cultivation and to formulate an eco-friendly management practice against the disease

5.1 Survey for assessment of incidence and severity of downy mildew

To begin with, a survey was conducted in Thrissur district during January December of 2015 by selecting nine different polyhouses located at Thanniyam, Peringottukara, Manaloor, Chendrapinm, Chavakkad and College of Horticulture, Vellanikkara The incidence and severity of downy mildew of cucumber in protected

structures and open condition were assessed using standard score chart and procedures The major meteorological factors *i e* temperature and relative humidity (RH) influencing the crop and the pathogen prevailing in the structures were also recorded using whirling psychrometer

During the survey, incidence of downy mildew was noticed in all the polyhouses where cucumber is cultivated irrespective of the season It was also noticed that, in all these polyhouses RH was found to be ≥79 per cent It is well documented that, if the RH is >75 per cent there is chance of occurrence of downy mildew inside the polyhouse (Ferguson, 2007) Both PDI and PDS of downy mildew were found to be high in the rainy season when the RH is high both inside and outside the structures Data collected during the survey shows that, high humidity and low temperature favoures the development of downy mildew of cucumber in polyhouse During the survey it was found that, temperature and RH are higher inside the polyhouse compared to outside The reason for high temperature inside the polyhouse is greenhouse effect brought about by the polythene roof *i e*, increase in temperature due to retention of major part of reflected radiation from the earth's surface by the polythene roof of the structure Thus, most of the energy which is transmitted into the polyhouse is retained inside and causes increase in atmospheric temperature (Board, 2004) Use of foggers, and increased plant transpiration due to high atmospheric temperature are the major reasons for high RH in polyhouses Studies also revealed that the closed environments enable the sporangia to stay inside the polyhouse for longer period so as to cause more infection Hence downy mildew develops in devastating proportions in cucumber under protected cultivation if microclimate is not maintained properly so that RH is less than 75 per cent (Labeda and Cohen, 2011) In naturally ventilated polyhouses, since complete control of microclimate is not possible, it is better to take prophylactic measures against downy mildew, when RH is ≥ 79 per cent

5.1.1 Correlation analysis of incidence and severity of downy mildew with meteorological factors

During the survey, the highest disease severity was observed in polyhouse (1) of Chentrapinni where PDI and PDS of downy mildew were 15 45 per cent and 35 75 per cent respectively It was also observed that RH was 97 per cent during this period Correlation analysis of disease incidence and severity with major meteorological parameters showed that there is a strong positive correlation between PDI and PDS with RH inside the polyhouse whereas they are negatively correlated with temperature The result confirms the findings already reported by Colucci (2008)

5.1.2 Soil nutrient status and other soil parameters

During the survey, out of the nine polyhouses visited, cucumber in three polyhouses exhibited symptoms of potassium, calcium and boron deficiency Hence the nutrient status of soil samples collected from these polyhouses was studied It was found that, compared to outside, K, Ca, B as well as other micro nutrients are present in sufficient/higher amounts in soil inside the polyhouses This is true for most of polyhouses in Kerala (KSCSTE, 2015) Eventhough nutrient deficiency symptoms are commonly found in cucumber under polyhouse cultivation (Carmona *et al*, 2015), there is sufficient amounts of nutrients in soil inside the polyhouses Further analysis on the weather parameters showed that, as the relative humidity increases, the transpiration rate decreases and plants are unable to absorb calcium through the transpiration flow Since Ca absorption and B availability are interrelated, B deficiency is also predominant in such plants Marginal necrosis of intermediate leaves is the main symptom of K deficiency and this is due to the draining out of K from intermediate leaves cucumber The reason may be either higher nutrient requirement of the crop or due to fast growth or lack of proper absorption Hence it is suggested that, separate nutrient requirement schedule may be fixed for crops under polyhouse

5.1.3 Population of soil microflora

In most of the polyhouses visited, population of soil bacteria and actinomycetes are more inside the structure whereas fungi are more in outside soil Soil bacteria prefer neutral to alkaline condition for growth (Rousk *et al* , 2009) In the present study also it was found that pH is more in soil inside the polyhouse compared to outside Moreover, when nutrient status of soil samples collected in survey were studied, it was found that soil inside polyhouse contain more nutrients compared to outside, which may be another reason for higher population of bacteria and actinomycetes Fungi prefer acidic pH (Rousk *et al* , 2009) and hence that may be the main reason why population of fungi was found to be less in soil inside polyhouse compared to outside

5 2 Symptomatology and characterization of the pathogen

Typical symptoms (Labeda, 1991) of downy mildew of cucumber were observed in cucumber cultivated in polyhouse Being a biotroph, the cucumber downy mildew pathogen cannot be cultured in artificial medium So the morphological characters of the organism present on the infected leaves were studied Slight variation in dimensions like increase in length and width of sporangia and sporangiophores were observed which may be attributed to high temperature inside the polyhouse as reported by Iwata (1942) and Waterhouse and Brothers(1981) However, all the morphological characters of pathogen (sporangiophores and sporangia) collected during the survey were found to be identical to those of *Pseudoperonospora cubensis* and hence it is confirmed (Voglmayr, 2003, Choi *et al* , 2005) that the symptoms found on cucumber in polyhouses are due to downy mildew caused by *Pseudoperonospora cubensis* (Berk & Curt)(Rostov)

5.3 Field experiments for management of downy mildew of cucumber under protected condition

Field experiments were conducted under polyhouse and rain shelter in the Department of Plant Pathology, College of Horticulture, Vellanikkara during November to May of 2015-2016 to evaluate different treatments for the management of downy mildew of cucumber under protected condition

5.3.1 Soil solarization inside polyhouse and rain shelter

Solarization in polyhouse and rain shelter was carried out for a period of three months before sowing cucumber seeds Soil temperature at 10 cm depth in solarized and nonsolarized beds was recorded Greenhouse solarization is extensively used in southern Europe and Japan to control diseases of strawberries, tomatoes, eggplants, cucumbers, and other intensively managed crops Solarization in greenhouses produces significantly higher soil temperatures than solarization in fields and therefore be more effective in cooler weather (Elmore *et al* , 1997)

Soil solarization is a method of heating soil by covering moistened soil with transparent polythene sheeting to control soilborne diseases The technique has been commercially exploited for growing high-value crops in diseased soil Moistened soil is covered with transparent polyethylene which allows the sun's radiant energy to be trapped in the soil where most of this energy is transmitted to the soil is not reflected back and thus increasing the soil temperature (Katan, 1981) This increase in soil temperature is the most important mechanism by which soil solarization controls diseases Solarization in greenhouse has been reported to be effective in controlling diseases, nematodes and weeds in tomato and melon (Camprubi *et al* , 2007 , Candido *et al* , 2008) In the present study also, it was found that in both polyhouse and rain shelter the temperature in solarized beds was higher than nonsolarized beds

