CHARACTERIZATION AND MANAGEMENT OF POWDERY MILDEW OF YARD LONG BEAN (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) UNDER PROTECTED CULTIVATION

By

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(2016-11-004)

THESIS

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DECLARATION

I, **Rahila Beevi M. H.** (2016-11-004) hereby declare that, this thesis entitled **"Characterization and management of powdery mildew of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) under protected cultivation"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title of any other University or Society.

Place: Vellanikkara

Date: 14-09-2018

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CERTIFICATE

Certified that this thesis entitled **"Characterization and management of powdery mildew of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) under protected cultivation"** is a record of research work done independently by **Mrs. Rahila Beevi M. H. (2016-11-004)** 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.

Place: Vellanikkara
Date: 14/9/2018

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Rahila Beevi M. H.

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Introduction

1. INTRODUCTION

Yard long bean or pole type vegetable cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) is an important vegetable widely cultivated in Kerala, which occupies prime position in coverage and popular preference subsequent only to bittergourd (KAU, 2004). Owing to the high protein content, nutritive value and palatability of the tender pods, the market demand of yard long bean has increased tremendously during the last decade, which made it a remunerative crop under protected cultivation in Kerala (Varghese and Celine, 2015).

Nowadays, protected cultivation and high-tech farming of vegetables is gaining importance because of increased commercialisation of agriculture. The season extension structures like polyhouse provide opportunity to grow vegetables in all the seasons by the manipulation of environmental conditions, which otherwise may restrict the crop growth. In Kerala, rain shelter which is a comparatively low cost protected structure is also considered as a promising alternative to grow vegetables especially in rainy seasons (Gokul and Hakkim, 2015). However, microclimate in enclosed structures is congenial for the multiplication and spread of pathogens. High density cropping and monocropping of high yielding varieties also makes the plants under polyhouse predisposed to various fungal diseases.

Yard long bean is susceptible to a wide range of pests and pathogens which can cause damage to the crop at all stages of growth among which powdery mildew is found to be the most serious disease inflicting huge economic loss especially under protected conditions. The disease under severe conditions affect the entire foliage and reduces the photosynthetic ability which in turn take a toll on the yield. So far much attention has not been paid to standardise management strategy for the disease under protected condition.

Management of powdery mildew is often achieved by means of chemical fungicides with high residual toxicity which is harmful to the environment as well as human health. Biological control is an alternative method to manage the disease, and is more effective under protected structures than in open fields. Moreover, use

of chemical fungicides pose threat to the naturally occurring biocontrol facilitated by the beneficial microbes on the foliage which in turn make the crop more vulnerable to pathogens. Continuous use of chemical fungicides exert high pressure on the pathogen to evolve which leads to the development of resistant strains. However, management of disease using biological means needs to be standardised to ensure satisfactory disease control before recommending the same to the farmers.

In this back ground, the present study was undertaken with the objective to assess the incidence and severity of powdery mildew of yard long bean under protected cultivation and to formulate an effective disease management strategy. The study was further grouped into following experiments.

1. Assessment of severity of powdery mildew of yard long bean in different locations of Thrissur district
2. Characterization of pathogen
3. Management of powdery mildew of yard long bean under polyhouse and rain shelter condition
4. Assessment of population of phylloplane microflora under protected condition
5. Survival of biocontrol agents on the leaves of yard long bean under protected condition
6. Persistence of chemical fungicide on yard long bean

Review of Literature

2. REVIEW OF LITERATURE

Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) is believed to be selected and developed in South East Asia from cowpea (*Vigna unguiculata* (L.) Walp.) for its long and tender pods. Cultivated cowpea consists of two main subspecies: subsp.*unguiculata,* which is grain type and subsp.*sesquipedalis,* which is consumed as a vegetable. Yard long bean is also known as asparagus bean, vegetable cowpea, snake bean, string bean, long-podded cowpea, Chinese long bean, pea-bean and sitao. The crop with chromosome number 2n = 22, belongs to the genus *Vigna* and family Fabaceae (Steele and Mehra, 1980). It is a highly nutritious vegetable containing good amount of protein (23.5-26.3%). Hence it is called 'poor man's meat'. Besides protein, the pods are rich source of other nutrients such as iron (2.5 mg 100 g^{-1}), calcium (80 mg 100 g^{-1}), phosphorus (74 mg 100 g^{-1}), vitamin A (941 IU 100 g^{-1}), vitamin C (13 mg 100 g^{-1}) and dietary fibre (2 g 100 g^{-1}) (Singh *et al.*, 2001; Ano and Ubochi, 2008).

Yard long bean is a warm season crop and can tolerate drought and high humidity. Moreover, being a legume, the crop has the capacity to fix nitrogen in the root nodules and thereby enhance soil fertility. Because of its quick growth habit and short duration, it has become an attractive component of crop rotation. In India, yard long bean is mostly cultivated in southern states particularly Kerala, Tamil Nadu, Andhra Pradesh and Odisha. The most extensive cultivation of yard long bean has been recorded in Kerala. Among the vegetables cultivated in the state, the crop occupies prime position in coverage and popular preference, next only to bitter gourd (KAU, 2004). However, the quality and productivity is low during the monsoon period due to heavy rainfall and unfavourable climatic conditions. Although the advent of protected cultivation made year round production possible, the micro climate in protected structures is congenial for the outbreak of diseases and sucking pests.

The major diseases hampering the yard long bean cultivation are powdery mildew (*Erysiphe polygoni* DC), anthracnose and die back (*Colletotrichum*

lindimuthianum), dry root rot (*Macrophomina phaseolina*), stem rot (*Pythium aphanidermatum*), leaf spots (*Cercospora cannescens*), rusts (*Uromyces appendiculatus*), bacterial blight (*Xanthomonas vignicola*) and Yellow Mosaic disease. Among these, the most dreaded disease under protected condition is powdery mildew causing yield reduction to the tune of 50 to 90 per cent (Chavan *et al*., 2014).

2.1 THE POWDERY MILDEWS

Powdery mildews are obligate parasites which cannot survive in the absence of a living host. It is one of the oldest plant diseases as evidenced by several Biblical references found in Old Testament (Moham, 2005). First historical account of the disease was given by Greek botanist Theophrastus. He described about powdery mildew on roses in 300 BC (Kiani *et al*., 2008).

Though powdery mildew diseases were most common, these were not considered to cause substantial damage to cultivated crops compared to other diseases like rust, root rot, downy mildews and viral diseases until 1840s when *Uncinula necator* inflicted serious economic loss to the grape vine cultivation of France and nearly shattered the grape production in the country. Thereafter, different powdery mildew pathogen causing serious economic losses in various cultivated crops across the globe captured attention. As an example, *Erysiphe* spp. cause powdery mildew on legumes, ornamentals, crucifers, cucurbits and beets. *Leveillula* spp. infects solanaceous crops. *Podosphaera* spp. infects apples, pears, stone fruits, some ornamentals and cucurbits. The genus *Sphaerotheca* spp. is generally pathogenic on berries, roses, cucurbits and also stone fruits. The genus *Uncinula* spp. causes powdery mildew on grapes. *Blumeria* spp. is the causal agent of powdery mildew on cereals and grasses. *Microsphaera* spp. affects many shade trees and woody ornamentals (Agrios, 2005).

2.1.1 Classification of Powdery Mildews

The powdery mildews belong to the kingdom Fungi; Phylum Ascomycota; Subdivision Pezizomycotina; Class Leotiomycetes; Order Erysiphales and Family Erysiphaceae. In 1753, Linneaus in his book "Species plantarum", recognised the genus Erysiphe and mentioned the first binomial species as "*Mucor erysiphe*" referring to a powdery mildew. De Candole in 1802 described many species of the genus. The first comprehensive monograph of powdery mildew pathogens was published by Salmon in 1900 as "The Monograph of Erysiphaceae". The second one "The Monograph of Erysiphales" appeared about 80 years later (Braun, 1987).

Morphological characteristics of anamorph and teliomorph had been used by many researchers for distinguishing different powdery mildew genera. Earlier, the main criteria for the classification were largely the teliomorph characters like morphology of chasmothecium and its appendages as well as number of asci. Later it was found that these characters are not as conserved as originally assumed. Moreover, the teliomorph stage may not be produced by these fungi during all seasons and ascal structures vary with environmental conditions. However, Hammet (1977) argued that the conidial dimensions within Erysiphaceae overlap and cannot be regarded as a conclusive evidence for differentiation. Contradicting this opinion, Cook *et al.* (1997) demonstrated the identification and classification of powdery mildew anamorphs using light microscopy and scanning electron microscopy. Description of conidial germination pattern was also proposed as a means for the rapid identification of powdery mildew anamorphs (Cook and Braun, 2009). However, with the leap in development of molecular techniques, information regarding the phylogeny and taxonomy of these pathogens increased immensely (Braun, 2011). Based on the DNA sequence data, taxonomy of powdery mildew underwent extensive revision recently. Currently, the authoritative monograph of Erysiphales is "Taxonomic Manual of Erysiphales" published by Braun and Cook in 2012, in which the criteria for classification include anamorphic, teliomorphic as well as molecular characters. According to this manual, the number of recognized

powdery mildew species is 820 including several revisions of existing species and descriptions of new species (Braun and Cook, 2012).

2.2 POWDERY MILDEW OF YARD LONG BEAN

No studies had been reported so far on the characterisation of powdery mildew of yard long bean. In cowpea, the pathogen reported to cause powdery mildew is *Erysiphe polygoni* DC. Another fungus *Sphaerotheca fuliginea* poll. had been reported to incite powdery mildew on cowpea from India (Jhooty and Munshi,1980). Soylu *et al.* (2004) reported *Podosphaera phaseoli* on cowpea for the first time in Turkey. According to the monograph published by Braun and Cook (2012) *Sphaerotheca fuiginea* and *Podosphaera phaseoli* were considered as synonym of *Podosphaera xanthii* (Castagne)U Braun & N Shishkoff. Both *E. polygoni* and *P. xanthii* produced hyaline, septate, branched and thin walled mycelia. Conidia were colourless, one celled, uninucleate, and were produced in chains on the conidiophores (Braun and Cook, 2012).

In *E. polygoni*, the conidia are cylindrical or barrel shaped. The foot cell of the conidiophore is straight, cylindrical and measured about 25 to 50 x 10 µm. The length and breadth of conidia ranged in between 30 to 45 x 10 to 20 µm. Fibrosin bodies are absent in the conidia of *E. polygoni*. The spores usually germinate apically with short germ tube and produce conspicuously lobbed appressoria (Gaetan and Madia, 2004; Dugan and Glawe, 2007).

In *P. xanthii*, conidia are hyaline with crenate edge lines (Shin and La 1993). Conidia are primarily ellipsoid to ovoid with length and breadth ranging between 20-39 x 12-22 µm (Chen *et al.,* 2008). Mature conidia of *P. xanthii* contain well defined fibrosin bodies. These are rod shaped structures measuring between 2-8 µm which can be visualised using standard light microscopy when conidia are mounted in three per cent aqueous KOH (potassium hydroxide) solution (Braun,1987). These distinctive refractive bodies appear yellow or blue under polarised light (Hammet, 1977). The true function of these bodies remain unclear,

nevertheless, they have been considered taxonomically important since first detected by Zopf in 1887 (Braun, 2011).

E. polygoni produce chasmothecia with several asci and the appendages are myceloid in nature. However, in case of *Podosphaera xanthii*, the chasmothecia with mycelioid appendages contain a single globular ascus bearing eight elliptical ascospores (Chen *et al.*, 2008). Price (1970) pointed out that chasmothecia mature on senescent leaves and woody stem towards the end of an epidemic but not all varieties of host necessarily support their production.

2.3 SYMPTOMATOLOGY OF POWDERY MILDEW OF YARD LONG BEAN

On legumes, powdery mildew usually occurs during the late stages of crop such as flowering, pod formation and harvesting. Initially, the disease appears on the upper surface of the older leaves as small white powdery spots which are visible. Later these spots enlarge and coalesce covering the entire upper leaf surface. Sometimes spots are seen on the abaxial surface of leaves also. The circular spots near the mid rib and veins later become elongated. The plant tissue underneath the mycelial growth is purplish in colour (Soylu *et al.*, 2004). The fungal hyphae and conidia are produced in abundance forming white powdery mycelium on the leaf surface. In advanced stages of infection, the powdery growth may become greyish in colour. Severely affected leaves become chlorotic and cause premature defoliation. Sometimes pods are also infected which cause shrivelling and drying (Chavan *et al.*, 2014).

2.4 DISEASE CYCLE AND EPIDEMIOLOGY

2.4.1 Disease Cycle

Powdery mildews are obligate parasites. Determination of primary source of powdery mildew is very difficult since the conidia are readily air borne and can travel very long distances. Possible sources of infection include crops grown earlier in the season, inoculum from greenhouses, ascospores formed in chasmothecia present in crop debris. However, the fungus spreads exclusively by conidia. The

conidia produced on infected leaves are disseminated readily by air movement. These wind borne conidia land on susceptible healthy leaves, germinate and produce abundance of mycelia and conidia which in turn infect new healthy plants and thus the disease perpetuates (Green *et al.*, 2002).

Upon landing on the susceptible plant surface, the powdery mildew conidia germinate within two hours and produce short germ tube which is preceded by the formation of appressorium, from which the feeding organ haustorium is sent into the epidermal cell. Once the pathogen has established itself, the growth is continued epiphytically by forming secondary hyphae, which penetrates further epidermal cells. At this stage, morphologically distinct conidiophores emerge from mycelium. At the tip of each conidiophore, conidia are formed in chains. Abundance of hyphae and conidia formed with the repeating cycles form dense white mycelium on leaf surface and this gives the characteristic powdery appearance (Glawe, 2008).

The powdery mildew fungus reproduces largely by asexual means. The sexual stage is controlled by a bipolar heterothallic mechanism and therefore requires two compatible hyphae of opposite mating type. Fruiting body chasmothecium (formerly known as cleistothecium) contain one or more ascus with ascospores. Chasmothecia are thought to be the overwintering structures of powdery mildew. Visually, these are brown to black conspicuous pin head like structures embedded superficially in the mycelium. However, the production of these structures vary greatly with the region. In some regions, these structures have not been observed or are rarely seen (Zitter *et al.*, 1996).

Yarwood (1957) stated that powdery mildew can overwinter through normal mycelium, dormant mycelium and dormant haustoria. Jhooty (1971) conducted a series of studies in India from which he concluded that in Indian conditions, powdery mildew overwintered as active mycelium in sheltered situations or on volunteer plants.

2.4.2 Epidemiology

The mere presence of susceptible host and virulent pathogen cannot incite a disease. The availability of favourable environmental condition is a pre-requisite (Agrios, 2005). Powdery mildew spreads rapidly under favourable conditions, requiring only three to seven days from infection to symptom development (Tetteh *et al.*, 2007). Large number of conidia are produced by these fungi in a relatively shorter time period with viability of seven to eight days. Unlike other fungi, powdery mildews are able to survive and sporulate on host tissue without the presence of free water. In contrast to most fungal spores, the powdery mildew conidia are fully hydrated and do not require exogenous water for its germination (Webster and Weber, 2007).

Many workers have studied the effect of free moisture on powdery mildew spore germination and concluded that presence of free water is inimical to the conidia (Yarwood, 1939; Zaracovitis, 1966). However, different powdery mildew conidia differ widely in their ability to germinate in water. Yarwood (1978) reported stimulatory effect of water on conidia of *Sphaerotheca* sp. However, Veena (1992) observed no germination of conidia of *Sphaerotheca fuliginea* when exposed to free water. Maximum germination of conidia was observed by her when the spore dusted slides were incubated in humid chamber with a water drop placed beneath the slide. Changes in internal structure of conidia are observed in many species when placed in water. The vacuoles coalesce and become granular and cytoplasmic integrity will be lost. Once the cytoplasmic integrity is lost, the conidia cannot germinate. Thus if conidia come in contact with water for a long time, they will either be rendered non-viable or will germinate abnormally and will exhaust the reserve food without forming appressoria that are necessary for infection (Perera and Wheeler, 1975). However, short period of exposure to water will not affect the ability of conidia to germinate (Sivapalan, 1993).

Powdery mildew epidemics are greatly influenced by the interactions of relative humidity and temperature. The optimum combinations of these parameters

for disease development vary in case of different powdery mildews (Yarwood, 1978). In general, the disease become more severe during periods of low rain fall in the winter and spring months. Optimum temperature varies for different powdery mildew pathogens. Yarwood and Gardner (1964) demonstrated that favourable temperature for spore germination of *P. xanthii* ranged from 9 to 34°C with optimum being at 22°C. Paulech (1969) observed that the minimum temperature for the germination of *E. polygoni* conidia was 7°C and maximum germination per cent was noticed at 25°C as against no germination at 35°C.

Podosphaera xanthii is frequently reported in warmer climates such as subtropical and tropical regions and under greenhouse conditions (Cohen, 1993; Shishkoff, 2000; Lebeda *et al.*, 2016). Season extension structures like green houses allow the persistence of inocula between seasons. Moreover, the conditions inside the polyhouse like moderate temperature (20-30^{0}C), high plant density and constant air movement are ideal for powdery mildew development and spread (Paulitz and Belanger, 2001). Though lower humidity is conducive for powdery mildew development, spore germination is favoured by relative humidity more than 90 per cent. However, infection can occur at humidity levels as low as 50 per cent (Belanger and Benyagoub, 1997). Powdery mildew incidence is positively correlated with sunshine hours and evaporation whereas negatively correlated with rainfall (Belanger and Labbe, 2002). In Indian conditions, powdery mildew is severe in January and February months when cool and dry weather prevails (Channaveeresh and Kulkarni, 2017).

2.5 MANAGEMENT BY CHEMICAL MEASURES

The powdery mildew fungi attack many economically important crops and the efforts for the control of disease often accounts for a high percentage of crop production cost (Glawe, 2008). In spite of the fact that, chemical control of disease by fungicides have negative impact on environment as well as human health, control of powdery mildews are often achieved by fungicides. Chemical control of the disease has been reported to be effective if applied at proper time (Jarial and

Sharma, 2011) and different fungicides have been tested for their efficacy against the disease from time to time.

2.5.1 Management by Contact Fungicides

Management of powdery mildew was done for many decades by sulphur and other contact fungicides. Sulphur dust was the first compound to successfully control powdery mildew in the history. Wettable sulphur also recorded 80 to 90 per cent control of powdery mildew of various crops. Sulphur exerts its fungicidal action at the surfaces of leaves, stems, flowers or fruits to which it is applied. It is re-distributed over such surface to a limited extent by vaporization. Because powdery mildew grows mainly on plant surface, applied sulphur can come into direct contact with existing mycelium and suppress its growth and sporulation. Powdery mildew fungus is vulnerable to the action of sulphur through most of its lifecycle except for the cleistothecial stage (Yarwood, 1957). The principal objection to the use of sulphur is its phytotoxic action.

Among mancozeb, ziram and copper oxychloride, better control of the disease was exhibited by mancozeb with 61.91 per cent reduction over control (Kunkalikar and Padagannur,1990). Dinocap is another fungicide widely recommended for the management of powdery mildew (Manojkumar *et al*., 2008). Amaresh *et al*. (2013) compared different contact fungicides including Bordeaux mixture, copper oxychloride, chlorothalonil, dinocap, mancozeb, thiram, wettable sulphur, zineb and ziram for their efficacy to manage powdery mildew of sunflower and found out that all the chemicals were effective in controlling the disease. However, wettable sulphur recorded the least disease severity of 54.67 per cent.

2.5.2 Management by Systemic Fungicides

During the last decade, systemic fungicides have been widely developed and used for powdery mildew management. Carbendazim (Methyl 2-benzimidazole carbamate) alone or in combination with mancozeb (Manganese ethylene bis-dithiocarbamate) gave better control of powdery mildew of green gram. In addition to fungicidal action, carbendazim possess some stimulatory effect

on plant growth (Kunkalikar and Padaganur, 1990). Veena (1992) also reported carbendazim as the best fungicide to manage powdery mildew of pumpkin with lowest disease severity of 7.2 per cent after two sprays which was on par with wettable sulphur and tridemorph. Water spray also resulted in reduced disease severity immediately after spraying, but there was no reduction during subsequent periods. Foliar spray with tridemorph (2,6-dimethyl 4-cyclododecyl morpholine) has been recommended by several workers (Khunti *et al.*, 2005; Loganathan, *et al.,* 2011; Chavan *et al.*, 2014; Ahir *et al.*, 2015) for controlling the disease.

Different triazole fungicides like hexaconazole, tebuconazole, difenoconazole, penconazole, propiconazole, flusilazole, triadimephon, myclobutanil were reported to be efficient to control the disease by different researchers (Hooda and Parashar, 1985; Khunti *et al.,* 2005; Prasad and Dwiwedi, 2007; Amaresh *et al.*, 2013). Prasad and Dwiwedi (2007) reported that the application of propiconazole resulted in the lowest disease severity. Tebuconazole also resulted in significant reduction of disease and provided better yield of pea (Jarial and Sharma, 2011). Among the six fungicides tested by Dahivelkar *et al.* (2017), hexaconazole 0.1 per cent resulted in lowest disease severity of 10.78 per cent followed by propiconazole with 11.85 per cent severity in mung bean powdery mildew. Amaresh *et al.* (2013) evaluated 12 systemic fungicides to control powdery mildew and revealed that difenoconazole gave better disease reduction combined with highest yield in sunflower compared to other fungicides. The efficacy of difenoconazole in controlling the disease was also discussed by Jarial *et al.* (2015) and reported that the chemical provided significant control of the disease.

These fungicides are demethylation inhibitors (DMI) and inhibit C-14 demethylase activity by preventing the demethylation of sterols and make them saturated. They are reported to possess preventive, curative and antisporulant action against powdery mildews. They interfere with biosynthesis of ergosterol which is an essential component of fungal cell wall and its absence causes irreparable damage to the cell wall leading to death of fungus. They also inhibit conidia and haustoria formation of the fungus. These changes in the sterol content

and saturation of polar fatty acids leads to alterations in membrane fluidity and thereby causing change in behaviour of membrane bound enzymes (Nene and Thapliyal, 1993). The efficacy of these fungicides may be attributed to their ability to eradicate established fungal infections and suppressing subsequent disease development (Loganathan *et al*., 2011).

Recently, Azoxystrobin, a strobilurin fungicide had been reported to possess very high efficiency against powdery mildew of green gram and chilly caused by *Erysiphe polygoni* and *Leveilulla taurica* respectively and ensured high yield. In addition they are easily dissipated leaving very less amount of residue on the crop. Strobilurins are quinine outside inhibitors (QoI) which cause inhibition of electron transport from cytochrome b to c and affect the cellular respiration of the fungus (Anand *et al*., 2010; Divyajyothi *et al*.,2014).

These systemic fungicides are more specific to the pathogen concerned and give very promising results in disease control. Owing to their single site activity, they act on certain specific points in metabolic pathways of the fungus. However, the problem with DMI fungicides and QoI fungicides is the development of resistance by the pathogens. Now there are a range of alternatives available including biocontrol agents, foliar fertilizers, natural mineral oils and botanicals many of which are effective in controlling powdery mildew (Jovicich *et al*., 2010).

2.6 MANAGEMENT BY BIOLOGICAL MEASURES

Biological control is an alternative means of management of foliar pathogens. Though the potential of biological approaches for disease control are advocated in general, there are only few commercialised products available for biocontrol of plant diseases especially those affecting foliar parts (Elad, 1990). Successful biological control methods of powdery mildew by fungal and bacterial antagonists have been proved under glasshouse conditions (Elad, 2000; Pertot *et al*., 2007).

2.6.1 Management by Fungal Biocontrol Agents

The most commonly used fungal biocontrol agents for the management of powdery mildew include *Ampelomyces quisqualis* and *Trichoderma* spp. *Ampelomyces quisqualis* is a natural hyperparasite of several powdery mildew fungi. Hyphae of *Ampelomyces* penetrate the hyphae of powdery mildews, continues to grow internally and produce their pycnidia in the hyphal cells, conidiophores and cleistothecia of the host. Secretion of an exo- β-1, 3-glucanase by *Ampelomyces quisqualis* was found to play a role during the later stages of hyperparasitism of powdery mildew. So these intracellular mycoparasites suppress the sporulation of the powdery mildew and eventually kill the parasitized cells, causing a gradual degradation of their cytoplasm (Falk *et al.*, 1995). A strain of *A. quisqualis*, isolated in the Hebrew University of Jerusalem, Israel (Kiss *et al.*, 2004), has been formulated and commercialized under the trade name AQ10 and is widely used to manage powdery mildew. Application of *Ampelomyces quisqualis* at ten days interval was found to be effective in controlling powdery mildew of black gram (Jayashekhar and Ebenezar, 2016).

Trichoderma isolates are known for their ability to control foliar pathogens especially powdery mildews (Elad and Freeman, 2002). The mechanisms involved in the biocontrol of foliar pathogens by *Trichoderma* sp. include mycoparasitism, antibiotic production, competition for space and nutrients, extracellular enzyme production and induction of host plant defence (Elad, 2000; Punja and Utkhede, 2003). Gliotoxin, gliovirin and viridin are well-known antibiotics produced by *Trichoderma* spp. which are effective in controlling different pathogens. In addition they are known to produce volatile compounds such as acetaldehyde, ethylene, acetone and carbon dioxide all with antifungal and antibacterial properties (Sawant, 2014).

Among these mechanisms, mycoparasitism is most common and extensively studied. Various stages of mycoparasitism involve coiling of the antagonist around the hyphae of pathogen, penetration of pathogen hyphae and

finally lysis. Lysis of fungal cell walls are facilitated by the release of series of enzymes including chitinase and 1,3-gluconase. However, these interactions between pathogen and mycoparasite require close physical proximity to each other. Powdery mildew being an ectoparasite, the hyphae are readily available for colonisation by *Trichoderma*. Though on phylloplane, *Trichoderma* is able to survive on the mycelium of pathogen, the population declines rapidly with time. Hence, frequent application of *Trichoderma* at an interval of one week is advocated for the establishment of sufficient population and efficient control of the disease (Punja and Utkhede, 2003).

In addition to direct antagonism, *Trichoderma* sp. triggers plant's latent defence mechanisms in response to infection by pathogen. Induction of systemic resistance by *Trichoderma* is evident from the experiment conducted by Elad (2000) where root zone application of *T. harzianum* T39 resulted in powdery mildew reduction up to 49 per cent on the upper leaves. Increased activity of defence related enzymes on leaves following the application of *Trichoderma* also provides evidence of induced resistance.

A study was conducted by El-Sharkawy *et al.* (2014) on biological control of powdery and downy mildews of cucumber under greenhouse conditions in which he evaluated bacterial and fungal biocontrol agents *viz*; *Pseudomonas fluorescens, Trichoderma harzianum*, *Bacillus subtilis* and *Derxia gummosa*. Activity of defence related enzymes peroxidase and polyphenol oxidase was measured and was found to be maximum in leaves treated with *T. harzianum* which is an indication of induced resistance. Chavan *et al.* (2014) obtained 52.94 per cent reduction in powdery mildew of cowpea using *T. viride* (0.2 %).

2.6.2 Management by Bacterial Biocontrol Agents

Among the currently available biocontrol agents, *Psedomonas fluorescens* is one of the most effective bacterial antagonist and is used primarily for seed, soil and foliar treatment due to its efficient antagonistic activity against various plant pathogens (Ramamoorthy *et al.*, 2002; Bharathi *et al.*, 2004). The mechanisms

involved in biocontrol of pathogens by fluorescent pseudomonads are competition for nutrients and space, antibiosis, production of siderophores and lytic enzymes. Induction of resistance by fluorescent pseudomonads is an additional mechanism by which these bacteria protect several crop plants against various diseases (Vivekananthan *et al.*, 2004; Viswanathan and Samiyappan, 2006; Saravanakumar *et al.*, 2007; Panpatte *et al.*, 2014). El-Sharkawy *et al.* (2014) in an attempt to control powdery mildew of cucumber observed shrivelling and lysis of conidiophores of *Podosphaera fusca* when treated with *P. fluorescens*. However, Chavan *et al.* (2014) opined that effect of *P. fluorescens* on cowpea powdery mildew was lower when compared to *T.viride.* This opinion parallels with that of Jayashekhar and Ebenezar (2016) where he could obtain only 9.22 per cent reduction in severity of powdery mildew of black gram.

A novel idea of integrating *P. fluorescens* with azoxystrobin to control powdery mildew of chilli was described by Anand *et al.* (2010). The inconsistent performance of *P. fluorescens* in controlling foliar pathogens was more or less overcome by combining with azoxystrobin.

2.7 MANAGEMENT BY BOTANICALS

Among the botanicals used against powdery mildew, most promising ones are neem products. The fungitoxic component in neem responsible for disease control is azadirachtin. If sprayed during early stages of disease incidence, neem oil and neem seed kernel extract (NSKE) could provide sufficient control of powdery mildew. Moreover, the reduced cost compared to fungicides also makes neem products preferable in disease control (Suryawanshi *et al.*, 2009).

A study was conducted by Surwase *et al.* (2009) on management of pea powdery mildew by botanicals and bio agents. The botanicals tested were NSKE, ginger extract, garlic extract, and *Lantana camera* extract of which maximum disease reduction of 71 per cent was recorded for NSKE. Suryawanshi *et al.* (2009) also demonstrated the effect of NSKE against powdery mildew of mung bean and obtained 44.64 per cent reduction over control. Karande *et al.* (2017) evaluated six

botanicals against powdery mildew of mango and results revealed that neem oil was best among the treatments with least powdery mildew severity of 27 per cent.

2.8 PHYLLOPLANE MICROFLORA

Since the mid 1950s, phyllosphere research has been recognised as a special field of microbial ecology. The term phyllosphere was introduced simultaneously by Last and Ruinen in 1955. The surfaces of aerial plant parts, especially leaves provide a habitat for epiphytic microorganisms, many of which are capable of influencing the growth of pathogens. Some of these organisms called residents (Andrews and Kinkel, 1986) may survive for longer periods on leaf surface and forms the phylloplane microflora.

Fungi, bacteria and yeast may form resident populations on leaves. Common phylloplane fungi include *Cladosporium* spp., *Phoma* spp., *Epicoccum* spp., *Alternaria* spp. and *Aureobasidium pullulans*. In addition to these, *Aspergillus* sp, *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* sp are also often encountered in the phylloplane. Among the bacteria, most predominant ones are Gram negative and include genera such as *Erwinia*, *Pseudomonas*, *Xanthomonas* and *Flavobacterium*. Gram positive bacteria such as *Bacillus*, *Lactobacillus* and *Corynebacterium* are less common in the phylloplane. However, the relative diversity of these organisms varies with age of the crop and season. Bacteria are more abundant on leaves early in the growing season whereas filamentous fungi become predominanat only towards the end of the season when leaf senescence begins (Blakeman, 1985).

Phylloplane microflora are usually enumerated by leaf washing technique. Here the leaf surface will be washed off in a wash buffer, which is then inoculated onto artificial growing media and incubated. Dilutions can be made to adjust the density of microorganisms in the plates. Phylloplane microorganisms can be described in terms of population density and species richness. The density of culturable microorganisms is commonly expressed as number of colony forming units (cfu) per unit of sample (Bakker, 2004). Another method used to study the

phylloplane microorganism is leaf impression technique (Lee and Hyde, 2002). Here both adaxial and abaxial surface of the leaves are gently pressed onto agar medium and plates are incubated. This gives an image on the location of organisms on the leaf surface and reduces the risk of excluding microorganism that does not survive a washing step. However, the resulting population on agar surface may be too dense to enable enumeration. Nevertheless, both these techniques exclude non culturable organisms.

These epiphytic microorganisms either non-pathogenic or saprophytic origin play an important role in controlling foliar pathogens (Leben, 1965) or they have attained prominent role in biological control (Bakker, 2004). *Cladosporium* sp. and *Ampelomyces* sp. which are frequently isolated from phylloplane of several plants are known to parasitize on powdery mildew and control the disease. Besides plant protection, these organisms favour plant growth by the secretion of phytohormones like indole -3- acetic acid (IAA) and cytokinin (Limtong and Koowadjanakul, 2012).

The population of microorganisms on the leaf surface and their activity is dependent on the microclimatological conditions like leaf temperature, humidity and leaf wetness as well as the biotic conditions which include the interaction between various microorganisms. In addition to these, the changes in chemical environment brought about by application of agrochemicals also have a major influence on microbial diversity and activity (Blakeman, 1985).

The phylloplane microflora and the effect of fungicide application on diversity and density of these organisms on various crops like potato, rice, saff flower, okra were studied by many workers (Dickinson, 1973, Gokulapalan, 1989; Mandhare and Suryawanshi, 2009; Ogwu and Osawaru, 2014). When plant protection chemicals are introduced into the environment of the plant to alleviate diseases, the disease may be prevented or cured but it may eliminate some harmless saprophytes which may ultimately lead to an imbalance in the natural ecosystem (Gokulapalan and Nair, 1984). The use of foliar pesticides to control diseases can

cause deleterious effect on phylloplane microorganism populations, often reducing the number and diversity of organisms. This can have a negative effect on naturally-occurring biological control, which in some cases, makes the plants more susceptible to other disorders (Hislop 1976; Bosshard *et al.* 1987). In some cases, microorganisms that were previously not considered plant pathogens may become pathogenic due to reduction of antagonist populations (Hislop 1976).

2.9 SURVIVAL OF BIOCONTROL AGENTS ON LEAF SURFACE

Apart from the antagonistic activity, the biocontrol agent should possess the ability to survive in the habitat where the agent is applied. Unfortunately, evaluation of biocontrol agents had been carried out by means of *in vitro* assays in most of the cases. It is obvious that the introduced biocontrol agent will not be active unless it gets established in the habitat. On the plant surface, apart from the pathogen, introduced biocontrol agent faces a variety of other microorganisms also. In order to be successful in controlling the pathogen, the biocontrol agent must compete with other microorganisms and establish an active population on the phylloplane (Elad, 1990).

The fungal biocontrol agent *T. viride* survives on the mycelium of the pathogen present on the surface of leaves whereas the bacterial biocontrol agent *P. fluorescens* survives on the surface wetness brought about by thin film of water on leaf surface or by leaf exudates (Hirano and Upper, 2000). The rate of survival of *Trichoderma* strains and *Pseudomonas* sp. may be quantified by serial dilution method on a semi selective medium (Elad and Kirshner, 1993). Known weight of leaf samples dispensed into 100 ml sterile water and after thorough shaking, population was estimated by dilution plating, using King's B medium for *P.fluorescens* and *Trichoderma* Selective Medium (TSM) for *T.viride* (Elad and Freeman, 2002).

A study conducted by Freeman *et al.* (2004) on the survival rates of *Trichoderma* strains T-39, T-105, T-161, and T-166 on strawberry phylloplane revealed that the population of *Trichoderma* declined rapidly to a lower level after

three days. Kurian and Mathew (2014) also noticed similar trend in the population of *T. viride* sprayed on bittergourd leaves where the antagonist survived up to three days on the phylloplane. Elad and Kirshner (1993) reported that survival of biocontrol agents on phylloplane was affected by different factors like environmental conditions, leaf exudates, natural microflora etc. and observed that the survival of introduced antagonist *Trichoderma harzianum* T-39 was either promoted or inhibited by other phylloplane microorganisms like bacteria, yeast and fungi depending on the microclimatic conditions and plant nutrition.

Cirvilleri *et al.* (1999) reported that *P. fluorescens* was able to survive up to 30 days after inoculation on the phylloplane of pepper, tomato, strawberry and egg plant. In an experiment evaluating the biocontrol potential of *P. fluorescens* against chilly powdery mildew Anand *et al.* (2010) found out that an active population of the bacteria sufficient enough to give satisfactory disease control survived up to seven days on the phylloplane. Gal *et al.* (2003) investigated the reasons for the ecological success of *P. fluorescens* on plant surfaces and found out that, anti-microbial activity along with efficient nutrient acquisition gives competitive advantage for the bacteria which enable them for active colonization.