In polyhouse there is an increase in soil temperature upto of 4^0C in solarized beds over nonsolarized beds at 2 30 pm

5 3.1.1 Enumeration of soil microflora

There is a drastic reduction in the population of fungi, bacteria and actinomycetes in solarized soil of polyhouse and rain shelter In nonsolarized soil also after three months, there was reduction in the population of soil microflora but it was to a lesser extent compared to solarized soil On comparing polyhouse and rain shelter, the per cent reduction in population of fungi, bacteria and actinomycetes was more prevalent in solarized beds of polyhouse This is because of higher temperature prevailing inside the polyhouse The results confirms the findings of Candido *et al* (2008) who reported that soil solarization under greenhouse condition also increases soil temperatures to levels lethal to many soil borne plant pathogens and weeds

5.3.2 Management of downy mildew of cucumber under protected condition

The foremost objective of the study was to assess the incidence and severity of downy mildew of cucumber under protected cultivation and to formulate an effective and eco-friendly management strategy against the disease Therefore it was essential to test the efficacy of selected plant protection chemicals and biocontrol agents against the downy mildew of cucumber under polyhouse and ram shelter condition.

Two experiments were conducted simultaneously during November to May, 2014 16 in the polyhouse and ram shelter in the Department of Plant Pathology, College of Horticulture, Vellanikkara The treatments included systemic and contact fungicides, biocontrol agents and biofungicides in different combinations

5.3.2.1 Effect of different treatments on downy mildew of cucumber under polyhouse

In the polyhouse, incidence of downy mildew was observed 49 days after sowing and there was a gradual increase in the severity of downy mildew throughout the period of experiment However, all the treatments recorded lower disease severity compared to control Lowest disease severity was recorded in the systemic fungicide, T_{10} (cymoxanil + mancozeb) and the per cent reduction was found to be 62 63 per cent after the third spray Cymoxanil is a cyanoacetamide oxime group fungicide which quickly penetrates into the leaf tissue and destroys the already emerging pathogens The compound differentially inhibits RNA synthesis of the pathogen at various development stages (Ziogas and Davidse, 1987) The result of the present study, further confirms the findings of Bhat *et al* (2013) who could obtain a minimum disease severity of 17 01 per cent in the management of downy mildew of cucumber over the untreated control Moreover, Gisi and Sierotzki, (2008) also reported cymoxanil as a major site-specific fungicide used in chemical control of *P cubensis* Call *et al* (2013) has also reported that systemic fungicides alone or in combination with protective fungicides, proved to be very successful in the control of cucumber downy mildew Cymoxanil allowed a 50 per cent reduction in the amount of mancozeb needed to control downy mildew when applied in a mixture with mancozeb, and treatment with this mixture at 10 day intervals provided satisfactory protection (Klopping and Delp, 1980) Treatment T_{10} is followed by T_{11} (mancozeb) which recorded 61 55 per cent reduction in disease severity The results agree with the findings of Call *et al* (2013) who found that mancozeb against downy mildew of cucumber and the results revealed that mancozeb caused a significant reduction in the germination of sporangia of the downy mildew pathogen However, treatment T_{10} and T_{11} were found to be on par after each spraying

Among the bio-fungicides, Calphomil and garlic recorded 55 50 and 54 95 per cent reduction of disease severity over control The key ingredient in Calphomil is

leaf extract of *Aegle marmalos* It contains different types of alkaloids (solasodine and solamne) and terpinoids which possess antifungal activity (Nigam and Nambiar, 2015) Further, allicin in garlic juice reduced the severity of cucumber downy mildew caused by *Pseudoperonospora cubensis* by approximately 50-100 per cent (Portz *et al* 2005) These results suggest the possibility of developing preparations from garlic for use in disease management in organic farming, under greenhouses

Among treatments involving biocontrol agents, *Trichoderma viride* along with soil solarization(T_1) gave the best result in the reduction of downy mildew which accounts for 60 99 per cent and this is followed by T_2 *i e* solarization + foliar spray of *Pseudomonas fluorescens* (59 34%) Biological control is an alternative means of management of plant pathogens Biocontrol agents suppress the foliar pathogens by the same mechanism as they do in the case of soil borne pathogens They control the foliar pathogens like *Pseudoperonospora cubensis* and *Sclerotinia sclerotiorum* in cucumber more effectively under commercial greenhouse (Elad, 2000) The mode of action of *Trichoderma viride* against foliar plant pathogens include myco-parasitism, antimicrobial activity, suppression of conidial growth and enzymes produced by the pathogen, competition for resources and induced systemic resistance (Sawant, 2014) Machenahalli *et al* (2013) reported that among biocontrol agents *Pseudomonas fluorescens* was found to be superior in inhibiting sporangial germination of downy mildew It has been reported by Panpatte *et al* (2014) that 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) Moreover, as a part of this investigation, survival of biocontrol agents on leaf surface was studied and it was found that *Trichoderma viride* and *Pseudomonas fluorescens* survived on leaf surface for 15 days after foliar appplication This confirms the activity of biological control in the management of foliar pathogens

As early as 1987, it has been reported that solarized soil are frequently more suppressive and less conducive to certain soil borne pathogens than non-solarized soils (Greenberger *et al* 1987) Since oospores of downy mildew survive in plant debris of cucumber in soil (Labeda and Cohen, 2011), solarization also helps to bring down the initial inoculum and inoculum build up in soil

Effect of treatments was reflected on yield of cucumber Eventhough the lowest PDS was recorded in T_{10} (cymoxanil + mancozeb), mean yield was found to be more in the treatments in which solarisation is carried out *i e*, in T_1 and T_2 Efficacy of soil solarisation on increasing yield of vegetables is well documented Elmore *et al* (1997) has reported that soil solarization can be successfully combined with the fungal biological control agent *Trichoderma* for disease management in greenhouse crops which ultimately increases the weight and yield of the commercial produce Soil solarization improves soil structure and increases the availability essential plant nutrients and further increases plant growth, harvestable yield, and crop quality The increased growth response of plants in solarized soil has been reported both in green house experiments and under field conditions (Candido *et al*, 2008)

5.3.2.2 Effect of different treatments on downy mildew of cucumber under rain shelter

In the rain shelter, incidence of downy mildew was observed 54 days after sowing and there was a gradual increase in the severity of downy mildew throughout the experiment However, all the treatments recorded lower disease severity compared to control as in polyhouse Lowest disease severity was recorded in the T_{10} (cymoxanil + mancozeb) and the per cent reduction after third spraying was 55 41 This result confirms the findings of Gisi and Sierotzki (2008), who reported that cymoxanil as a major site-specific fungicide against *P cubensis* Treatment T_{10} is followed by T_{11} (mancozeb) which recorded 54 05 per cent reduction in disease

severity The efficacy of contact fungicide, mancozeb against downy mildew of cucumber has already been reported by Call *et al* (2013)