2.10 PERSISTENCE OF FUNGICIDE RESIDUES

Agricultural produce particularly vegetables are often highly contaminated with pesticide residues owing to their indiscriminate use by the farmers. Since most of the vegetables are either consumed raw or after minimum cooking, the presence of chemical fungicides or their degradation products as residues may pose a threat to human health. Prolonged intake of chemical fungicides through fruits and vegetables causes changes in metabolic pathways, hepatotoxicity and subsequent liver damage in human. To evaluate the potential risk associated with the use of pesticides, it is important to know the fate of pesticide on a plant. The dissipation rate of same chemical varies according to the crop to which it is applied. This is because the dissipation of fungicides is affected by the naturally occurring compounds on the leaf, interacting synergistically or antagonistically with the

fungicide degradation (Dik, 1991). The study on persistence and dissipation of hexaconazole on french bean pods applied @ 525 and 1050 g ai ha^{-1} conducted by Ahuja and Awasthi (2002) recorded an initial deposit of 0.195 and 0.485 mg/kg of residues. Since the dissipation of residues showed half-lives of 2.59 and 4.21 days, they have prescribed a pre harvest waiting period of 6.5 and 13 days for safe bean consumption. Tebuconazole residue of 0.21 mg/kg was recorded in peas with a half life of seven days (Amer *et al.*, 2007). However, the field trials conducted to evaluate the harvest residues of tebuconazole in paddy and ground nut revealed that the residue was below detection limit (Kundu *et al.*, 2011). Vijayaraghavan *et al.* (2017) analysed the persistence of tebuconazole (0.15%) on bhindi. Since presence of residue was detected even on seventh day, they advocated to restrict the spray at least 10 days before harvest.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Characterization and management of powdery mildew of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) under protected cultivation" was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara (10°31'N latitude and 76°17'E longitude and at an elevation of 40 m above sea level), during 2016-2018. The details of materials used and techniques adopted for the investigation are described below.

3.1 SURVEY FOR ASSESSMENT OF SEVERITY OF POWDERY MILDEW IN FARMER'S FIELDS

Purposive sampling surveys were conducted during September 2017 to January 2018 in both open and protected structures where yard long bean was being cultivated at different locations of Thrissur district *viz.,* Koratty, Elanad, Chelakkara, Mannuthy, Vadanappally, Punnayur and Vellanikkara. The crops at these locations were observed for the symptoms of powdery mildew. The severity of powdery mildew was assessed using standard score chart and procedures.

In each field, five locations were selected and from each of these five locations, 10 plants were selected randomly. 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 (Wu *et al.*, 2014).

Table 1. Disease severity chart for powdery mildew

Grade	Percentage leaf area infection
0	No infection
1	Below 10% infection
2	>10 -25% infection
3	>25 – 50% infection
4	>50 – 75 % infection
5	Above 75 % infection

Per cent disease severity was calculated based on the percentage of leaf area infected using the following formula (Wheeler, 1969)

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

3.2 SYMPTOMATOLOGY AND CHARACTERIZATION OF THE PATHOGEN

Symptomatology was studied in detail during the survey under natural and artificial conditions. The course of development of symptom was documented periodically. The variation in symptoms observed and stage of the crop at different locations were also recorded. The infected leaves showing typical symptoms of powdery mildew were collected from each of the survey locations and the samples were subjected to microscopic examination. Morphological characters of the pathogen like hyphal characters, conidial and conidiophores characters were recorded. Conidia and conidiophores were examined using sticky tape method (Yang, 2003) as well as normal slide preparation method. In slide preparation, clean glass slides were taken, on which a drop of cotton blue stain was added. The powdery growth of the fungus from the leaves was transferred to the stain using a needle. Then cover slip was gently placed over this. In sticky tape method, an infected leaf was taken over which a piece of sticky tape was placed and pressed gently. The piece of sticky tape having conidia and conidiophores imprinted on the sticky surface was then pressed on a clean glass slide and viewed through 400X magnification. Conidial characters like shape of conidia, length and breadth of conidia, presence or absence of fibrosin bodies were recorded. Morphology of conidiophores and length of foot cell of conidiophores was also recorded. The pattern of germination of conidia was also noted. The images viewed in Olympus microscope at 400X magnification were captured using image analyser and maintained for further comparison if necessary.

3.2.1 Molecular Characterisation

3.2.1.1 Sample Preparation for DNA Isolation

Since powdery mildew pathogens are obligate parasites which cannot be cultured on artificial media, it is not possible to obtain the pure culture of the pathogen. Hence, the sample for DNA isolation was prepared as follows. Infected leaves were collected from the field. Measured volume (1 ml) of sterile water was pipetted onto the portion of leaves with abundant powdery growth of the pathogen and was gently scraped using a sterile blade. The mycelial growth suspended in sterile water was transferred to a sterile eppendorf tube and sealed with parafilm.

3.2.1.2 DNA Isolation and Sequencing

The isolation, PCR amplification and sequencing of complete internal transcribed spacer (ITS) region of rDNA was done at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. The details of the universal primers ITS 1 and ITS 4 (White *et al.*, 1990) used for the amplification of ITS region are given in Table 2.

Table 2. Primers used for PCR amplification

Target	Primer Name	Direction	Sequence (5' → 3')
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTGCGG
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC

The nucleotide homology of the sequence thus obtained was analysed through the nucleotide blast (BLASTn) programme of NCBI (blast.ncbi.nlm.nih.gov/Blast.cgi). Similarity of sequence was studied and the pathogen was identified.

3.2.2 Pathogenicity

Pathogenicity of powdery mildew was tested by inoculating the conidia artificially on healthy leaves of live plants. The conidia from infected leaves were dusted onto healthy leaves of four week old plants kept inside the polyhouse. Humid

chamber for incubation was created by covering the inoculated leaf with polythene cover containing moist cotton. Non inoculated plants grown under same condition served as control. The symptoms developed on inoculated leaf was observed and compared with that of natural infection. The morphological characters were also observed for similarity.

3.3 *IN VITRO* EVALUATION OF FUNGICIDES, BIO AGENTS AND BOTANICALS

In vitro evaluation of fungicides, bio agents and botanicals were carried out by spore germination method as well as detached leaf method. The treatment details are furnished in Table 3.

3.3.1 Spore Germination Method

Since spore germination of different powdery mildew fungi requires different conditions, the set of conditions which provide maximum spore germination was standardised for powdery mildew of yard long bean.

3.3.1.1 Standardisation of Spore Germination Method

Spore germination assay was carried out using 5 per cent sucrose solution (Manojkumar *et al.*, 2008). Mature conidia from infected leaves were transferred to 100 µl of sterilised sucrose solution (5%) taken in cavity slides kept inside Petri dishes and incubated at different conditions given below.

a. Temperature (28±2°C)

b. Temperature (20° C)

c. Moist chamber + Temperature (28±2°C)

d. Moist chamber + Temperature (20° C)

The spore germination was standardised in sterile water also. Here the conidial suspension made in sterile water was subjected to the same incubation conditions. The observation on conidial germination was taken after 24 hours of incubation.

Table 3. Treatment details for *in vitro* evaluation

Treatments	Trade name/ manufacturer	Concentration (%)
T_1 Difenoconazole 25 EC	Score	0.025
T_2 Difenoconazole 25 EC	Score	0.05
T_3 Tebuconazole 250 EC	Folicur	0.05
T_4 Tebuconazole 250 EC	Folicur	0.1
T_5 Mancozeb 75 WP	Indofil	0.1
T_6 Mancozeb 75 WP	Indofil	0.2
T_7 Wettable sulphur 80 WP	Thionutri	0.1
T_8 Wettable sulphur 80 WP	Thionutri	0.2
T_9 *Trichoderma viride* (TF)(2x 10^6 cfu/g)	KAU	1.0
T_{10} *Trichoderma viride* (TF)(2x 10^6 cfu/g)	KAU	2.0
T_{11} *Trichoderma viride* (LF)(2x 10^6 cfu/ml)	KAU	0.25
T_{12} *Trichoderma viride* (LF) (2x 10^6 cfu/ml)	KAU	0.5
T_{13} *Pseudomonas fluorescens* (1x 10^8 cfu/g)	KAU	1.0
T_{14} *Pseudomonas fluorescens* (1x10^8 cfu/g)	KAU	2.0
T_{15} Neem oil	Nimbicidin	1.0
T_{16} Neem oil	Nimbicidin	2.0
T_{17} Control		

LF- Liquid formulation TF – Talc Formulation

3.3.1.2 Evaluation of Treatments Using Spore Germination Method

Fungicides, bioagents and botanicals (Table 3) at different concentrations were evaluated under *in vitro* condition against powdery mildew fungus using standardised spore germination method. Required concentrations of compounds were prepared separately in sterile water. From that, 100 µl of each treatment was transferred to separate cavity slides. Then conidia from infected leaves were transferred to the solution. All the cavity slides were kept in moist chamber and incubated at 20°C for 24 hours. The observation on spore germination was taken at 24 hours after incubation.

3.3.2 Evaluation of Treatments Using Detached Leaf Method

Fresh healthy leaves of yard long bean were collected from the field and washed thoroughly with tap water. The treatments at specified concentrations (Table 3) were sprayed on these leaves with a hand sprayer and were allowed to air dry. The treated leaves were then placed in Petri dishes lined with moist filter paper. Conidia were then gently brushed from the diseased leaves on the top of the treated leaves. The plates were incubated at room temperature for five days. After incubation, the leaves were observed for the symptoms of powdery mildew (Tenhovirta, 2012). Number and size of the lesions formed was recorded and per cent leaf area infected was calculated. Inoculated leaves without treatment application served as control. Based on this, promising treatments from systemic fungicides, contact fungicides, biocontrol agents and botanical were selected for field evaluation.

3.4. FIELD EXPERIMENT FOR MANAGEMENT OF POWDERY MILDEW OF YARD LONG BEAN UNDER POLYHOUSE AND RAIN SHELTER CONDITION

Field experiments were conducted under poly house and rain shelter of Department of Plant Pathology, simultaneously from October to March of 2017-18. Polyhouse and rain shelter with gable type roof had a size of 300 m^2 and 200 m^2 respectively. Both the structures were equipped with drip irrigation system and fertigation system and were constructed in North – South direction in the Department of Plant Pathology, College of Horticulture, Vellanikkara. The experimental site had a well-drained sandy loam soil and experienced a warm humid tropical climate. The details of the experiment are as follows.

Design : RBD

Treatments : 9

Replication : 3

Plot size : 3m x 1m

Spacing : 1m x 0.45m

Variety : Vellayani Jyothika

Season : October to March (2017-18)

3.4.1 Treatment Details

The treatments used for the control of powdery mildew of yard long bean consisted of systemic fungicides, contact fungicides, biocontrol agents and botanicals. The details are furnished in Table 4.

Table 4. Treatments selected for field experiment

Treatment	Description	Concentration (%)	Type
T_1	Difenoconazole	0.05	Systemic fungicide
T_2	Tebuconazole	0.1	Systemic fungicide
T_3	Wettable sulphur	0.1	Contact fungicide
T_4	Wettable sulphur	0.2	Contact fungicide
T_5	*T. viride* ($2x10^6$cfu/g)	2.0	Fungal biocontrol agent
T_6	*T. viride* (LF)($2x10^6$cfu/ml)	0.5	Fungal biocontrol agent
T_7	*P. fluorescens* ($1x10^8$ cfu/g)	2.0	Bacterial biocontrol agent
T_8	Neem oil	2.0	Botanical
T_9	Control		

LF- Liquid formulation

3.4.2 Field Preparation

The land inside these structures was thoroughly ploughed and prepared using power tiller. Beds of size 3.0 x 1.0 m^2 were taken. Farmyard manure was incorporated into the soil by ploughing and the beds were levelled and irrigated. Then seeds of yard long bean variety Vellayani Jyothika were sown in the beds at spacing of 1.0m x 0.45 m and 3-4 cm depth. Over this, a thin layer of soil was spread. Irrigation and fertigation were provided with drip irrigation system.

3.4.2.1 Cultural Operations

Nylon nets were fixed for the vines to trail over. Hand weeding inside the structures was carried out as and when required. Fertilizers were given as per KAU package of practices recommendation.

3.4.3 Management of Powdery Mildew of Yard Long Bean Under Protected Cultivation

The selected plant protection chemicals, biocontrol agents and botanicals were tested for their efficacy in controlling powdery mildew of yard long bean under protected condition. First foliar spray was given with the onset of disease and next spray was scheduled 15 days after the first spray. While spraying, plots were separated using plastic screens to avoid drift. Disease incidence and severity were recorded using 0-5 scale (Table 1) before and ten days after each spray. For this, four plants were selected randomly from each plot, and a total of five leaves, two from bottom, two from middle and one from top were observed from each plant. Disease severity was calculated based on the percentage of leaf area infected as described under 3.1.

3.4.4 Biometric Observations

Biometric observations such as vine length, days to flowering, days to harvest, pod weight, pod length and shelf life of yard long bean were recorded to assess the effect of treatments on these parameters.

3.4.5 Assessment of Crop Loss

To assess the crop loss due to powdery mildew, the yield from plots which recorded maximum and minimum yield was used. Percentage yield loss was estimated using the formula (Simon, 1996) as shown below.

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

Yield in treatment = Yield of treatment with maximum yield

Yield in control = Yield of plot without fungicide spray

3.4.6 Economic Analysis

Total expenditure incurred and total returns were calculated separately for each treatment in polyhouse as well as rain shelter. The benefit : cost ratio was worked out at market price (Rs. 40 kg^{-1}) of yard long bean for all treatments as well as at 20 per cent premium price for organic treatments alone.

3.4.7. Incidence of Other Diseases and Pests

Incidence of pests and diseases apart from powdery mildew in polyhouse and rain shelter was recorded during the field experiment.

3.5 METEOROLOGICAL PARAMETERS

Temperature and relative humidity inside the polyhouse and rain shelter was recorded daily at 7.30 am and 2.30 pm throughout the experiment using temperature and moisture meter which is installed in these structures. Correlation analysis was performed between major meteorological factors and disease severity. Per cent disease severity in control and average temperature and relative humidity during the previous week of observation on disease was used for the analysis.

3.6 ENUMERATION OF PHYLLOPLANE MICROFLORA OF YARD LONG BEAN

Enumeration of phylloplane microflora (fungi, bacteria and actinomycetes) of the crop was carried out by following the method devised by Elad and Kirshner (1993). Leaves were collected from all the treatments in polyhouse and rain shelter before spraying as well as one day after and seven days after spraying. Area of the leaves collected was measured using graph paper method. Then the leaves were cut into small pieces and dispensed into 100 ml sterile water and agitated well for one minute. Then one ml of these leaf washings were pipetted into Petri dishes and suitable media were poured. Martin's rose bengal streptomycin

agar (MRBSA), nutrient agar (NA) and Ken Knights agar was used for fungi, bacteria and actinomycetes respectively. The plates were rotated gently in clock wise and anti-clock wise direction to ensure even mixing and then incubated at room temperature. Observations were taken after 24 hours for bacteria, 48 hours for fungi and seven days for actinomycetes. Population of phylloplane microflora was expressed as number of colony forming units per unit area (cfu cm^{-2}) of leaf.

3.7 SURVIVAL OF BIOCONTROL AGENTS ON THE PHYLLOPLANE OF YARD LONG BEAN

The biocontrol agents on leaves were enumerated before and after the treatment application to know the period of survival on the phylloplane. *Trichoderma viride* and *Pseudomonas fluorescens* were isolated using TSM (*Trichoderma* selective agar medium) and King's B agar medium respectively. Leaves were collected from the field before, one day after and seven days after treatment application. Biocontrol agents were isolated in the same way as described for the isolation of phylloplane microflora. The colonies were counted at 24 hours and 48 hours of incubation for *Pseudomonas* and *Trichoderma* respectively and the population was expressed as number of colony forming units per unit area (cfu cm^{-2}) of leaf.

3.8. RESIDUE ANALYSIS OF MOST EFFECTIVE CHEMICAL FUNGICIDE

Residue analysis of pods collected from plots sprayed with difenoconazole was carried out at Pesticide Residue Research and Analytical Laboratory of the All India Network Project on Pesticide Residue, KAU Centre, College of Agriculture, Vellayani, Thiruvananthapuram.

3.8.1 Sampling

Mature pods of yard long bean were collected from plots sprayed with difenoconazole at periodical intervals such as two hours, one day, three days, five days and seven days after spraying. Samples were sent to the laboratory in polythene bags and processed for residue analysis.

3.8.2 Residue Extraction and Estimation

Extraction and clean-up was done by QuEChERS method (Anastassiades, 2003). The residue of difenoconazole was estimated using liquid chromatography-mass spectrometry (LC- MS/MS).

3.9 STATISTICAL ANALYSIS

The data collected during experiments were subjected to analysis of variance (ANOVA). Data sets were analysed using web agri-stat package (WASP 2.0). Multiple comparison of treatment means were done using Duncan's multiple range test where F test was significant.

Results

4. RESULTS

The study entitled "Characterization and management of powdery mildew of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) under protected cultivation was conducted at Department of Plant Pathology, College of Horticulture, Vellanikkara during 2017-2018. The study consisted of survey for assessing severity of the disease in different locations of Thrissur district, characterization of powdery mildew pathogens collected from different locations, *in vitro* evaluation of fungicides, bioagents and botanicals and field experiments in polyhouse and rain shelter for the management of the disease. The results are presented below.

4.1 SURVEY FOR ASSESSMENT OF SEVERITY OF POWDERY MILDEW

Purposive sampling surveys were conducted in different locations of Thrissur district, where yard long bean was being cultivated in both open and protected structures. The different locations surveyed were Koratty, Elanadu, Chelakkara, Punnayur, Vadanappally, Mannuthy and Vellanikkara (Plate 1). The severity of powdery mildew of yard long bean was assessed using standard score chart (Plate 2) and procedures. Per cent disease severity varied from 1.67 to 67.33 in different locations surveyed (Table 5). Highest disease severity was recorded at Elanadu (67.33%) followed by Koratty (54.33%). The crop was at pod formation and harvesting stage at both these locations. In Vellanikkkra, the crop at harvesting stage recorded a disease severity of 43.41 per cent. Comparatively low disease intensity was observed at Mannuthy (5.33%) and Punnayur (2.31%) during the vegetative stage of the crop. Lowest disease severity of 1.67 per cent was recorded at Vadanappally and the crop was at flowering stage. From the survey conducted, it was found that powdery mildew severity was high during the months of September, November and December. The severity also varied with the age of the crop and was more during the late stages like pod formation and harvesting.