The bio-fungicides, Calphomil and garlic were also effective against the disease and they recorded 45 95 and 44 60 per cent reduction of disease severity respectively over control which confirmed the results obtained in the polyhouse experiment

Among the biocontrol agents, *Trichoderma viride* along with soil solarization(T_1) and *Pseudomonas fluorescens* along with soil solarisation (T_2)gave the significant reduction of downy mildew However, compared to polyhouse the reduction was slightly lesser *i e* 52 70 and 51 35 per cent respectively over control Under rain shelter also, the biocontrol agents *Trichoderma viride* and *Pseudomonas fluorescens* survived on leaf surface upto 15 days after spraying

The efficacy of soil solarisation on yield was found to be true in the case of rain shelter experiment also and the yield increase for treatments T_1 and T_2 was 54 92 and 48 per cent respectively over control

5.3.3 Effect of treatments on the biometric parameters of cucumber in polyhouse and rain shelter

In the polyhouse experiment there was no significant difference among the treatments with regard to biometric observations except shelf life However, fruit weight and vine length were more in cucumber grown in solarized beds Shelflife was the highest in T_8(Calphomil) followed by T_{10}(cymoxanil + mancozeb) Calphomil is a botanical preparation and the key ingredient is leaf extracts of *Aegle marmalos*(bael) It is reported that the terpinoids and alkaloids alkaloids (solasodine and solanine) in bael increases nitrate reductase activity and chlorophyll content when applied on leaves and fruits It also reduces the phylloplane microflora which helps to further increase the shelf life of the produce (Azhagumurukan *et al*, 2013) Reduction in

epiphytic microflora brought about by cymoxanil + mancozeb may be the reason for increased shelf life in T_{10} As in later in the present study it was found that the chemical reduces the phylloplane fungi from 69 61 to 17 14 x 10 cfu cm^2 and phylloplane bacteria from 18 10 to zero immediately after spraying

However in the case of rain shelter experiments there was no significant difference in any of the biometric characters and also shelf life It may be concluded that the efficacy to increase the shelf life by Calphomil and cymoxanil + mancozeb was lesser in in rain shelter compared to polyhouse It was observed that fruit weight, fruit length and vine length were more in cucumber grown in treatments T_1 and T_2 and this may be due to the growth promoting effect of biocontrol agents as well as soil solarization

5.3.4 Economic analysis of cucumber in polyhouse and rain shelter

B C ratio was calculated by giving same price of Rs 30/- kg^{-1} of cucumber for all the treatments and at a premium of 20 per cent for organic produce From the study it was found that the organic production of cucumber in protected condition will be remunerative than the conventional production only if it fetches a minimum of 20 per cent premium price In polyhouse, B C ratio is found to be the highest in T_{10}(cymoxanil + mancozeb) which is followed by T_2(solarization+ *Pseudomonas fluorescens)* when calculated at the same price (Rs 30/- kg^{-1}) If 20 per cent premium price is obtained, T_2(solarization+ *Pseudomonas fluorescens*) is the best treatment on economic point of view which is followed by T_1(solarization+ *Trichoderma viride*)

Among the various treatments, same trend was observed in rain shelter also However cucumber cultivation in polyhouse is more remunerative compared to rain shelter and this is brought about by various factors like higher growth rate, early germination and higher yield of the parthenocarpic variety used in the polyhouse

5.4 Meteorological factors influencing the crop and the pathogen during the experiment

On analyzing the major meteorological parameters during the field experiment, it was observed that temperature is more in rain shelter whereas relative humidity is higher in the polyhouse This is found to be the reason for early (49 days) incidence of downy mildew in polyhouse As closed plastic polyhouse causes increase in RH which is favoured by the disease as described by Labeda and Cohen (2011) Since temperature was higher in rain shelter comparatively lesser incidence of downy mildew was observed Accordingly earlier incidence and higher severity of downy mildew was noticed in polyhouse

5 4.1 Correlation analysis of incidence and severity of downy mildew with meteorological factors

Correlation analysis was performed utilizing the data collected during the field experiments It was found that observed that inside the polyhouse and rain shelter, there is a strong positive correlation between PDS and RH whereas PDS is negatively correlated with temperature The results confirm those obtained from the survey

5.5 Enumeration of phylloplane microflora of cucumber 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) Some of these microorganisms called residents(Andrews and Kinkel, 1986), may survive on the leaf surface for a longer period and together form the microflora of the phylloplane The effect of treatments on the phylloplane microflora *ie* fungi, bacteria

and actinomycetes of cucumber in polyhouse and rain shelter were studied under this session

In the present study it was found that there is a drastic reduction in the population of phylloplane fungal and bacterial flora in polyhouse and rain shelter immediately after spraying chemical fungicides T_{10}(cymoxanil + mancozeb), T_{11}(mancozeb) and T_9 (potassium phosphonate + hexaconazole) Eventhough it gradually increased after 15 days of spraying it did not reach the initial level till 15 DAS The result indicates the non-selectivity of the fungicides on general fungal and bacterial population on the leaves Since the effect persisted for a long period of 15 days, it is an indication on the residual effect of the chemical The residue of both systemic and contact fungicides persisted upto 15 DAS and thus it affected the re-emergence of the phylloplane microflora The use of foliar pesticides to control diseases can cause major disruption of phylloplane microorganism populations, often reducing the number and diversity of organisms This is in confirmation with the findings of Bosshard *et al* (1987) who reported that fungicides cause a negative effect on naturally-occurring biological control, which in some cases, makes the plants more susceptible to other disorders Moreover, the microorganisms that were previously not considered plant pathogens, may become pathogenic due to reduction of antagonist populations by the chemical treatments The result of the present study, gave an indirect indication of residual activity of chemical fungicides Bakker (2004) expressed that a single isolated foliar application of a chemical product can affect the phylloplane microbial population densities and species richness