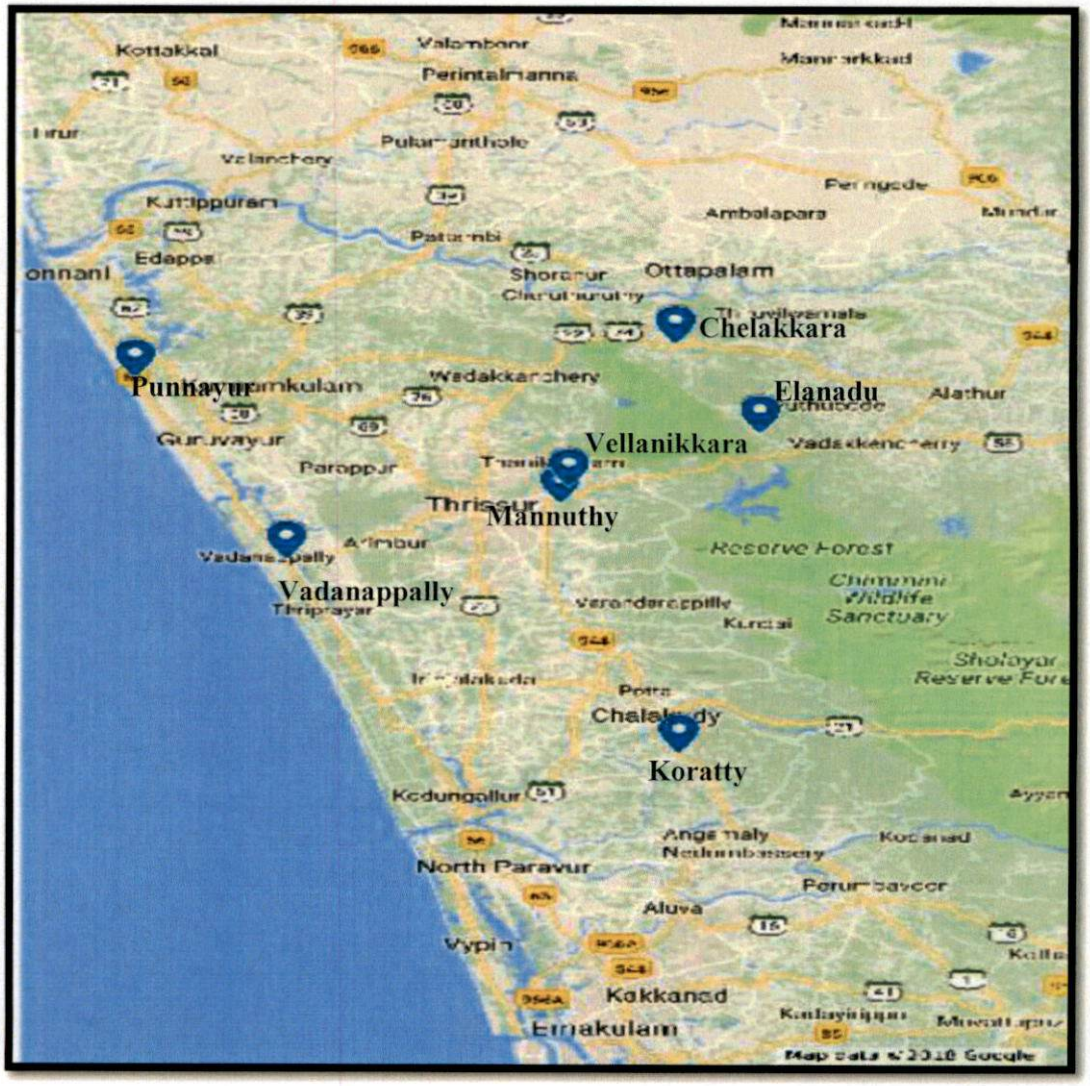


Plate 1. Locations of purposive sampling survey

0 (No infection)

1 (below 10% infection)

2 (10 -25% infection)

3 (> 20 -50% infection)

4 (>50 -75% infection)

5 (above 75% infection)

Plate 2. Score chart for powdery mildew of yard long bean

Table 5. Severity of powdery mildew of yard long bean in different locations of Thrissur district

Location	Geographical position	Period	Stage of crop	PDS
Koratty	10.267205° N 76.350388°E	September 2017	Pod formation and harvesting	54.33
Vellanikkara	10.5484° N, 76.2857° E	November 2017	Harvesting	43.41
Mannuthy	10.5285° N, 76.2682° E	December 2017	One month after sowing	5.33
Elanadu	10.6275° N, 76.3956° E	December 2017	Pod formation and harvesting	67.33
Chelakkara	10.68981° N 76.340398o E	December 2017	Harvesting	3.67
Punnayur	10.6584° N, 75.9783° E	January 2018	One month after sowing	2.31
Vadanappally	10.4730° N, 76.0682° E	January 2018	Flowering	1.67

PDS – Per cent Disease Severity

4.2 SYMPTOMATOLOGY AND CHARACTERISATION OF THE PATHOGEN

4.2.1 Symptomatology of Powdery Mildew of Yard Long Bean

During the survey, symptomatology was studied in detail. Variation was observed in symptoms. The first type of symptom was seen on young plants of about 10 to 15 leaf stage. The infection started as small white powdery spots on the lower leaves. However, within a week, white powdery growth disappeared and the lesions turned necrotic. No further spread of the disease occurred to nearby leaves as well as plants (Plate 3a). The second infection occurred on plants at pod bearing stage. Here also, the disease initiated as very small but visible white powdery spots on upper surface of older leaves. Later, these white powdery lesions composed of

mycelium and conidia of the pathogen enlarged, forming conspicuous spots and covered the entire leaf surface. During late stage of the crop, the white powdery mass turned light grey in colour. Further with the advancement of disease, it became dark grey covering the whole leaf surface. Under heavy infection, the leaves especially the lower ones turned yellow and defoliation occurred (Plate 3b). On petioles, sparse to effused powdery growth was seen. However, no symptoms were observed on pods. Another variation regarding the pattern of spread of mycelial growth on the leaves was observed in samples collected from Koratty. The white mycelial growth initiated as spots were then seen restricted along the veins of leaves (Plate 3c).

4.2.2 Morphological Characterisation

Since the powdery mildews cannot be cultured on artificial media, morphological characters were studied by microscopic examination of the fungus from infected leaves. The hyphal characters, conidial and conidiophores characters were studied using sticky tape method (Yang, 2003) and the results are presented in Table 6.

Dense epiphytic mycelium was observed under the microscope. Hyphae were hyaline, straight or flexuous, branched and septate. The length and breadth of hyphal cells varied from 35.81 to 44.73 µm and 5.44 to 10.48 µm respectively.

Conidiophores erect, straight, unbranched, hyaline and septate. Distinct difference was noticed in the conidia formed in chains over the conidiophores. Prominent constriction was seen at the junction between the cells of conidiophore in the samples collected from Vellanikkara whereas this constriction was absent in the samples collected from all other locations. The length of the foot cell of conidiophore ranged from 40.12 to 60.28µm.

Shape of the conidia was cylindrical with smooth edges in all the locations except Vellanikkara where it was elliptical to ovoid in shape with crenate edge lines. Distinct rod shaped fibrosin bodies were observed in the conidia collected from Vellanikkara. Length and breadth of conidia ranged from 20.26 to 36.25 µm and

12.38 to 19.26 µm respectively. The germ tube formed from conidia was lateral in case of samples collected from Vellanikkara and was apical in all other cases. Nipple shaped appressorium was observed in Vellanikkara isolate (Plate 4).

Based on molecular characterization, the isolates collected were placed in two groups. All the isolates except that from Vellanikkara were similar and hence placed in one group, PM 1. The pathogen found in Vellanikkara was morphologically distinct from PM1 and hence placed in another group *ie.,* PM2. The characters of PM1 type were in line with that of *Erysiphe polygoni*, which is the widely known pathogen causing powdery mildew of legumes while the characters of PM 2 were similar to that of members of genus *Podosphaera.* Since this pathogen was reported rarely on pulses, molecular characterization was carried out to confirm the identity.

4.2.3 Molecular Characterization

The powdery growth suspended in sterile water was subjected to molecular characterization at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram. Complete internal transcribed spacer (ITS) region of rDNA of the pathogen was amplified and sequenced. The sequence obtained was deposited in NCBI Genbank nucleotide database with accession number MH645799. The ITS sequence (Plate 5) was subjected to *in silico* analysis through nucleotide blast (BLASTn) programme of NCBI. The 524 base pair long sequence showed 100 per cent homology with *Podosphaera xanthii* isolate HUVU-08 (MH143485.1) from china (Plate 6).

Based on morphological and molecular characteristics, the pathogen causing powdery mildew of yard long bean at Vellanikkara was identified as *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff.

Table 6. Morphological characters of powdery mildew pathogen

Location	Hypha			Conidiophore		Conidia				
	Colour	Septation	Size of cell* (µm)	Conidiogenesis	Foot Cell* (µm)	Shape	Size* (µm)	Edges	Fibrosin body	Germ tube
Koratty	Hyaline	+	37.64 x 6.76	No constriction	40.12x 12.21	Cylindrical	32.21 x 12.38	Smooth	Absent	Apical
Vellanikkara 1	Hyaline	+	35.81 x 7.42	Constriction between cells	52.21 x 11.18	Elliptical to ovoid	20.26 x 16.47	Crenate	Present	Lateral
Vellanikkara 2	Hyaline	+	40.86 x 10.41	Constriction between cells	56.13 x 14.22	Elliptical to ovoid	27.10 x 18.23	Crenate	Present	Lateral
Mannuthy	Hyaline	+	38.93 x 5.44	No constriction	60.28 x 10.32	Cylindrical	30.87x 15.47	Smooth	Absent	Apical
Elanadu	Hyaline	+	37.88 x 6.82	No constriction	58.34 x 11.45	Cylindrical	34.35 x 15.15	Smooth	Absent	Apical
Chelakkara	Hyaline	+	38.12 x 10.48	No constriction	42.56 x 13.48	Cylindrical	30.85 x 19.26	Smooth	Absent	Apical
Punnayur	Hyaline	+	44.73 x 10.32	No constriction	59.28 x 14.37	Cylindrical	32.86 x 15.63	Smooth	Absent	Apical
Vadanappally	Hyaline	+	38.21 x 5.87	No constriction	49.27 x 14.86	Cylindrical	36.25 x 19.23	Smooth	Absent	Apical

*Mean of 20 observations

55

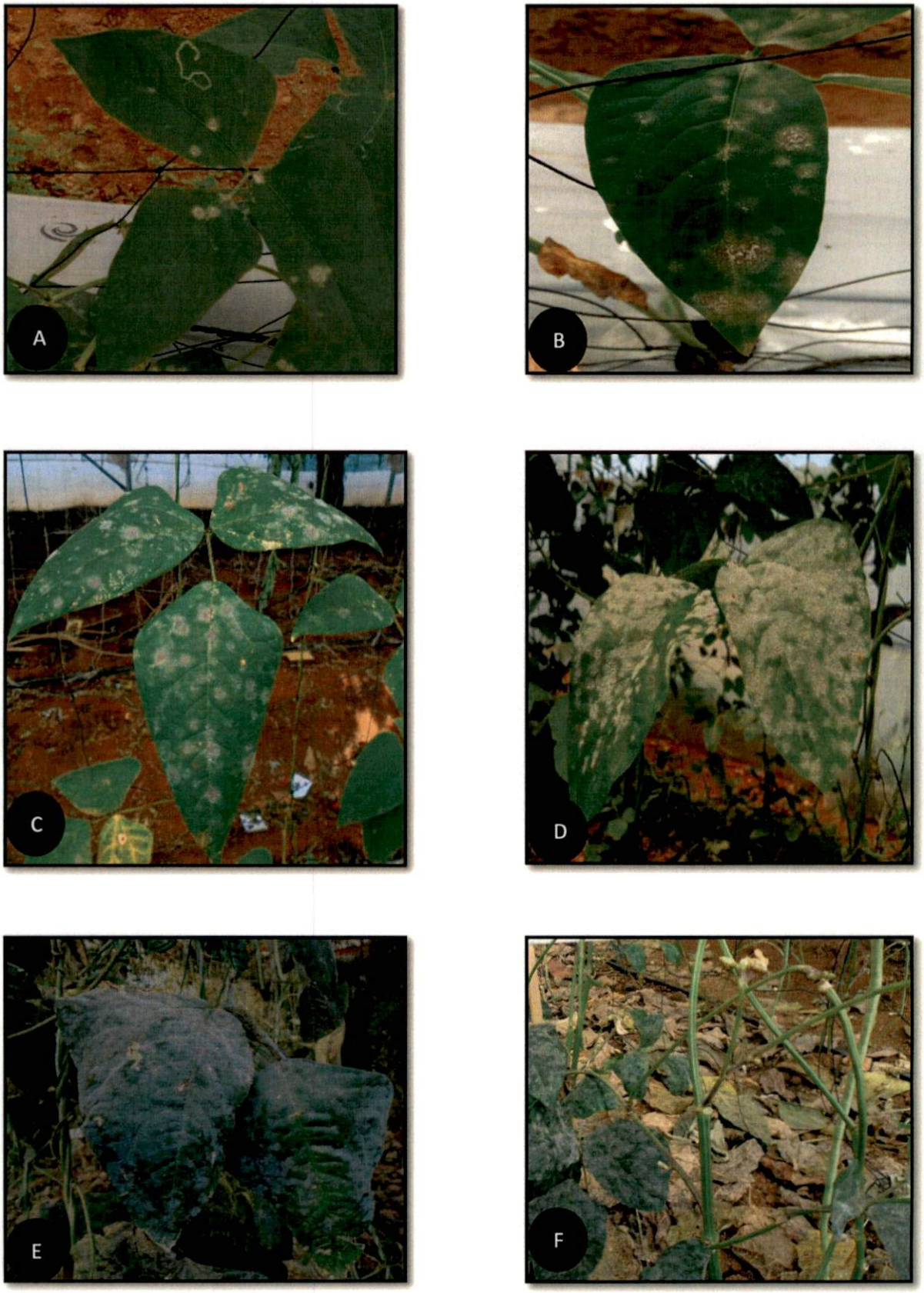


Plate 3b. Powdery mildew symptoms on plants at pod bearing stage: A: Initiation of white powdery lesions, B: Enlarged conspicuous lesions, C, D: Powdery growth covering entire leaf lamina, E:Greyish discoloration of mycelia, F: Defoliation of lower leaves

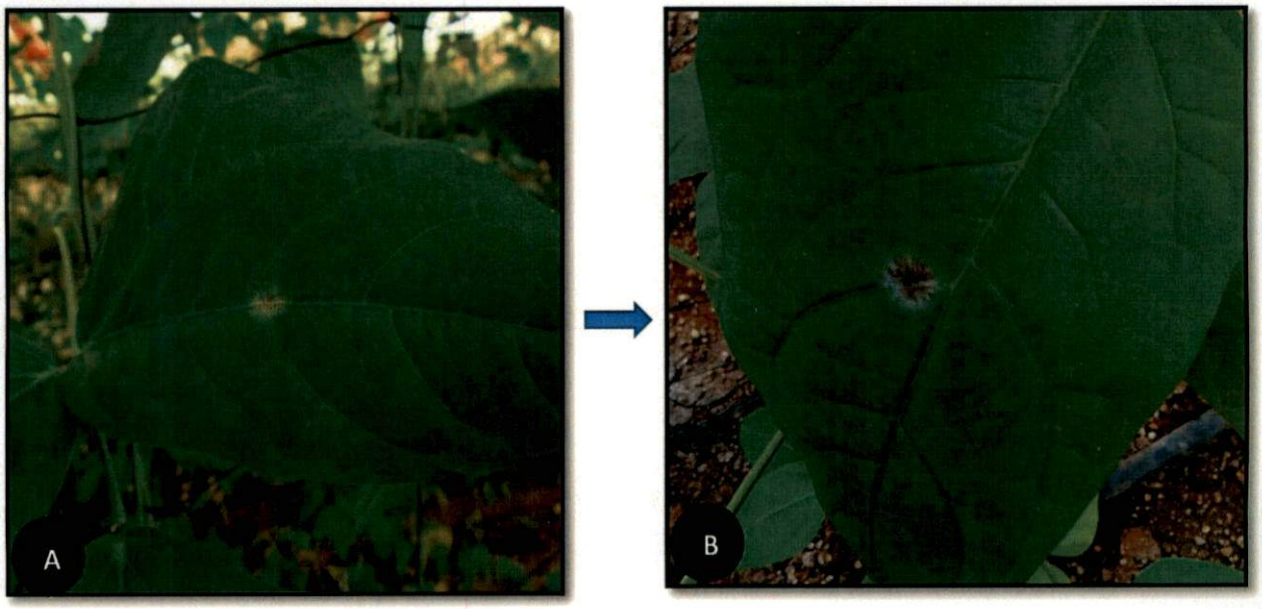


Plate 3a. Powdery mildew symptoms on young plants: A: Initiation of white powdery lesions, B: Dried up lesion after one week

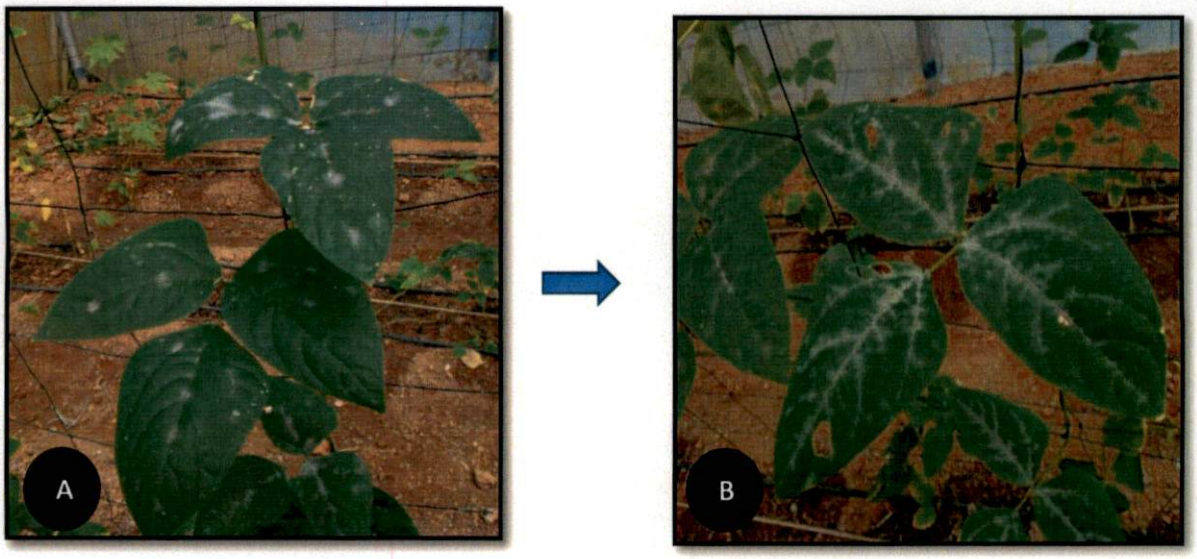


Plate 3c. Variation in symptoms at Koratty, A: White powdery lesions on the leaves, B: Spread of the lesions restricted along the veins