In organic treatments, *i e*, in treatments T_1 and T_3, where *Trichoderma* was sprayed on leaves, there is a drastic increase in the population of phylloplane fungal flora immediately after spraying and this may be due to the additive effect of *Trichoderma* sprayed on leaves However, the population reaches the original level at 15DAS Moreover, according to Harman *et al* (2004), *Trichoderma* sp , have a wide application in controlling foliar plant pathogens by increasing the population of non-

pathogenic phylloplane microflora The same trend is observed in both polyhouse and rain shelter immediately after the first spray In treatment T_6 (cowdung +*Pseudomonas*) also, there was a tremendous increase in the population of phylloplane bacteria in polyhouse and rain shelter Similarly in T_2 and T_4, where *Pseudomonas* was sprayed on leaves, there was an increase in the population of phylloplane bacteria The biocontrol agents, *T viride* and *P fluorescens* increases the population of non-pathogenic phylloplane microflora and by the mechanism of competition, reduce the foliar diseases to a greater extent (Harman *et al* , 2004)

Biofungicides, garlic and Calphomil also showed a reduction in the population immediately after spraying But it was less compared to chemical fungicides and after 15 days, the population gradually got build upto original level However the reduction in phylloplane microflora by these treatments shows their broad spectrum activity against fungi and bacteria These results suggest a potential for developing preparations from garlic for use in specialised aspects of organic farming, e g for reducing pathogen inoculum potential in greenhouses (Portz *et al* , 2008) It has been also reported that Calphomil having key ingredient *Aegle marmalos* (bael) increases biochemical constituents such as nitrate reductase activity and chlorophyll content when applied on leaves and fruits and thereby indirectly reduce the phylloplane microfloia (Azhagumurukan *et al* , 2013) However their effect on beneficial organisms is lesser compared to chemical treatments, as it was found that the reduction of population was to a lesser extent

5.6 Survival of biocontrol agents on the phylloplane of cucumber in protected structures

On spraying biocontrol agents, *Trichodei ma vi ide* and *Pseudomonas fluoi escens,* total microflora in general increased after spraying but it came down to initial level This may be due to the additive effect of population of propagules of biocontrol agents which survive on the phylloplane Moreovei, later in this study it

was found out that biocontrol agents once sprayed on leaves survive there for 15 days or more The results of the present study confirms the findings of Freeman *et al* (2004), where the survivability/viability of four *Trichoderma* strains T-39, T-105, T-161 and T-166, applied individually on strawberry leaf surfaces, was assessed and he reported that the *Trichoderma* declined rapidly to a lower level after 3 days, but less rapidly, 7-14 days after application In the present study naturally occurring *Trichoderma* isolates was detected on plates from control which varied from 1 80 to 2 76 x 10 cfu cm^2 This is in contrast with the findings of Freeman *et al* (2004) where they couldnot isolate naturally occurring *Trichoderma* population from control

The fungal biocontrol agent *T viride* survive mainly on the hyphae of the pathogen present on the leaf surface or else it may get transferred to resting spores whereas 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) The results of the present study, confirms the findings of Cirvilleri *et al* (1999), who reported that the population densities of *P fluorescens* strains declined within 30 days after inoculation on plant species such as pepper, tomato, eggplant and strawberry

Summary

6. SUMMARY

Cucumber is one of the most preferred vegetable grown under protected conditions in the developed world It is used as salad, pickle and also as cooked vegetable because of its low calorie content The crop is devastated by various diseases and among them, one of the major foliar disease is downy mildew Cucurbit downy mildew, caused by the oomycete *Pseudoperonospora cubensis* (Berk & Curt)(Rostov), is a devastating, disease of cucurbit crops in the open field and under protected structures Annual loss to the crop at global level due to the pathogen has been estimated to be $ 246 2 million Farmers are using toxic chemical fungicides against the disease Since the use of chemical fungicides leaves pesticide residue in the commercial produce which is consumed as raw vegetable (salad), biological control is being promoted in Kerala Hence, the present study was conducted with the major objective of formulating an ecofriendly management practice against the disease The experiment entitled "Management of downy mildew (*Pseudoperonospora cubensis* (Berk & Curt) Rostov) of cucumber under protected cultivation" was conducted in the department of Plant Pathology, College of Horticulture, Vellanikkara during the period from October to April 2015-16 The study consisted of survey on incidence and severity of downy mildew of cucumber in polyhouses and field experiments for management of the disease in protected structures

1 Incidence of downy mildew was noticed in all nine polyhouses in different locations in Thrissur district irrespective of the season and the disease severity varied from 11 33 to 35 75 per cent
2 The disease severity of cucumber was higher in the polyhouse where relative humidity was higher and the RH was ≥ 79 per cent in all the polyhouses visited
3 Temperature and relative humidity was found to be higher inside the polyhouse compared to outside

4 It was found that there is a significant positive correlation between disease incidence and severity of downy mildew with relative humidity (RH) inside the polyhouse whereas as it is negatively correlated with temperature

5 Symptoms of K, Ca and B deficiencies were observed in cucumber in polyhouses However, when soil samples collected were subjected to nutrient analysis it was found that these nutrients were available in sufficient quantities in the soil

6 Population of fungi was found to be higher in soil outside polyhouse whereas population of bacteria and actinomycetes was found to be higher in soil inside polyhouse

7 Leaves having symptoms of downy mildew collected during the survey were subjected to microscopic examination using sticky tape method for characterization of the pathogen It was observed that sporangiophores are dichotomously-branched at acute angles which taper to curved pointed tips Sporangia are large lemon shaped having brownish yellow colour borne at the tip of the sporangiophore

8 Field experiments were conducted simultaneously in the polyhouse and rain shelter for the management of downy mildew with 12 treatments and three replications The treatments included two biocontrol agents, cowdung supernatant, two biofungicides and two systemic and one contact fungicide

9 Soil solarization inside the protected structures was carried out as part of treatments, T_1 (soil solarization + seed treatment and soil application + foliar spray with *Trichoderma viride*) and T_2 (soil solarization + seed treatment and soil application + foliar spray with *Pseudomonas fluorescens*)and it was found that soil temperature at 10 cm depth in solarized soil is higher compared to nonsolarized soil by 4°C and 3 5°C respectively in polyhouse and rain shelter

10 Population of soil microflora was reduced upto 79 34 per cent due to solarization in protected structures

11 Foliar spray with cymoxanil + mancozeb (T_{10}) was the most effective treatment for the management of downy mildew in both polyhouse and rain shelter followed

by foliar spray with mancozeb (T_{11}) and soil solarization + seed treatment and soil application + foliar spray with *Trichoderma viride*(T_1)and these were on par

12 Results obtained from the field experiment suggest that downy mildew of cucumber inside the protected structures can be effectively managed by biocontrol agents which give a reduction in disease severity comparable to that of systemic fungicide

13 Highest yield was recorded in T_1 (soil solarization + seed treatment and soil application + foliar spray with *Trichoderma viride*) followed by T_2 (soil solarization + seed treatment and soil application + foliar spray with *Pseudomonas fluorescens*

14 Meteorological parameters (temperature and RH) inside the structures were recorded daily during the experiment Correlation analysis was performed between the meteorological data and PDS at periodic intervals and it was confirmed that there is significant positive correlation between PDS and RH and negative correlation between PDS and temperature

15 Shelf life was found to be higher in calphomil(T_8) followed by cymoxanil + mancozeb (T_{10}) in cucumber grown in polyhouse

16 Economic analysis of the field experiments showed that foliar spray with cymoxanil + mancozeb (T_{10}) gave the highest benefit cost ratio But if the produce from treatments T_1 to T_8 (biocontrol agents and biofungicides) fetches a 20 per cent premium price, T_2 (soil solarization + seed treatment and soil application + foliar spray with *Pseudomonas fluorescens*) gives the highest benefit cost ratio in both polyhouse and rain shelter

17 Inorder to study the effect of foliar treatments on phylloplane microflora inside the structures, enumeration of fungi and bacteria on the leaf surface of cucumber was done using serial dilution plating of leaf washings There was a drastic reduction in phylloplane fungi and bacteria after spraying with chemical fungicides whereas the population increased on spraying the biocontrol agents The effect persisted upto 15 days after spraying in systemic and contact fungicides but the increase in the population in biocontrol treatments, did not

persist upto this period The study indicates that chemical fungicides cause loss of valuable phylloplane microbes Moreover, the result gives an indirect indication of residual effect of fungicides sprayed on leaves

18 Survival of biocontrol agents on the phylloplane of cucumber was also studied and it was found that both *T viride* and *P fluorescens*, survived on leaf surface upto 15 days after foliar application

References

7. REFERENCES

Abawi, G S and Widmer, T L 2000 Impact of soil health management practices on soil borne pathogens nematodes and root diseases of vegetables *Appl Soil Ecol* 15 37-47

Abd-El-Moity, T H, Abed-El-Moneim, M L, Tia, M M M, Aly, A Z and Tohamy, M R A 2003 Biological control of some cucumber diseases under organic agriculture *Acta Hort* 608 227-236

Andrews, J H and Kinkel, L L, 1986 Colonization dynamics The island theory In Fokkema, N J and van den Heuvel, J (eds), *Microbiology of the Phyllosphere*, Cambridge University Press, New York pp 63-76

Aujla, I S, Thind, T S, Kumar, K S, Tiwary, K N, and Madan, K J 2010 Design and technology for cooling in tropical and subtropical regions *Energy and buildings* 41(12) 1269-1275

Azhagumurukan, C, Pavaraj, M, and Rajan, M K 2013 Efficacy of leaf extract of *Aegle marmelos* on the biochemical changes on Horse gram *Macrotyloma uniflorum* infected by root-knot nematode *Melodogyne incognita American-Eurasian J Scientific Res* 8(3) 124-127

Bains, S S and Jhooty, J S 1976 Host-range and possibility of pathological races in *Pseudoperonospora cubensis*- cause of downy mildew of muskmelon *Indian Phytopathol* 29 214-216

Bakker G R 2004 The effects of disease management practices on phylloplane microorganism population of Apple M Sc thesis, Lincoln University, New Zealand, 4p

Berkeley, M S and Curtis, A 1868 *Peronospora cubensis Phytopathol* 61 736–737

Bhat, J A , Thind, T S , Bala, A and Kumar, P 2013 Factors affecting development of downy mildew of cucumber grown under plastic low tunnel and its management with fungicides *Plant Dis Res* 28(1) 58-63

Blakeman, J P , 1985 Ecological succession of leaf surface microorganisms in relation to biological control In Windels, E and Lindow, S E (eds), *Biological Control on the Phylloplane*, The American Phytopathological Society, St Paul Minnesota, USA, pp 6-30

Board, N 2004 *Cultivation of fruits vegetables and floriculture* National institute of Industrial research Kamala nagar, New Delhi, 453p

Bonnen, A M and Hopkins, C 1997 Fungicide resistance and population variation *Verticillium fungicola,* a pathogen of the button mushroom, *Agaricus bisporus Mycological Res* 101 89-96

Bosshard, E , Schiiepp, H , and Siegfried, W 1987 Concepts and methods in biological control of diseases in apple orchards *Plant Pathol* 17 655-663

Call, A D , Wehner, T C , Holmes, G J , and Ojiambo, P S 2013 Effects of host plant resistance and fungicides on severity of cucumber downy mildew *Hort Sci* 48 53-59

Camprubi, A , Estaun, V , El- Bakali, M A , Garcia-Figueres, F , and Calvet, C 2007 Alternative strawberry production using solarization, metham sodium and beneficial soil microbes as plant protection methods *Agron Sustain Dev* 27 179–184

Candido, V , D'Addabbo T , Basile, M , Castronuovo D , and Miccolis, V 2008 Greenhouse soil solarization effect on weeds, nematodes and yield of tomato and melon *Agron Sustain Dev* 28(2) 221-230

Carmona V V , Coata, L C , and Filho, A B C 2015 Symptoms of nutrient deficiencies in cucumbers *Int J Plant Soil Sci* 8(6) 1-11

Chaube, H S and Sharma, J 2002 Integration and interaction of solarization and fungal and bacterial bioagents on disease incidence and plant growth response of some horticultural crops *Pl Dis Res* 17 201-211

Cirvilleri, G , Catara, V , Bella, P , Marchese E 1999 Isolation and characterization of rhizosphere bacteria of potential interest for biological control of soil borne pathogens *Phytophag Palermo* 9 79-87

Choi, Y J , Hong, S B , and Shin, H D 2005 A re-consideration of *Pseudoperonospora cubensis* and *P humuli* based on molecular and morphological data *Mycological Res* 109 841–848

Cohen, Y 1981 Downy mildew of cucurbits D M Spencer (ed), *The Downy Mildew* Academic Press, New York, pp 341-354

Cohen, Y and Rotem, J 1971 Field and growth chamber approach to epidemiology of *Pseudoperonospora cubensis* on cucumbers *Phytopathol* 61 736–737

Cohen, Y , Meron, I , Mor, N , and Zuriel, S 2003 A new pathotype of *Pseudoperonospora cubensis* causing downy mildew in cucurbits in Israel *Phytoparasitica* 31 458-466

Colucci, S 2008 Host range, fungicide resistance and management of *Pseudoperonospora cubensis*, causal agent of cucurbit downy mildew M Sc thesis, North Carolina State University Raleigh, USA, 156p

Ferguson, G 2007 Downy mildew in greenhouse cucumber *Ministry of Agriculture and Rural Affairs* pp 1-4

Fravel, D R , Rodes, D J , and Larkin, P R 1994 Production and commercialization of biocontrol products Kluwer Academic Publishers, Wageningen, The Netherlands, pp 365-376