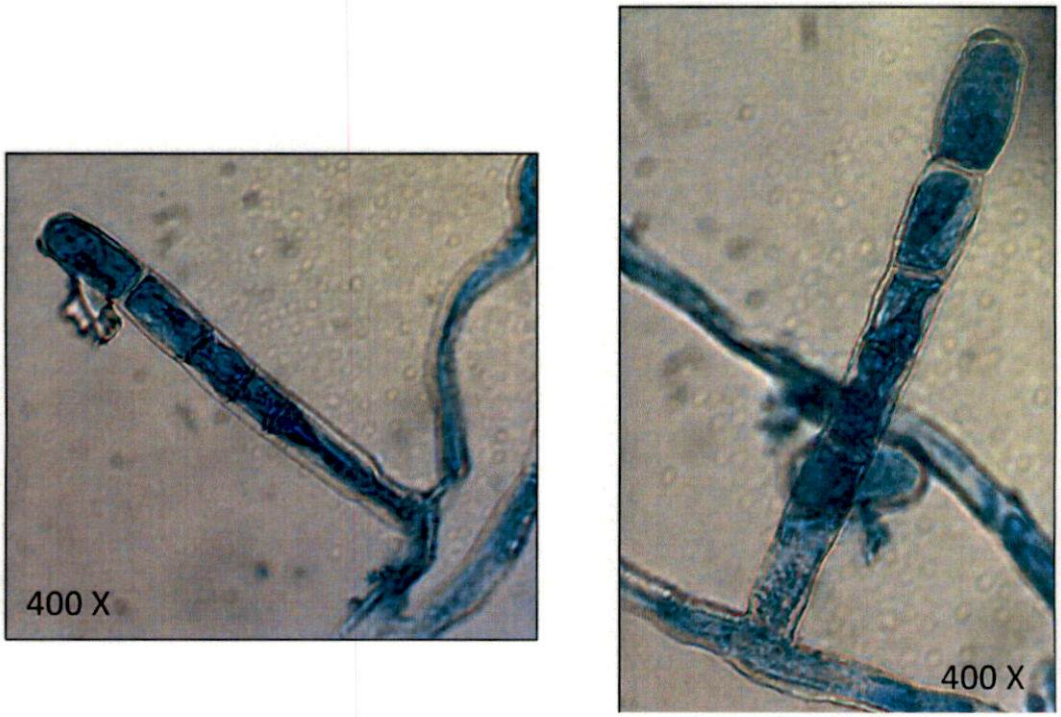


Plate 4a: Conidiophores of *Erysiphe polygoni* with conidia formed in chains

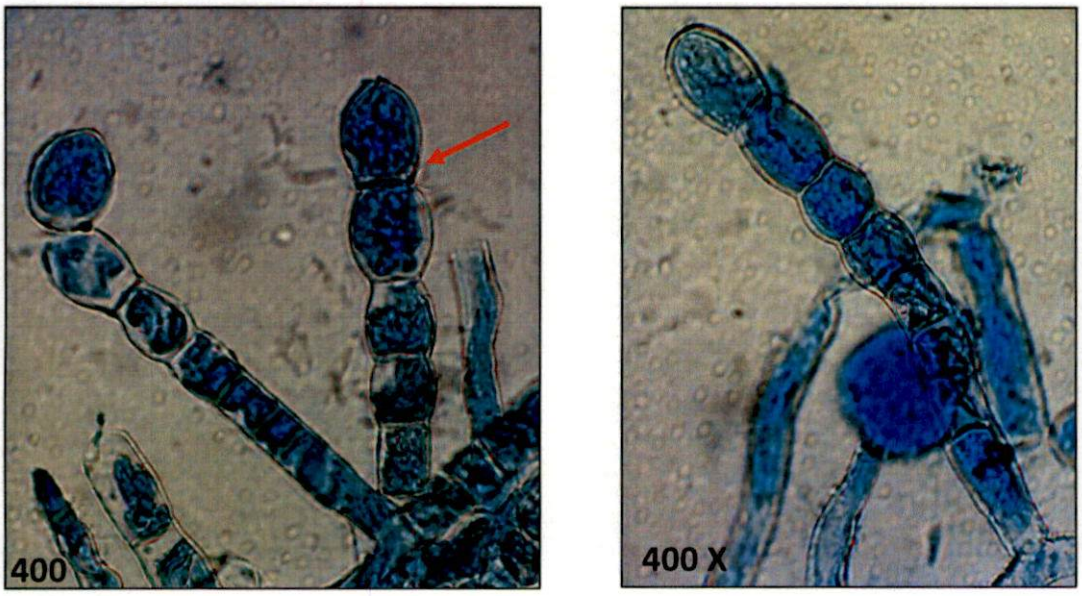


Plate 4b: Conidiophores of *Podosphaera* sp. with prominent constriction at the junction of each conidia born in chains

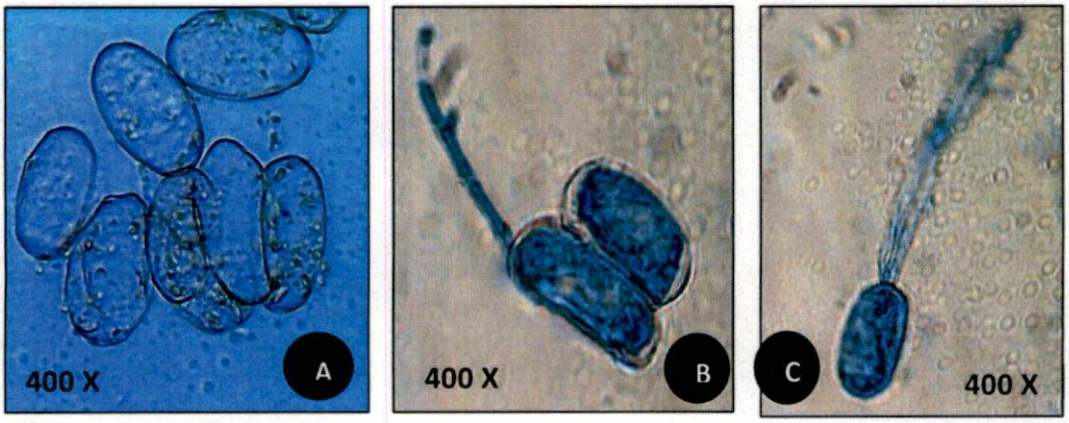


Plate 4c: Conidia of *Erysiphe polygoni*, A: Cylindrical to ovoid smooth walled conidia, B&C: Apical germination of conidia

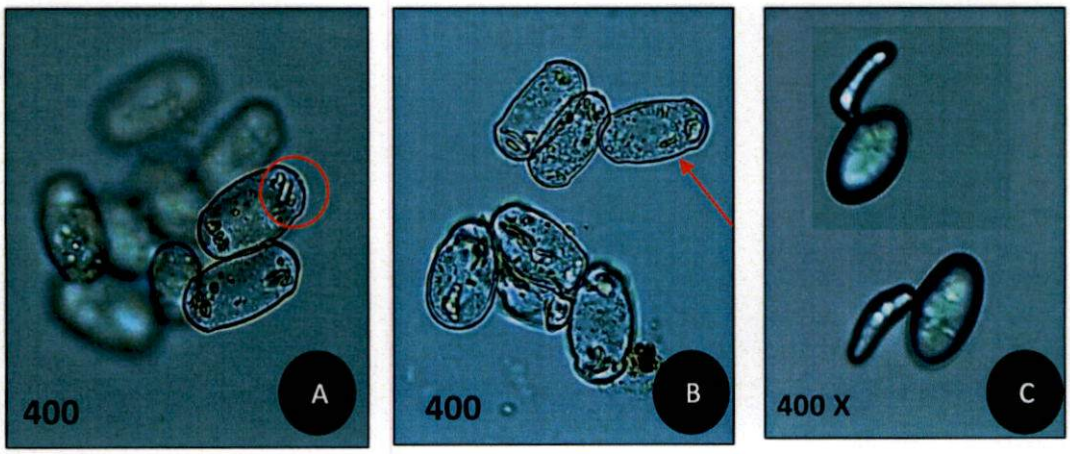


Plate 4d: Conidia of *Podosphaera* sp. A: Conidia with rod shaped fibrosin bodies, B: Elliptical conidia with crenate outer edges C: Lateral germination of conidia

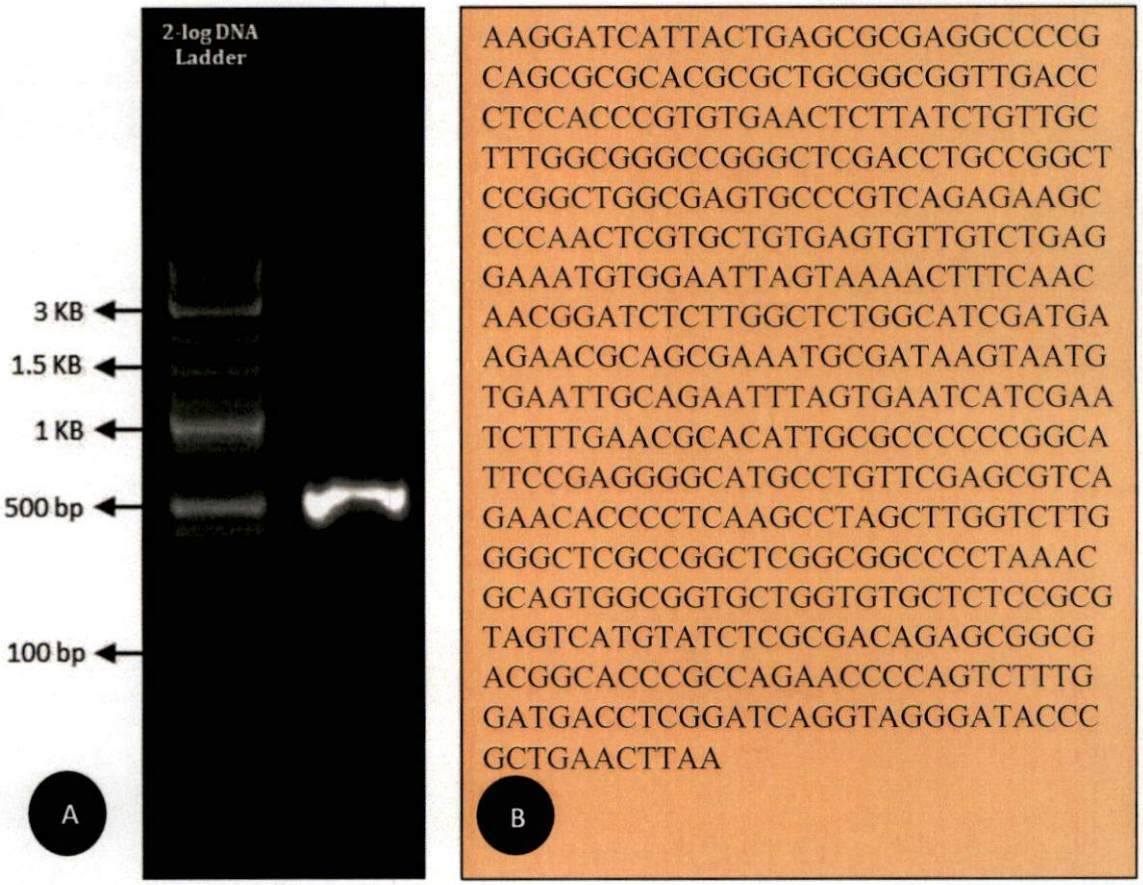
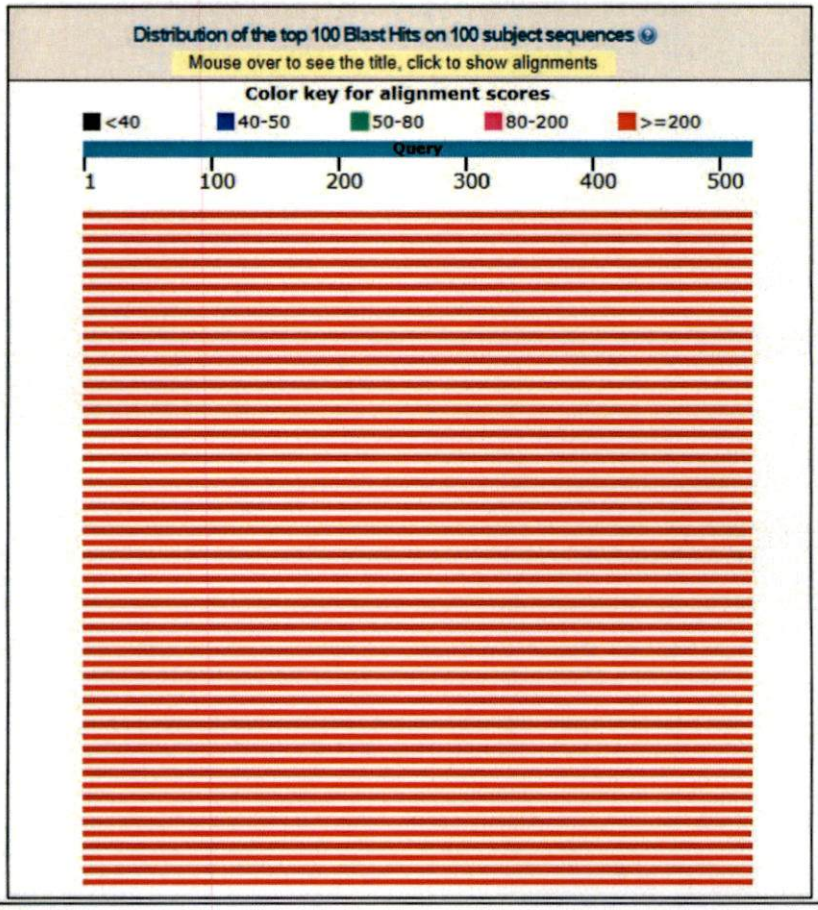


Plate 5: Molecular characterisation, A: PCR gel profile of *Podosphaera* sp. (Vellanikkara isolate) with amplification near 500bp, B: Nucleotide sequence of rRNA-ITS region of *Podosphaera* sp.



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Podosphaera xanthii isolate PM1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large s	968	968	100%	0.0	100%	MH645799.1
Podosphaera xanthii isolate HUVU-08 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed	968	968	100%	0.0	100%	MH143485.1
Podosphaera hibiscicola genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	968	968	100%	0.0	100%	AB040308.1
Podosphaera xanthii isolate HUCP-09 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed	963	963	100%	0.0	99%	MH143487.1
Podosphaera xanthii isolate HUCS-07 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed	963	963	100%	0.0	99%	MH143483.1
Podosphaera xanthii genes for ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, specimen_voucher: MUMH</JPN>:3284	963	963	100%	0.0	99%	LC270779.1
Podosphaera xanthii genes for ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, specimen_voucher: MUMH</JPN>:6947	963	963	100%	0.0	99%	LC270778.1
Uncultured Podosphaera clone MBG20170613 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tra	963	963	100%	0.0	99%	MG516581.1
Uncultured Podosphaera clone MBG20170612 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal tra	963	963	100%	0.0	99%	MF405168.1
Podosphaera xanthii strain HMJAU91759 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed space	963	963	100%	0.0	99%	KY860728.1
Podosphaera xanthii isolate BJ1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed space	963	963	100%	0.0	99%	MG928388.1
Podosphaera xanthii voucher KUS:F28385 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spac	963	963	100%	0.0	99%	KY947513.1
Podosphaera xanthii voucher KUS-F30094 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcr	963	963	100%	0.0	99%	MG754404.1
Podosphaera xanthii isolate race 2F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed sp	963	963	100%	0.0	99%	MG719984.1
Podosphaera xanthii isolate race 2F small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed sp	963	963	100%	0.0	99%	MG706136.1
Podosphaera xanthii isolate FUCN internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large	963	963	100%	0.0	99%	MG270574.1
Podosphaera sp. clone Mc-1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,	963	963	100%	0.0	99%	MF183114.1

Plate 6: BLASTn graphical and text output of ITS rRNA sequence of *Podosphaera* sp. (Vellanikkara isolate)

4.2.4 Pathogenicity

Pathogenicity of powdery mildew was tested by inoculating conidia from infected leaves onto healthy leaves of live plants. Symptoms were observed seven days after inoculation. Dispersed white powdery growth was seen above the leaf lamina on inoculated leaves whereas no symptoms developed on the control plants (Plate 7). The symptoms developed were similar to that of natural infection. The morphological characters of conidia observed under the microscope revealed similarity with that of the original pathogen. Thus pathogenicity of powdery mildew was proved.

4.3 *IN VITRO* EVALUATION OF FUNGICIDES, BIO AGENTS AND BOTANICALS

In vitro evaluation of treatments were done using spore germination method as well as detached leaf method

4.3.1 Spore Germination Method

4.3.1.1 Standardisation of spore germination method

Since different powdery mildews require varying conditions for spore germination, the condition giving maximum germination was standardised for powdery mildew of yard long bean.

Conidial suspension in five per cent sucrose solution was incubated under four different conditions described in 3.3.1.1. However, none of the conidia germinated. Under microscopic observation, it was found that the conidia were collapsed and its cytoplasmic integrity was lost (Plate 8). When the experiment was repeated using conidial suspension in sterile water, up to 30 per cent germination was obtained when incubated at 20°C in moist chamber (Plate 9). Therefore, incubation of conidial suspension in sterile water at 20°C was selected for the evaluation of treatments.