Freeman, S , Minz, D , Kolesnik, I , Barbul, O , Zveibil, A , Maymon, M , Nitzani, Y , Kirshner, B , Rav-David, D , Bilu, A , Dag, A , Shafir, S , and Elad, Y 2004 *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea* and survival in strawberry *Eur J Plant Pathol* 110 361–370

Gaddeyya, G , Niharika, P S , Bharathi, P , and Kumar, P R 2012 Isolation and identification of soil microflora in different crop fields at Salur Mandal *Adv Appl Sci Res* 3(4) 2020-2026

Gisi, U 2002 Chemical control of downy mildews In Spencer-Phillips, P T N , Gisi, U , and Lebeda, A (eds), *Advances In Downy Mildew Research* Dordrecht Kluwer Academic Publishers, pp 119-159

Gisi, U and Sierotzki, H 2008 Fungicide modes of action and resistance in downy mildews *Eur J Plant Pathol* 122 157-167

Goker, M , Voglmayr, H , Riethmuller, A , and Oberwinkler, F 2007 How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews *Fungal Genet Biol* 44 105-122

Gowdu J B, and Balasubramanian, R 1988 Role of phylloplane microorganisms in the biological control of foliar plant diseases *J Plant Dis Prot* 95 310-331

Greenberger, A , Yogev, A , and Katan, J 1987 Induced suppressiveness in solarized soil *Phytopathol* 77 1663-1667

Dik, A J 1991 Interactions among fungicides, pathogens, yeasts and nutrients in the phyllosphere In Andrews, J H and Hirano, S S (eds), *Microbial Ecology of Leaves*, Springer-Verlag, New York, pp 412-429

Dik, A and Pelt van, I A 1992 Interaction between phyllosphere yeasts, aphid honeydew and fungicide effectiveness in wheat under field conditions *Plant Pathol* 41 661-675

Elad, Y 1994 Biological control of grape grey mould by *Trichoderma harzianum* *Crop protect* 13 35–38

Elad, Y 2000 Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action *Crop prot* 19 709-714

Elad, Y and Freeman, S 2002 Biological control of fungal plant pathogens In Kempken, F (ed), *The Mycota, A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research* Springer, Heidelberg, Germany, pp 93–109

Elad, Y and Kirshner, B 1993 Survival in the phylloplane of an introduced biocontrol agent (*Trichoderma harzianum*) and populations of the plant pathogen *Botrytis cinerea* as modified by abiotic conditions *Phytoparasitica* 21 303-313

Elmore, C L , Stapleton, J J , Bell, C E , and Devay, J E 1997 Soil Solarisation A Nonpesticidal Method For Controlling Diseases, Nematodes And Weeds University of California Division of Agriculture and Natural Resources USA pp 6

Fenoll, J , Ruiz, E , Hellin, P , Navarro, S , and Flores, P 2010 Solarization and biosolarization enhance fungicide dissipation in the soil *Chemosphere* 79(2) 216-220

Harman, G E, Howell, C R, Viterbo, A, Chet, I, and Lorito, M 2004 *Trichoderma* species-opportunistic, avirulent plant symbionts *Nat Rev Microbiol* 2 43-56

Hickman, G W 2016 Greenhouse vegetable production statistics a review of current data on the international production of vegetables in greenhouses Cuesta Roble greenhouse consultants, Mariposa, pp 72

Hirano, S S and Upper, C D 2000 Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringe*- a pathogen, ice nucleus and epiphyte *Microbiol Mol Biol Rev* 64(3) 624-653

Iwata, Y 1942 Specialization in *Pseudoperonospora cubensis* (Berk and Curt)Rostov Comparative studies of the morphologies of the fungi from *Cucumis sativus* L and *Cucurbita moschata* Duchesne *Ann Phytopathological Society* 11 172-185

Jasuja, N D, Saxena, R, Chandra, S and Joshi, S C, 2013 Isolation and identification of microorganism from polyhouse agriculture soil of Rajasthan *African J Microbiol Res* 7(41) 4886-4891

Jensen, M H 2002 Controlled environment agriculture in deserts tropics and temperate regions – A world review *Acta Hort* 578 19-25

Jovicich, E, Akem, C, and MacManus, G 2010 Integrated Management of Powdery and Downy mildewa in Zuchini Agri-Science Queensland, Queensland, Australia, pp 1-15

Karthikeyan, V, Sankaralingam, A, and Nakkeran, S 2006 Management of groundnut root rot with biocontrol agents and organic amendments *Archives Phytopathol Plant Prot* 39(3) 215-223

Katan, J 1981 Solar heating (solarisation) of soil for control of soil borne pests *Annu Rev Phytopathol* 63 211-236

Keinath, A P , Holmesb, G J , Evertsc, K L , Egeld, D S , Langston Jr , D B 2007 Evaluation of combinations of chlorothalonil with azoxystrobin, harpin, and disease forecasting for control of downy mildew and gummy stem blight on melon *Crop Prot* 26 83-88

Klopping, H L and Delp, C J 1980 2-Cyano-N-[(ethylamino) carbonyl]-2-(methoxyimino)acetamide, a new fungicide *J Agric Food Chem* 28 467-468

Kowalska, J 2010 The use of natural substances and microorganisms in organic potatoes production In Rembialkowska E (ed), *The Impact of Organic Production Methods on the Vegetable Product Quality*, Agencja Rekl -Wyd A Grzegorczyk, Warszawa, pp 41-52.

Kristkova, E , Lebeda, A and Sedlakova, B 2009 Species spectra, distribution and host range of cucurbit downy mildews in the Czech Republic, and in some other European and Middle Eastern countries *Phytopathasitica* 37 337-350

KSCSTE [Kerala State Council for Science, Technology and Environment] 2015 *Annual Progess Report 2014-2015* Base line studies on vegetable crops under protected cultivation in Kerala as a prelude to precise disease management, Kerala, 32p

Lebeda, A 1991 Resistance in muskmelons to Czechoslovak isolates of *Pseudoperonospora cubensis* from cucumbers *Scientia Horticulturae* 45 255-260

Lebeda, A , J Pavelkova, J , Urban, and Sedlakova, B 2011 Distribution, host range and disease severity of *Pseudoperonospora cubensis* on cucurbits in the Czech Republic *J Phytopathol* 159 589-596

Lebeda, A and Cohen, Y 2011 Cucurbit downy mildew (*Pseudoperonospora cubensis*)- biology, ecology, epidemiology, host-pathogen interaction and control *Eur J Pl Pathol* 129 157-192

Leben, C 1965 Epiphytic microorganisms in relation to plant disease *Annu Rev Phytopathol* 3 209-230

Machenahalli, S , Rao, M S L , and Nargund, V B 2013 Effect of fungicides botanicals, bioagents and organics on inhibition of sporangial germination of *Pseudoperonospora cubensis* (Berk and Curt) Rostov *BIOINFOLET* 10(2) 588-592

Menon, J S 2010 Concept of rain shelter gaining popularity *The New Indian Express*, 20 Sep 2010, p 12

Morris, C E , Monier, J M and Jacques, M A 1997 Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganisms *Appl Environ Microbiol* 63 1570-1576

Nigam, V , and Nambiar, V S 2015 Therapeutic potential Of *Aegle marmalos*(L) correa leaves as antioxidant and anti-diabetic agent A review *Inter J Pharma Sci Res* 6(2) 611-621

Oerke, E , Steiner, U , Dehne, H , and Lindenthal, M 2006 Thermal imaging of cucumber leaves affected by downy mildew and environmental conditions *J Exp l Bot* 57 2121–2132

Palti, J and Cohen, Y 1980 Downy mildew of cucurbits *(Pseudoperonospora cubensis) The* fungus and its hosts, distribution, epidemiology and control *Phytoparasitica* 8 109-147

Panpatte, D G , Shelat, H N , Vyas, R V and Jhala, Y K 2014 Plant growth promoting rhizobacteria- a promising tool for ecofriendly agriculture *J Pure Appl Microbiol* 8(6) 4947-4959

Portz, D , Koch, E , and Slusarenko, A J 2008 Effects of garlic (*Allium sativum*) juice containing allicin on *Phytophthora infestans* and downy mildew of cucumber caused by *Pseudoperonospora cubensis Eur J Plant Pathol* 122(1) 197-206

Pradeepkumar, T , Bhardwaj, A , Roch, V C , and Geethu 2015 New parthenocarpic F1 hybrid in cucumber for polyhouse from KAU In *New age of protected cultivation* Vol 1 Agritech, Banglore, India, pp 14-15

Pucarevic, M , Bursic, V , Pankovic, D , Nebojsa, R M , Cara, M , and Kecojevic, I 2013 Superficial fluid extraction of tebupirimphos residues in sugar beet *J Anim Plant Sci* 23 277-280

Rangaswami, G and Bagyaraj, D J 2005 *Agricultural Microbiology*, (2nd Ed) Prentice Hall of India, Pvt Ltd , New Delhi, 47p

Rini, C R and Sulochana, K K 2006 Management of seedling rot of chilli (*Capsicum annuum L*) using *Trichoderma* spp and fluorescent pseudomonads (*Pseudomonas fluorescens*) *J Trop Agric* 44 79–82

Rotem, J , Cohen, Y and Bashi, E 1978 Host and environmental influences on sporulation on vivo *Ann Rev Phytopathol* 16 83-101

Rousk, J , Brookes, P , and Baath, E 2009 Contrasting soil pH effects on fungal and bacterial growth suggests functional redundancy in carbon mineralization *Appl Environ microbial Sci* 75 1589-1596

Runge, F and Thines, M 2011 Host matrix has major impact on the morphology of *Pseudoperonospora cubensis Eur J Plant Pathol* 129 147-156

Russell, P 2004 Sensitivity baselines in fungicide resistance research and management *Monogr* 3 1-6

Sabir, N and Singh, B 2013 Protected cultivation of vegetables in global arena A review *Indian J Agric Sci* 82(2) 123-135

Savory, E , Gramke, L , Quesada-Ocampo, L , Varbanova, M , Hausbeck, M , and Day, B 2010 The Cucurbit Downy Mildew Pathogen *Pseudoperonospora cubensis Mol Plant Pathol* 12 217-226

Sawant, I S 2014 *Trichoderma*- Foliar pathogen interactions *The Open Mycol J* 8 58-70

Simon, M 1996 Varietal screening host range and control of downy mildew of bittergourd (*Momordica charantia* L) caused by *Pseudoperonospora cubensis* (Berk and Curt)Rostov M Sc,(Ag) thesis, Kerala Agricultural University, Thrisur, 82p

Singh, A and Banyal, D K 2007 Occurrence of different diseases of capsicum, tomato and cucumber under protected cultivation in Himachal Pradesh *Plant Dis Res* 26(2) 167-188

Singh, B and Kumar, M 2006 Techno-economic feasibility of Israeli and indigenously designed naturally ventilated greenhouses for year-round cucumber cultivation *Acta Hort* 710 535-538

Skalicky, V 1961 *Pseudoperonospora cubensis Agric Phytopathol* 3 390-393

Srivastava, B K and Singh, M P 1997 Boosting off season production of vegetables in low cost polyhouse In *Proceedings of Third Agricultural Science Congress*, 12-15 March 1997, PAU, Ludhiana pp 12-15

Umesha, S , Dharmesh, S M , Shetty, S A , Krishnappa, M , and Shetty, H S 1999 Biocontrol of downy mildew disease of pearl millet using *Pseudomonas fluorescens Crop Prot* 17(5) 387-392

Urban, J and Lebeda, A 2006 Fungicide resistance in cucurbit downy mildew-methodological, biological and population aspects *Ann Appl Biol* 149 63-75

Vivekananthan, R , Ravi, M , Ramanathan, A , and Samiyappan, R 2004 Lytic enzymes induced by *Pseudomonas fluorescens* and other biocontrol organisms mediate defence against the anthracnose pathogen in mango *World J Micro Biotech* 20 235-244

Voglmayr, H 2003 Phylogenetic study of *Peronospora* and related genera based on nuclear ribosomal ITS sequences *Mycol Res* 107 1132-1142

Voglmayr, H 2008 Progress and challenges in systematics of downy mildews and white blister rusts new insights from genes and morphology *Eur J Pl Pathol* 122 3-18

Vukovic, G , Stereva, D , Bursic, V , Mladenova, R , and Lazic, S 2012 Application of GCMSD and LC-MS/MS for the determination of priority pesticides in baby food in Serbian market, LWT *Food Sci Technol* 49 312-319

Wang, H C , Zhou, M G , Wang, J X , Chen, C J , Li H X , and Sun, H Y 2009 Biological mode of action of dimethomorph on Pseudoperonospora cubensis and its systemic activity in cucumber *Agri Sci China* 8(2) 172-181

Waterhouse, G M and Brothers, M P 1981 The taxonomy of *Pseudoperonospora Mycol Pap* 148 1-18

Wehner, T C and Shetty, N V 1997 Downy mildew resistance of the cucumber germplasm collection in North Carolina field tests *Crop Sci* 37 1331- 1340

Wheeler, B E J 1969 *An introduction of Plant Disease* John Wiley & Sons Ltd London, 301p