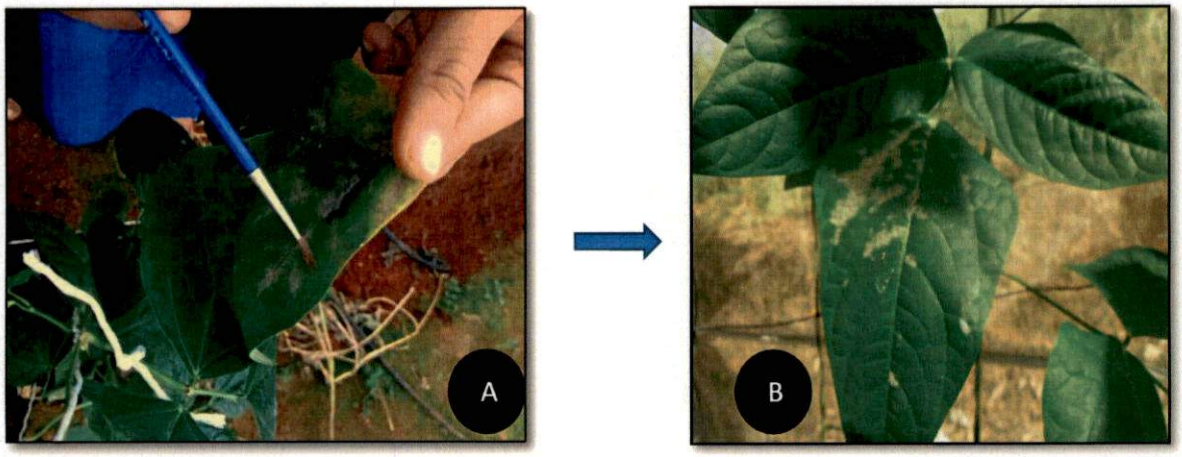


Plate 7. Pathogenicity test, A: Artificial inoculation of conidia from infected leaf to healthy leaf, B: White powdery lesions developed on inoculated leaf

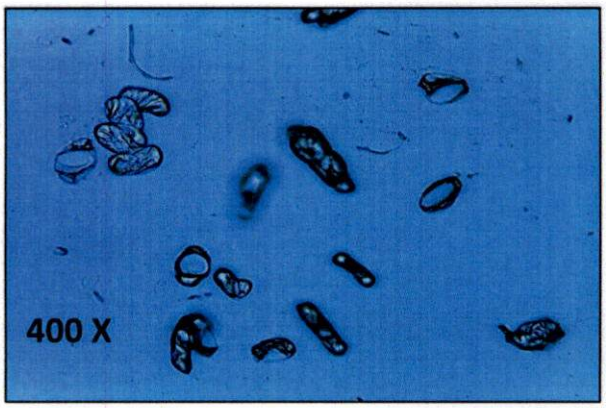


Plate 8 : Collapsed conidia in sucrose solution

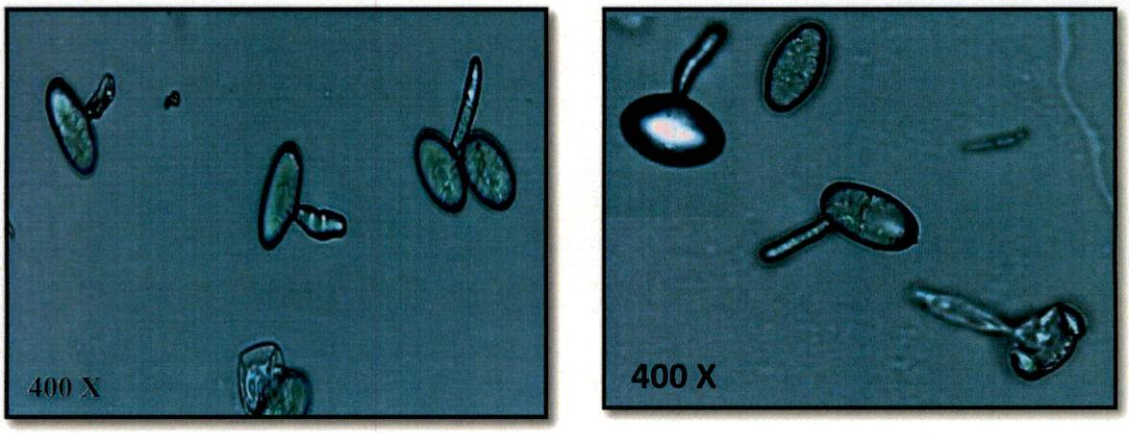


Plate 9a: Germinated conidia in sterile water

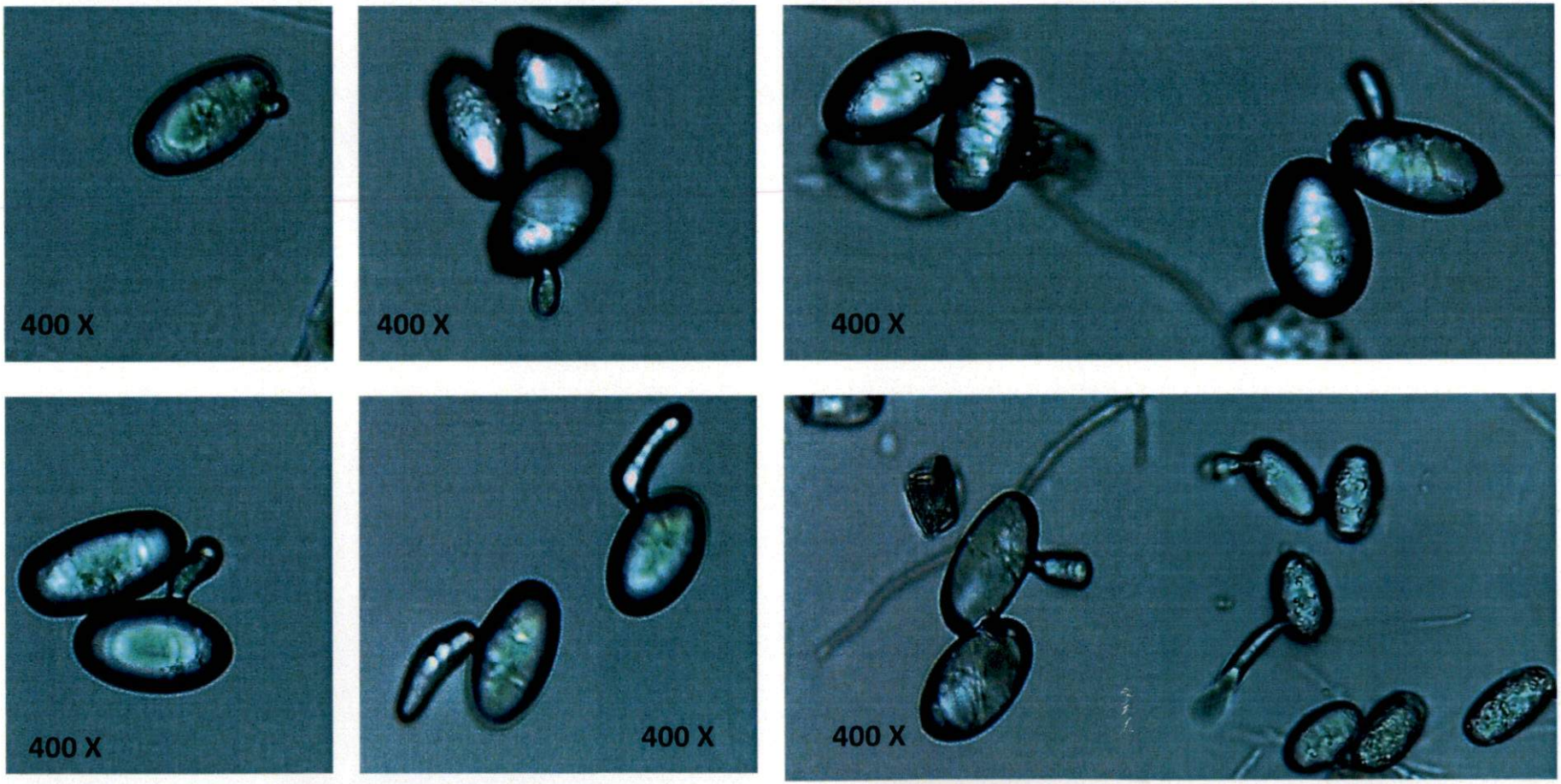


Plate 9b : Different stages of conidial germination in sterile water

4.3.1.2 Evaluation of Treatments Using Spore Germination Method

Required concentrations of fungicides, bioagents and botanicals were prepared in sterile water. A measured volume (100 µl) of the treatments was placed in the cavity slide onto which conidia from infected leaves were transferred. Slides were kept in moist chamber and incubated at 20°C for 24 hours. Cent per cent inhibition of conidial germination was obtained in all the treatments as against 30 per cent germination in control.

4.3.2 Evaluation of Treatments Using Detached Leaf Method

Treatments were evaluated by inoculating conidia from infected leaves on sprayed leaves as described in 3.3.2. Small white lesions of powdery mildew were observed after five days of incubation (Plate 10). The lesions were smaller in size and powdery growth was not profuse unlike in case of natural infection. The size and number of the lesions were recorded and per cent leaf area infected (LAI) was calculated. The results are furnished in Table 7.

The treatments consisted of four different classes of compounds *viz*., systemic fungicides, contact fungicides, biocontrol agents and botanicals. So representatives from all the four categories were selected for evaluation under field conditions. Among the systemic fungicides, T2 and T4 showed lowest leaf area infection (0.09%). In treatments with contact fungicides, the two treatments of wettable sulphur (T7and T8) were found to be superior over mancozeb. Among biocontrol agents, T10, T12 and T14 were found to be superior with 5.45, 5.52 and 5.45 per cent leaf area infection. Among the two treatments of neem oil, the higher concentration resulted in lower per cent leaf area infection (5.68%). However, all the treatments were superior to control. Thus eight treatments were selected along with control for field experiment and new treatment numbers were assigned as given below (Table 8).

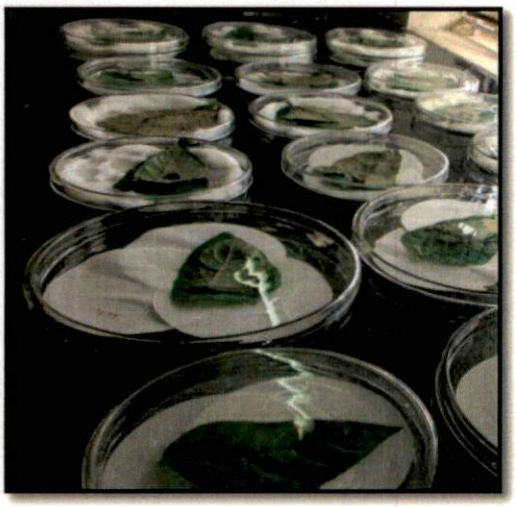
Table 7. Effect of different treatments on powdery mildew by detached leaf method

Treatment	Concentration (%)	LAI (%)
T1 Difenoconazole	0.025	1.09
T2 Difenoconazole	0.05	0.09
T3 Tebuconazole	0.05	0.91
T4 Tebuconazole	0.1	0.09
T5 Mancozeb	0.1	6.14
T6 Mancozeb	0.2	4.54
T7 Wettable sulphur	0.1	1.59
T8 Wettable sulphur	0.2	2.18
T9 *Trichoderma viride*	1.0	6.00
T10 *Trichoderma viride*	2.0	5.45
T11 *Trichoderma viride* (LF)	0.25	8.18
T12 *Trichoderma viride*(LF)	0.5	5.52
T13 *Psudomonas fluorescens*	1.0	6.90
T14 *Pseudomonas fluorescens*	2.0	5.45
T15 Neem oil	1.0	9.54
T16 Neem oil	2.0	5.68
T17 Control		10.9
CD		0.125

LAI : Leaf Area Infection

A. Application of treatments on detached leaves

B. Inoculation of conidia on treated leaves



C. Incubation

D. Lesions developed 5 DAI

Plate 10: *In vitro* evaluation of treatments by detached leaf method

Table 8. Details of treatments selected for field experiment

Sl. no.	Treatments	Label assigned
1	T2- Difenoconazole -0.05%	T1
2	T4- Tebuconazole- 0.1%	T2
3	T7- Wettable sulphur 0.1%	T3
4	T8- Wettable sulphur -0.2%	T4
5	T10- *Trichoderma viride* -2% (2x 10^6cfu/g)	T5
6	T12- *Trichoderma viride* LF-0.5% (2x10^6cfu/ml)	T6
7	T14 - *Pseudomonas fluorescens* - 2% (1x10^8 cfu/ml)	T7
8	T16- Neem oil-2%	T8
9	T17- control	T9

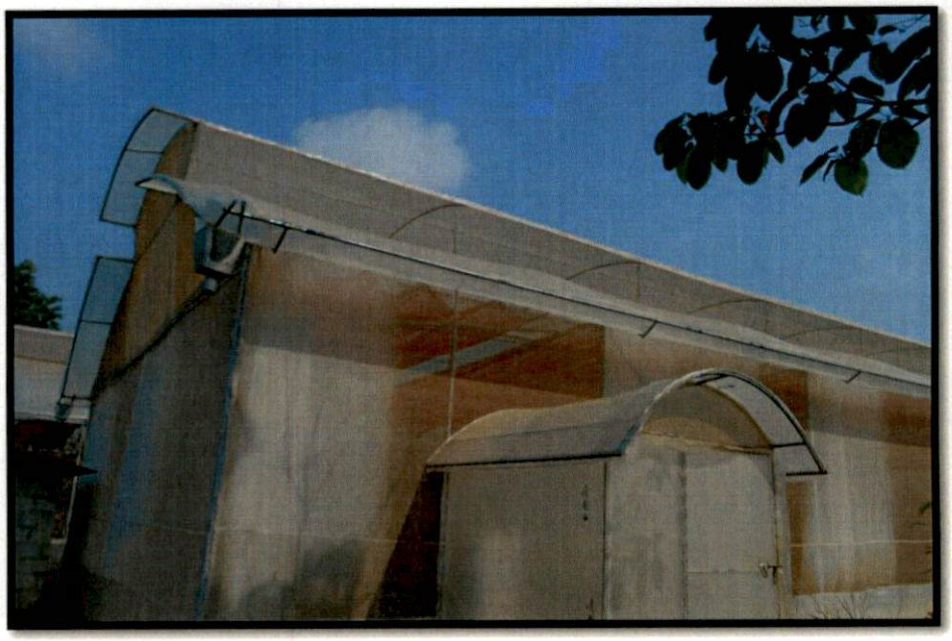
4.4 FIELD EXPERIMENTS FOR MANAGEMENT OF POWDERY MILDEW OF YARD LONG BEAN UNDER POLYHOUSE AND RAIN SHELTER CONDITION

The effectiveness of selected fungicides, biocontrol agents and botanicals were tested against the powdery mildew of yard long bean under polyhouse and rain shelter (Plate 11). The crop was raised from October 2017 to March 2018. Incidence of powdery mildew was noticed at 53 days after sowing (DAS) in rain shelter and 55 days after sowing in polyhouse. The first foliar application was given at the onset of disease. The second spray was given after 15 days of first spray. Disease severity was recorded using 0-5 scale before spraying and 10 days after each spraying.

4.4.1 Effect of Different Treatments on Powdery Mildew of Yard Long Bean Under Polyhouse

Results of the field experiment for management of powdery mildew of yard long bean under polyhouse are presented in Table 9 (Fig. 1). Incidence of powdery mildew was noticed 55 DAS in polyhouse. Before treatment application there was no significant difference in disease severity among the treatments and the severity ranged from 5.00 per cent to 7.67 per cent. After first spraying, an increase in

Plate 11. Protected structures for field experiments



Polyhouse

Rain shelter

disease severity was observed in all the treatments. However all treatments were superior in controlling the disease compared to control. After second spraying, fungicidal treatments showed a reduction in disease severity compared to that after first spraying. Lowest disease severity was observed in T1 (difenoconazole 0.05%) at all stages of observation and reduction over control was 91.10 per cent after both the sprays. This was followed by T2 (tebuconazole 0.1%) which showed 84.25 per cent reduction in disease severity over control after two sprays. At all stages of observation, the efficacy of these two systemic fungicides were on par with each other.

In case of biocontrol agents, after first spray, T7 (*Pseudomonas fluorescens* 2%) showed lowest per cent disease severity (15.67%) compared to T5 (*Trichoderma viride* 2%) and T6 (*Trichoderma viride* 0.5% liquid formulation). However after second spray, the best disease control among biocontrol agents was given by T5 *Trichoderma viride* 2% with 41.10 per cent reduction in disease severity compared to control. The treatment T5 was followed by T6 (*Trichoderma viride* 0.5% liquid formulation) and T7 (*Pseudomonas fluorescens* 2%) where the reduction was 39.04 per cent and 36.99 per cent respectively. The botanical neem oil 2 % (T8) also gave significant reduction (35.62%) in disease severity compared to control. From the disease severity data after two sprays, it was found that all the three biocontrol agents and neem oil were on par in their efficacy in controlling powdery mildew of yard long bean.

Effect of treatments was reflected on yield of yard long bean also. All the treatments recorded higher yield compared to control. Highest yield of 4.98 kg was recorded in T1. This was followed by T5, T2and T7 with 4.66 kg, 4.46 kg and 4.40 kg. All these treatments were on par. Lowest yield (3.03 kg $plot^{-1}$) was recorded in control.