Wittwer, S H and Castilla, N 1995 Protected cultivation of horticultural crops worldwide *Hort Technol* 5(1) pp 6-23

Yang, C S 2003 Microscopic examination of sticky tape or bulk samples for the evaluation and identification of fungi EMLab P&K pp 1-3

Yang, X T , Li, M , Zhao, C J , Zhang, Z , and Hou, Y L , 2007 Early warning model for cucumber downy mildew in unheated greenhouses *J Agric Res* 50 1261-1268

Yohalem, D S 2000 Research in antagonistic microorganisms with potential for control of pathogenic foliar fungi *Hortic* 12 31-35

Yucel, S , Kececi, M , Yurtmen, M , Yildiz, R C , Ozarslandan, A , and Can, C 2013 Integrated pest management of protected vegetable cultivation in Turkey *Eur J Pl Sci Biotechnol* 7(1) 7-13

Zhan-Bin Sun, Xing- Fang Yuan, Hui Zhang, Lei- Fei Wu, Chen Liang, and Yong Jun Feng 2013 Isolation, screening and identification of antagonistic downy mildew endophytic bacteria from cucumber *Eur J Plant Pathol* 137 847-857

Zhang, X , Chen, Y , Zhang, Y J , and Zhou, M G 2008 Occurrence and molecular characterization of azoxystrobin resistance in cucumber downy mildew in Shandong province in China *Phytoparasitica* 36 136–143

Ziogas, B N and Davidse, L C 1987 Studies on the mechanism of action of cmoxamil in *Phytophthora infestans Pesticide Biochem Physiol* 29(2) 89-96

Appendix

APPENDIX

1. Martin's Rose Bengal Agar medium

Dextrose	10 0 g
Peptone	5 0 g
KH_2PO_4	1 0 g
$MgSO_4$	0 5 g
Agar	20 0 g
Rose Bengal	0 03 g
Streptomycin	30 0 mg (added asceptically)
Distilled water	1000 ml

2. Nutrient Agar medium (NA)

Peptone	5 0 g
Beef extract	1 0 g
Sodium chloride	5 0 g
Agar	20 0 g
Distilled water	1000 ml
pH	6 5 to 7

3. Kenknight's Agar medium (pH 7.0)

Dextrose	1 0 g
KH_2PO_4	0 1 g
$NaNO_3$	0 1 g
KCl	0 1 g
$MgSO_4$	0 1 g
Agar	20 0 g
Distilled water	1000 ml

4. King's B Agar medium

Peptone	20 0 g
Glycerol	10 0 ml
K_2HPO_4	10 0 g
$MgSO_4$ $7H_2O$	0 1 g
Agar	20 0 g
Distilled water	1000 ml
pH	7 2-7 4

5. *Trichoderma* Selective Agar medium (TSM)

$MgSO_4$	2 0 g
K_2HPO_4	0 9 g
NH_4NO_3	1 0 g
KCL	0 15 g
Glucose	3 0 g
Metalaxyl	0 3 g
PCNB	0 2 g
Rose Bengal	0 15 g
Chloramphenicol	0 25 g
Agar	20 0 g
Distilled water	1000 ml

MANAGEMENT OF DOWNY MILDEW (*Pseudoperonospora cubensis* (BERK. & CURT.) (ROSTOV.) OF CUCUMBER UNDER PROTECTED CULTIVATION

By

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ABSTRACT OF A THESIS

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ABSTRACT

The present study entitled "Management of downy mildew (*Pseudoperonospora cubensis* (Berk & Curt) Rostov) of cucumber under protected cultivation" was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara during the period from October to April 2015-16 The major objective was to assess the incidence and severity of downy mildew of cucumber under protected cultivation and to formulate eco-friendly management package against the disease

A survey was conducted in Thrissur district during January to December 2015 in nine polyhouses During the survey, incidence of downy mildew was noticed in all the polyhouses where cucumber was cultivated irrespective of the season and the disease severity varied from 11 33 to 35 75per cent There is a significant positive correlation between the disease incidence and severity with relative humidity (RH) inside the polyhouse and negative correlation with temperature

Morphological characterization of the pathogen present on the diseased leaves collected during survey revealed that sporangiophores are dichotomously-branched at acute angles that tapered to curved pointed tips Large lemon shaped, brownish yellow sporangia are borne singly at the tip of the sporangiophore

Field experiments were conducted simultaneously inside the polyhouse and rain shelter for management of downy mildew with 12 treatments and three replications The treatments included two biocontrol agents (*Trichoderma viride* and *Pseudomonas fluorescens*), cowdung supernatant, two biofungicides (garlic and calphomil) and two systemic (potassium phosphonate + hexaconazole and cymoxanil + mancozeb) and one contact (mancozeb) fungicide Soil solarisation inside the protected structures was included as part of treatments *viz* T_1 (soil solarisation + seed treatment and soil application + foliar spray with *T viride*) and T_2 (soil solarisation + seed treatment and soil application + foliar spray with *P fluorescens*)

It was found that soil temperature at 10 cm depth was higher in solarized soil when compared to nonsolarised soil by 4 °C and 3 5 °C inside polyhouse and rain shelter respectively It was also recorded that the population of soil microflora was reduced due to solarisation in protected structures Among the treatments, T_{10} (foliar spray with cymoxanil + mancozeb- 0 2%) was the most effective for management of downy mildew in both polyhouse and rain shelter followed by T_{11} (foliar spray with mancozeb-0 2%) and T_1 (soil solarisation + seed treatment and soil application + foliar spray with *T viride*) and these were statistically on par Moreover, highest yield was recorded in T_1 followed by T_2 (soil solarisation + seed treatment and soil application + foliar spray with *P fluorescens*)

Correlation analysis was performed with the meteorological data and per cent disease severity (PDS) at periodic intervals and it was confirmed that there is significant positive correlation between PDS and RH and negative correlation between PDS and temperature inside the structure Economic analysis of the field experiments suggested that the treatments with biocontrol agents recorded the highest B C ratio

Analysis of population of phyllolane microflora proved that there was drastic reduction in the population of phylloplane fungi and bacteria after spraying with chemical fungicides whereas the population increased after spraying with biocontrol agents Survival of biocontrol agents on the phylloplane of cucumber was also studied and it was found that both *T viride* and *P fluorescens*, survived on the leaf surface upto 15 days after foliar application

Results of the survey and field experiments indicated that, there is a chance of incidence of downy mildew inside the structure if the RH is $\geq$ 79 per cent Downy mildew of cucumber inside the protected structures could be effectively managed by biocontrol agents, which gave a reduction in disease severity comparable to that of systemic fungicide