Table 9 . Effect of different treatments on powdery mildew of yard long bean under polyhouse

Treatment	Per cent disease severity*					Mean yield (kg plot^{-1})**	Per cent increase over control
	Before spray	After first spray	Per cent reduction over control	After second spray	Per cent reduction over control		
T1- Difenoconazole -0.05%	5.00	7.33^{a}	78.64	4.33^{a}	91.10	4.98^{a}	39.15
T2- Tebuconazole- 0.1%	6.00	9.67^{a}	71.84	7.67^{ab}	84.25	4.46^{ab}	32.06
T3- Wettable sulphur 0.1%	6.68	16.33^{bc}	52.43	16.67^{c}	65.75	4.10^{bc}	26.09
T4- Wettable sulphur -0.2%	6.33	15.67^{b}	54.37	14.00^{bc}	71.23	4.21^{bc}	28.02
T5- *Trichoderma viride* -2%	6.00	19.67^{c}	42.72	28.67^{d}	41.10	4.66^{ab}	34.98
T6- *Trichoderma viride* LF-0.5%	7.67	18.33^{bc}	46.60	29.67^{d}	39.04	4.06^{d}	25.36
T7 - *Pseudomonas fluorescens* - 2%	7.67	15.67^{b}	54.37	30.67^{d}	36.99	4.40^{abc}	31.14
T8- Neem oil-2%	7.00	23.67^{d}	31.07	31.33^{d}	35.62	4.09^{cd}	25.92
T9- control	6.33	34.33^{e}	-	48.67^{e}	-	3.03^{e}	-
CV	15.30	12.31		18.13		4.51	
CD	NS	3.8		7.38		0.58	

*Mean of three replications, Values followed by same superscript are not significantly different by DMRT (P=0.05) ** Plot size 3m^2
LF- Liquid Formulation

Table 10. Effect of different treatments on powdery mildew of yard long bean under rain shelter

Treatment	Per cent disease severity*					Mean yield (kg plot^{-1})**	Per cent increase over control
	Before spray	After first spray	Per cent reduction over control	After second spray	Per cent reduction over control		
T1- Difenoconazole -0.05%	5.33	10.33^{a}	78.17	7.67^{a}	85.98	4.54^{a}	31.93
T2- Tebuconazole- 0.1%	5.00	14.33^{ab}	69.72	10.67^{a}	80.49	4.42^{ab}	30.10
T3- Wettable sulphur 0.1%	6.33	18.67^{c}	60.56	22.67^{b}	58.54	3.67^{cd}	15.80
T4- Wettable sulphur -0.2%	6.00	17.33^{bc}	63.38	19.67^{b}	64.02	3.87^{bc}	20.15
T5- *Trichoderma viride* -2%	6.00	26.00^{d}	45.07	38.67^{c}	29.27	4.21^{ab}	26.60
T6- *Trichoderma viride* LF-0.5%	6.00	29.33^{de}	38.03	38.67^{c}	29.27	3.78^{cd}	18.25
T7 - *Pseudomonas fluorescens* - 2%	6.33	30.67^{e}	35.21	36.33^{c}	33.54	4.34^{ab}	18.20
T8- Neem oil-2%	5.33	32.67^{e}	30.99	40.33^{c}	26.22	3.72^{cd}	16.93
T9- control	6.66	47.33^{f}	-	54.67^{d}	-	3.09^{d}	-
CV	11.50	9.48		10.09		4.56	
CD	NS	4.13		5.23		0.34	

*Mean of three replications, Values followed by same superscript are not significantly different by DMRT (P=0.05) ** Plot size 3m^2
LF- Liquid Formulation

4.4.2 Effect of Different Treatments on Powdery Mildew of Yard Long Bean Under Rain Shelter

The results of field experiment in rain shelter are depicted in Table 10 and Fig.2. The first incidence of powdery mildew was noticed 53 days after sowing in rain shelter. As in case of polyhouse there was no significant difference among the treatments initially and disease severity ranged from 5.00 to 6.66 per cent. After first treatment application all the treatments recorded an increase in disease severity, but showed significant reduction when compared to control. However, after second spraying T1 (difenoconazole 0.05%) and T2 (tebuconazole 0.1%) showed reduced disease severity compared to first spraying. Other treatments showed gradual increase in disease severity. All treatments at all stages of observation were found to be superior over the control in reducing the rate of increase of the disease. After both the sprays, T1 (difenoconazole 0.05%) recorded the lowest PDS (7.67) and this treatment gave disease reduction of 85.98 per cent over control. It was followed by T2 (tebuconazole 0.1%) which gave 80.49 per cent disease reduction over control. These two treatments were statistically on par. The treatments T4 (wettable sulphur 0.2%) and T3 (wettable sulphur 0.1%) also showed significant reduction in disease severity with a reduction of 64.02 per cent and 58.54 per cent respectively and were on par.

Among the biocontrol agents, T7 (*Pseudomonas fluorescens* 2%) gave the best reduction of disease severity with 33.54 per cent reduction over control. This was followed by T5 (*Trichoderma viride* 2%) and T6 (*Trichoderma viride* liquid formulation 0.5%), which recorded the same per cent disease severity after two sprays with a reduction of 29.27 per cent over control. The treatment T8 (neem oil 0.2%) also succeeded in reducing the disease severity and recorded 26.22 per cent reduction over control. However, all the bio control agents and neem oil were on par in their efficacy to control powdery mildew of yard long bean in rain shelter.

Fig 1. Effect of treatments on per cent disease severity of powdery mildew of yard long bean under polyhouse

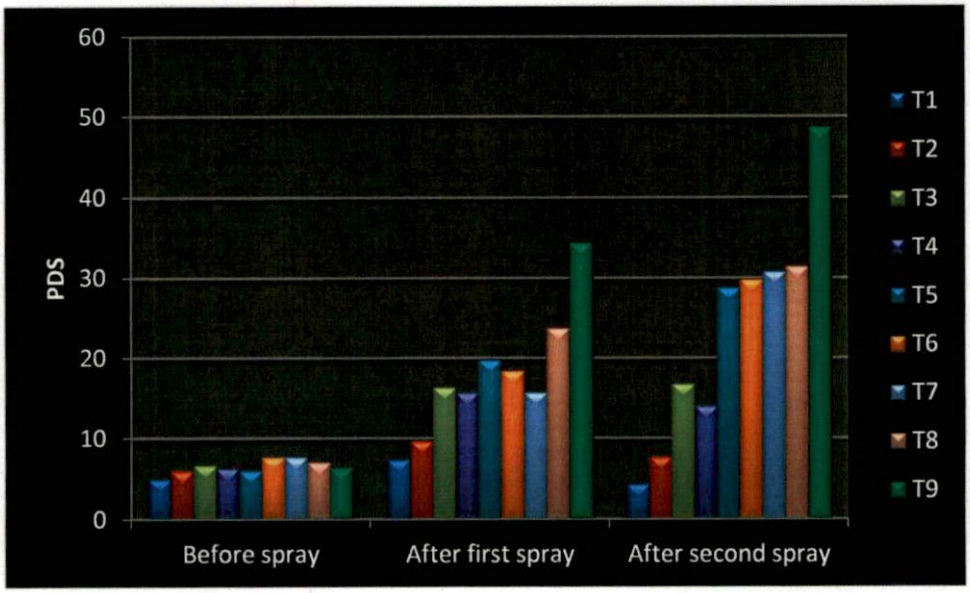
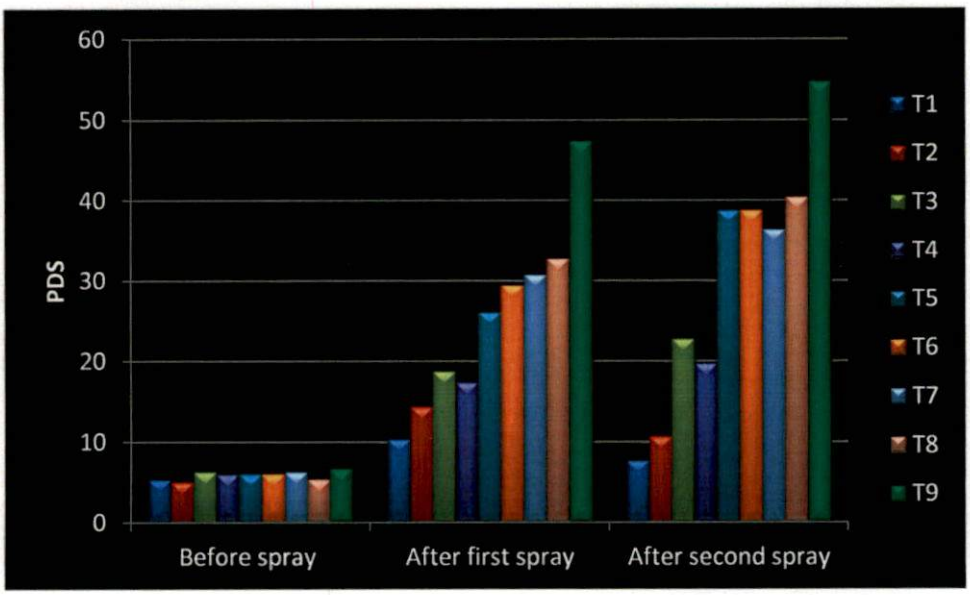


Fig 2. Effect of treatments on per cent disease severity of powdery mildew of yard long bean under rain shelter



T1- Difenoconazole 0.05%

T2- Tebuconazole 0.1%

T3-Wettable sulphur- 0.1%

T4- Wettable sulphur- 0.2%

T5- *Trichoderma viride* 2%

T6- *Trichoderma viride* (Liquid formulation)-0.5%

T7- *Pseudomonas fluorescens* 2%

T8- Neem oil 2%

T9- Control

In rain shelter, all the treatments recorded lower yield compared to polyhouse. Highest yield of 4.54 kg/plot was obtained in T1 (difenoconazole 0.05%) and an increase of 31.93 per cent was obtained over control. The treatments T2 (tebuconazole 0.1%), T7 (*P. fluorescens* 2%) and T5 (*T. viride* 2%) recorded 4.42 kg, 4.34 kg and 4.21 kg respectively and were on par. The lowest yield of 3.09 kg was recorded in control.

4.4.3 Effect of Treatments on the Biometric Characters

Biometric characters of the crop like days to flowering, days to harvest, vine length, fruit weight, fruit length, shelf life and marketable yield were recorded in both polyhouse and rain shelter during the field experiments.

Table 11. Effect of treatments on biometric characters of yard long bean in polyhouse

Treatment	Biometric characters*					
	Days to flowering	Days to harvest	Vine length (m)	Pod weight (g)	Pod length (cm)	Shelf life (days)
T1- Difenoconazole -0.05%	36.33	47.67	5.10	25.17	63.33^{a}	3.67
T2- Tebuconazole- 0.1%	36.67	47.67	5.00	24.73	61.00^{ab}	3.67
T3- Wettable sulphur 0.1%	37.67	48.33	5.18	24.41	57.67^{abc}	4.00
T4- Wettable sulphur -0.2%	38.67	49.67	5.18	25.03	59.67^{abc}	3.67
T5- *Trichoderma viride* -2%	35.00	47.00	5.10	24.9	55.33^{bcd}	3.67
T6- *Trichoderma viride* LF-0.5%	35.00	46.67	5.30	24.18	51.67^{cde}	3.67
T7-*Pseudomonas fluorescens* - 2%	36.33	46.67	5.28	24.93	59.00^{cde}	3.33
T8- Neem oil-2%	37.33	48.33	5.19	25.02	55.33^{de}	3.67
T9- control	38.33	49.00	5.14	23.94	55.33^{e}	3.67
CV	5.85	3.41	2.70	3.352	4.48	18.18
CD	NS	NS	NS	NS	4.45	NS

*Mean of three replications, LF-Liquid Formulation, Values followed by same superscript are not significantly different by DMRT (P=0.05)

In polyhouse experiment (Table 11), there were no significant difference among the treatments in any of the biometric characters except pod length (Plate 12). In polyhouse, flowering commenced from 35 days of sowing and the plants in all treatments flowered within 39 days. However, a slight earliness in flowering and harvest was observed in case of *Trichoderma* treatments T5 and T6. The time from flowering to harvest was about 10 to 11 days in all the treatments. Vine length of yard long bean in polyhouse ranged from 5 to 5.3 m and was slightly high in treatments with biocontrol agents. Pod weight recorded in different treatments ranged from 23.94 to 25.17g. Pod length showed a variation from 51.67 to 63.33 cm and was highest in case of T1. At room temperature, irrespective of treatments, yard long bean pods remained fresh for 3 to 4 days after which yellowing and drying was observed. Maximum shelf life of four days was observed in T3 (wettable sulphur 0.1%).

The observations on biometric characters of yard long bean under rain shelter were depicted in Table 12. No significant difference was observed among the treatments in any of the biometric characters. Flowering in rain shelter commenced five days later (40DAS) compared to poly house. The plants in all treatments flowered within 40 to 43 days. Pods were mature for harvest after 10 to 11 days of flowering in all the treatments. Vine length of yard long bean ranged between 4.86 m to 5.02 m in rain shelter. Weight of pods in different treatments varied from 24.78 to 26.83 grams. Pod length in different treatments was in the range of 52.67 to 59cm in rain shelter. However the difference was insignificant unlike that of polyhouse. The shelf life of pods in rain shelter also varied from 3 to 4 days (Plate 13).

Table 12. Effect of different treatments on biometric characters of yard long bean in rain shelter

Treatment	Biometric characters*					
	Days to flowering	Days to harvest	Vine length (m)	Pod weight (g)	Pod length (cm)	Shelf life (days)
T1- Difenoconazole -0.05%	40.33	51.33	4.96	26.83	59	3.33
T2- Tebuconazole- 0.1%	41.00	51.33	5.01	25.90	57.33	3.67
T3- Wettable sulphur 0.1%	41.00	51.67	4.86	25.89	57.00	3.33
T4- Wettable sulphur -0.2%	42.33	52.33	4.91	25.95	58.00	4.00
T5- *T. viride* -2%	40.67	51.33	4.89	25.40	56.33	3.67
T6- *T. viride* LF-0.5%	42.00	52.33	5.02	26.43	56.67	3.33
T7-*P. fluorescens* - 2%	40.00	51.33	4.86	26.47	58.00	3.67
T8- Neem oil-2%	41.67	52.00	4.88	24.78	52.67	3.67
T9- control	41.33	52.00	4.98	25.29	54.33	3.67
CV	4.46	3.51	2.24	3.77	4.64	20.13
CD	NS	NS	NS	NS	NS	NS

*Mean of three replications, LF-Liquid Formulation, Values followed by same superscript are not significantly different by DMRT (P=0.05)

4.4.4 Assessment of Crop Loss

Yield from the plot having maximum yield T1 (difenoconazole 0.05%) and minimum yield T9 (control) were recorded and per cent yield loss in polyhouse and rain shelter was worked out and presented in Table 13.

Table 13 . Per cent yield loss in yard long bean due to powdery mildew disease

Protected structure	Yield (Kg plot $^{-1}$)*		
	T1(difenoconazole 0.05%)	T9 (control)	Per cent yield loss
Polyhouse	4.98	3.03	39.15
Rain shelter	4.54	3.09	31.93

*plot size-3m^2

Plate 12. Different stages of crop in polyhouse

Plate 13. Different stages of crop in rain shelter

In polyhouse, the yield loss was found to be 39.15 per cent, whereas in rain shelter, it was 31.93 per cent.

4.4.5 Economic Analysis

Benefit: cost ratio was calculated at market price of Rs.40 per Kg for all the treatments and also with 20 per cent premium price for organic treatments.

Table 14. Economic analysis of treatments in polyhouse

Treatment	Benefit : Cost ratio					
	Total cost (Rs.)	Yield (kg)	@ Rs.40 kg^{-1}		@ 20 % premium price for organic produce	
			Total returns	B : C ratio	Total returns	B : C ratio
T1- Difenoconazole - 0.05%	408	14.94	597	1.46	597	1.46
T2- Tebuconazole- 0.1%	429	13.38	535	1.24	535	1.24
T3- Wettable sulphur 0.1%	355	12.30	492	1.38	492	1.38
T4- Wettable sulphur -0.2%	363	12.65	506	1.39	506	1.39
T5- *Trichoderma viride* -2%	408	13.98	559	1.37	671	1.65
T6- *Trichoderma viride* LF-0.5%	380	12.18	487	1.28	585	1.53
T7-*Pseudomonas fluorescens* - 2%	391	13.20	528	1.35	634	1.63
T8- Neem oil-2%	434	12.27	491	1.13	589	1.14
T9- control	350	9.09	364	1.04	437	1.24

LF- Liquid formulation

Benefit cost ratio for different treatments in polyhouse was furnished in Table 14. At the market price of Rs.40 kg^{-1}, B:C ratio was found to be highest (1.46:1) for the treatment T1 (difenoconazole 0.05%). However, if it is possible to obtain 20 per cent premium price for organic produce, T5 (*T. viride* 2%) will be the most remunerative treatment with B:C ratio of 1.65:1.

Table 15. Economic analysis of treatments in rain shelter

Treatment	Benefit : Cost ratio					
	Total cost (Rs.)	Yield (kg)	@ Rs.40 kg^{-1}		@ 20 % premium price for organic produce	
			Total returns	B : C ratio	Total returns	B : C ratio
T1- Difenoconazole - 0.05%	408	13.62	545	1.34	545	1.34
T2- Tebuconazole- 0.1%	429	13.26	530	1.23	530	1.23
T3- Wettable sulphur 0.1%	355	11.01	440	1.24	440	1.24
T4- Wettable sulphur -0.2%	363	11.61	464	1.27	464	1.27
T5- *Trichoderma viride* -2%	408	12.60	504	1.23	605	1.48
T6- *Trichoderma viride* LF-0.5%	380	11.34	454	1.19	545	1.43
T7-*Pseudomonas fluorescens* - 2%	391	13.02	521	1.33	625	1.59
T8- Neem oil-2%	434	11.16	446	1.02	535	1.23
T9- control	350	9.27	370	1.05	445	1.27

LF- Liquid Formulation

Benefit : cost ratio of treatments in rain shelter (Table 15) shows that the treatment T1 (difenoconazole 0.05%) recorded the highest B:C ratio of 1.34 at market price of Rs.40 kg^{-1}. However, when calculated with 20 per cent premium price for organic produce, the most remunerative treatment in rain shelter was T7 (*Pseudomonas fluorescens* 2%) with B:C ratio 1.59.

4.4.6 Incidence of Other Diseases and Pests

Incidence of other diseases and pests on the crop were recorded during the experiment. Minor incidence of mosaic disease was noticed in polyhouse and rain shelter. *Cercospora* leaf spot incidence was observed in polyhouse. Major infestation of red spider mites (*Tetranichus* sp.) was noticed in polyhouse.

Infestation of serpentine leaf miner (*Liriomyza trifolii*) was noticed from 17 and 27 days after sowing in rain shelter and polyhouse respectively. Aphid (*Aphis craccivora*) infestation was noticed in rain shelter and poly house and was severe during pod formation stage. Pod bug (*Riptortus pedestris*) infestation was noticed in rain shelter during harvesting period. Thrips (*Thrips tabaci*) was also found in polyhouse at late stage of the crop (Plate 14).

4.4.6.1 Population of mites

Since mite infestation was noticed in all the treatments in polyhouse, the population of mites was recorded using window count method before and after treatment application to know the effect of treatments (Table 16).

Table 16. Population of mites in polyhouse

Treatment	Number of mites per cm^2 of leaf	
	Before treatment application	After treatment application
T1- Difenoconazole -0.05%	9.50	16.00^{bc}
T2- Tebuconazole- 0.1%	10.33	15.67^{bc}
T3- wettable sulphur 0.1%	8.50	13.67^{ab}
T4- Wettable sulphur -0.2%	8.33	12.00^{a}
T5- *Trichoderma viride* -2%	9.17	16.33^{bc}
T6- *Trichoderma viride* LF-0.5%	8.67	17.00^{c}
T7 - *Pseudomonas fluorescens* - 2%	9.67	15.67^{bc}
T8- Neem oil-2%	9.17	12.33^{a}
T9- control	10.50	16.00^{bc}
CD(0.05)	NS	3.07

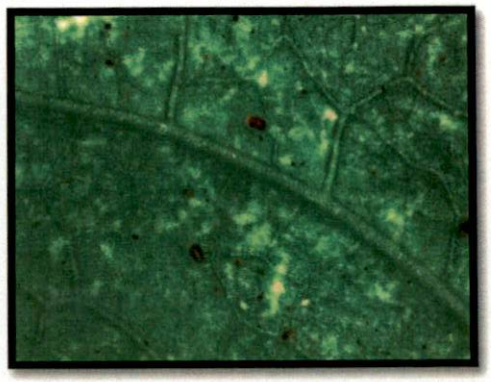
First incidence of mite infestation was observed 50 days after sowing in polyhouse. The population of mites ranged in between 8.33to 10.50 per cm^2 of leaf in various treatments before treatment application. An increase in mite population

Plate 14. Incidence of pests and diseases during field experiments

Pod bug - *Riptortus pedestris*

Serpentine leaf miner- *Liriomyza trifoli*

Aphids- *Aphis craccivora*



Red spider mite- *Tetranichus* sp.

Plate 14 b. Incidence of diseases other than powdery mildew

Leaf spot – *Cercospora* sp.

Cowpea mosaic virus

was recorded in all the treatments after spraying. However, comparatively low population was observed in T4 (wettable sulphur 0.2%) and T8 (neem oil 2%).

4.5 METEOROLOGICAL PARAMETERS

Temperature and relative humidity inside the polyhouse and rain shelter was recorded daily at 7.30 am and 2.30 pm during the experiment (Table 17). Temperature inside the polyhouse was found to be higher compared to that in rain shelter. Inside the polyhouse, the temperature at 7.30 am varied from 22.25°C to 29°C and that at 2.30 pm was in the range of 32.36°C to 37.24°C. The relative humidity in poly house ranged from 56.75 per cent to 71.36 per cent at 7.30 am and at 2.30 pm the relative humidity range was in between 36 per cent to 93 per cent.

However, in rain shelter, the temperature at 7.30 am ranged between 23 to 28.6°C and that at 2.30 pm was in the range of 31.4°C to 36.86°C. The relative humidity at 7.30 am ranged from 36 per cent to 93 per cent. At 2.30 pm, The temperature in rain shelter was in between 31.4 to 36.86°C. The relative humidity at this time varied from 39 to 87.9 per cent. In both polyhouse and rain shelter temperature at 7.30 am was lower during December–January months.

Table 17 . Meteorological data during the period of experiment in poly house and rain shelter

Std. week *	Polyhouse				Rain shelter			
	7.30 am		2.30pm		7.30 am		2.30pm	
	Temp (°C)	RH(%)	Temp (°C)	RH(%)	Temp (°C)	RH(%)	Temp (°C)	RH(%)
43	29.00	65.00	36.00	91.00	28.60	72.00	32.50	85.00
44	28.52	69.00	36.40	93.00	27.54	76.30	33.00	86.00
45	26.20	68.00	34.00	83.00	26.10	77.00	34.70	87.90
46	24.80	73.00	35.00	85.00	24.70	81.00	34.60	82.00
47	24.32	75.00	37.00	79.36	24.12	86.40	36.80	77.00
48	24.10	68.24	36.34	71.54	23.90	78.40	33.20	76.00
49	23.72	66.23	36.90	69.42	23.50	74.00	34.00	63.00
50	23.68	66.15	35.20	55.36	23.46	71.20	32.70	52.00
51	23.24	65.23	34.72	52.95	23.12	67.50	31.80	49.00
52	23.20	68.74	32.36	49.85	23.00	63.40	31.60	48.00
1	23.08	64.32	32.54	46.32	23.09	68.90	31.40	46.80
2	22.86	57.63	33.41	44.00	23.45	61.30	32.60	46.00
3	22.25	63.45	34.38	39.20	23.18	68.40	32.40	39.00
4	25.42	60.51	35.69	36.00	24.89	64.30	33.80	44.00
5	25.34	56.75	34.98	44.00	24.74	61.45	32.00	51.00

6	25.41	61.23	36.39	45.6	25.23	63.75	34.00	53.00
7	26.34	66.00	36.78	51.20	25.90	68.68	36.12	54.00
8	26.84	68.43	37.00	53.00	26.31	69.78	36.86	57.00
9	26.98	70.23	37.21	52.80	26.42	71.45	35.41	61.00
10	28.49	71.36	37.24	51.67	27.34	75.60	35.78	59.40

*average of seven days

Table 18. Correlation analysis of disease severity with major meteorological parameters

Meteorological parameters	Correlation coefficients	
	Polyhouse	Rain shelter
Temperature	-0.879*	-0.933*
Relative humidity	-0.896*	-0.704*

*Correlation significant at 0.05 per cent level

Correlation analysis was performed between the per cent disease severity and the meteorological data collected during the field experiment (Table 18). It was found that the powdery mildew severity exhibited significant negative correlation with temperature and relative humidity in polyhouse and rain shelter (Fig. 3,4).

4.6 ENUMERATION OF PHYLLOPLANE MICROFLORA UNDER PROTECTED CONDITION

Phylloplane microflora (fungi and bacteria) of the crop was enumerated before and after treatment application using serial dilution plating method in both polyhouse and rain shelter (Plate 15). Actinomycetes could not be isolated from the phylloplane of any of the treatments.

4.6.1 Population of Phylloplane Fungi in Polyhouse

Changes in fungal population due to foliar application of treatments were furnished in Table 19. First foliar application was given 55 DAS subsequent to the incidence of powdery mildew. Before spraying, more or less uniform population of phylloplane fungi was observed inside the polyhouse which ranged from 39.33 to 42.67 cfu cm^{-2}. However, immediately after treatment application, drastic changes were observed in population of phylloplane fungal flora receiving different treatments. It is evident from the results that population in chemical treatments was reduced drastically and highest reduction was caused by T1 (difenoconazole 0.05%) from 42 to 2.67 cfu cm^{-2}. This was closely followed by T2 (tebuconazole 0.1%) in

Fig 3. Influence of meteorological parameters on disease severity in polyhouse

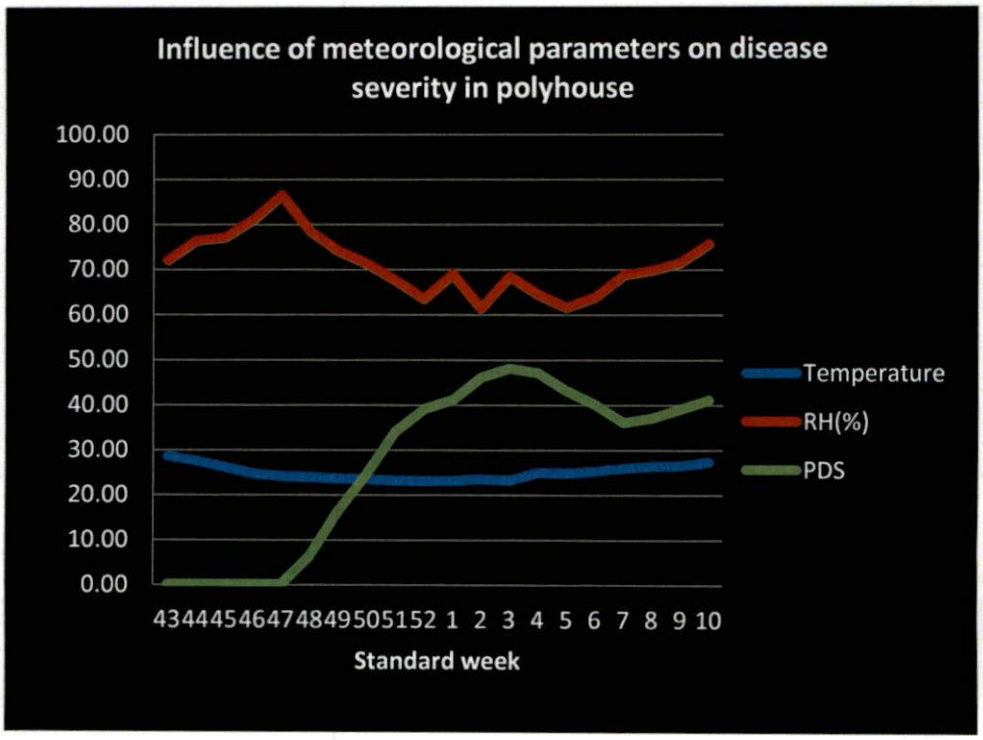


Fig 4. Influence of meteorological parameters on disease severity in rain shelter

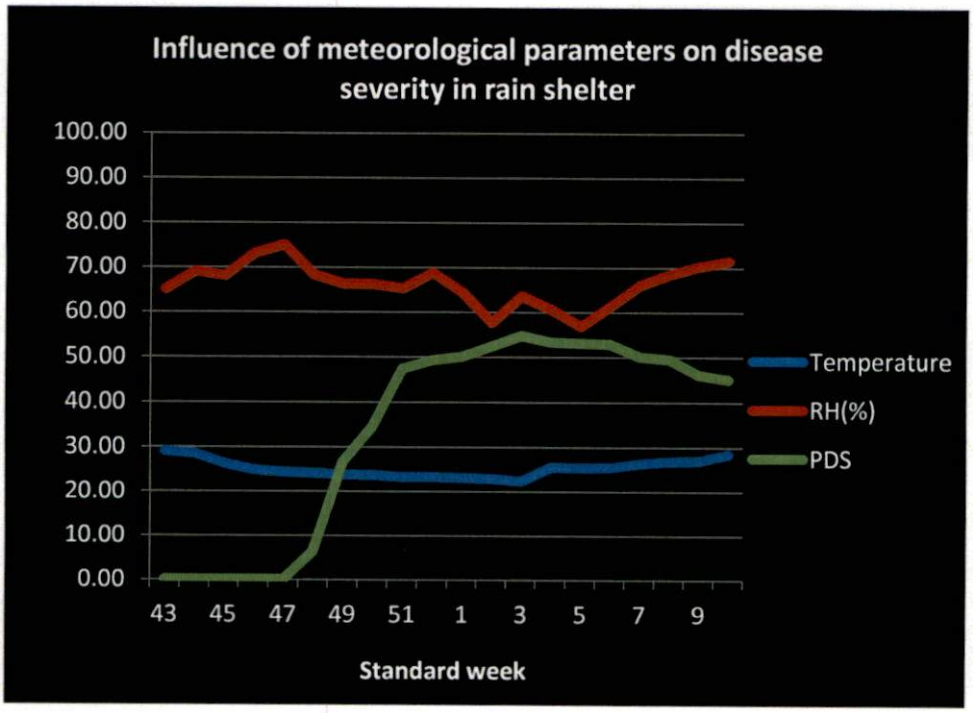
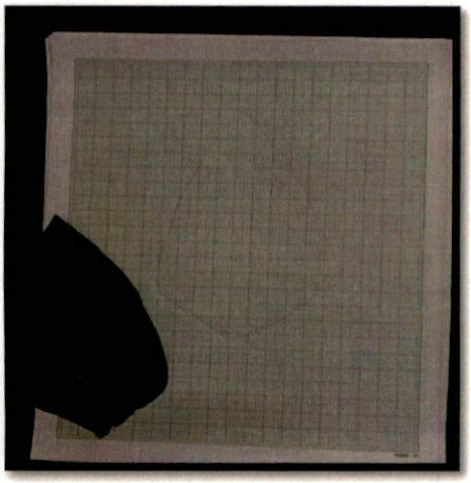


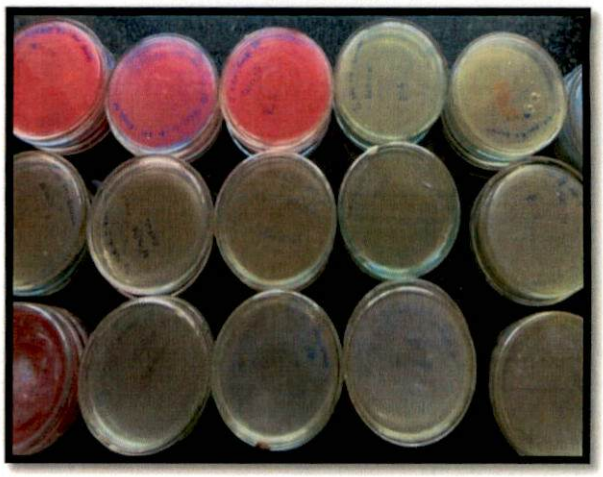
Plate 15. Enumeration of phylloplane microflora



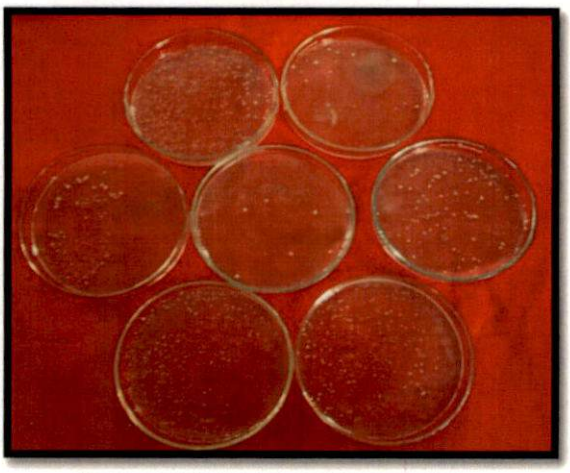
A. Assessment of leaf area

B. Extraction of phylloplane microflora in sterile water

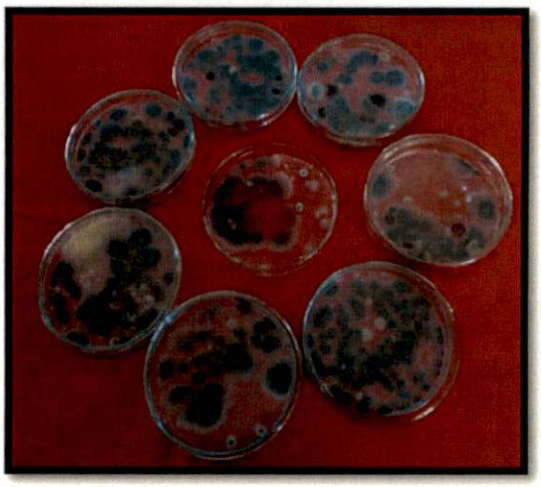
C. Media used
Bacteria- Nutrient Agar
Fungi- Marin's Rose Bengal Agar
Actinomycetes- Ken Knights Agar



D. Incubation of plates



E. Phylloplane bacteria



F. Phylloplane fungi

which, the reduction was from 40.33 to 5.67cfu cm^{-2}. The treatments of wettable sulphur (T3 and T4) also reduced the fungal population on the phylloplane. However, the population slightly increased to 31 and 27 cfu cm^{-2} in T1 and T2 respectively after seven days of spraying. The revival of fungal population was more in case of T3 (34 cfu cm^{-2}) and T4 (32 cfu cm^{-2}). Though *P.fluorescens* and neem oil also caused reduction in fungal population, the reduction was not sharp as fungicides and after seven days, the population revival was also more.

Application of *T. viride* on the foliage (T5 and T6) caused a radical increase in fungal population from 40.33 to 69.67 cfu cm^{-2} in T5 and from 41 to 54 cfu cm^{-2} in T6. However after seven days, the population got reduced to 57.33 and 49.33 respectively in T5 and T6 (Fig. 5).

Table 19 . Effect of different treatments on phylloplane fungi in polyhouse

Treatments	Fungi (cfu cm^{-2})*		
	Before spray	1DAS	7 DAS
T1- Difenoconazole -0.05%	42.00	2.67^{f}	31.00^{e}
T2- Tebuconazole- 0.1%	40.33	5.67^{ef}	27.00^{f}
T3- wettable sulphur 0.1%	41.00	10.00^{e}	34.00^{de}
T4- Wettable sulphur -0.2%	41.00	9.33^{e}	32.00^{e}
T5- *Trichoderma viride* -2%	40.33	69.67^{a}	57.33^{a}
T6- *Trichoderma viride* LF-0.5%	41.00	54.00^{b}	49.33^{b}
T7 - *Pseudomonas fluorescens* - 2%	42.67	36.00^{c}	41.67^{c}
T8- Neem oil-2%	42.67	19.00^{d}	36.33^{d}
T9- control	39.33	38.33^{c}	43.00^{c}
CV	6.27	10.1	5.52
CD	NS	4.75	3.73

*Mean of three replications; DAS-Days after spraying; LF –Liquid formulation

4.6.2 Population of Phylloplane Fungi in Rain Shelter

The trend in variation of phylloplane fungal flora in rain shelter was same as that of poly house (Table 20). However, the initial population was slightly higher in rain shelter compared to polyhouse and varied from 45 to 49.33 cfu cm^{-2}. First foliar application was given at 53 days after sowing with the onset of disease. Immediately after spraying, there occurred a momentous reduction of fungal population in T2 (tebuconazole – 6.33 cfu cm^{-2}) and T1 (difenoconazole -6.67cfu cm^{-2}). However, population slightly increased to 28.33 and 28.67 cfu cm^{-2} respectively after seven days of spraying. In treatments T3 and T4, where wettable sulphur was sprayed on leaves, immediately after treatment application, the population got reduced to 12.33 and 11 cfu cm^{-2} respectively. After seven days of spraying the population in T3 and T4 gradually increased to 35.33 and 31.67 cfu cm^{-2} respectively.

Table 20 . Effect of different treatments on phylloplane fungi in rain shelter

Treatments	Fungi (cfu cm^{-2})*		
	Before spray	1 DAS	7 DAS
T1- Difenoconazole -0.05%	46.33	6.67^{g}	28.67^{e}
T2- Tebuconazole- 0.1%	45.00	6.33^{g}	28.33^{e}
T3- Wettable sulphur -0.1%	45.33	12.33^{f}	35.33^{cd}
T4- Wettable sulphur -0.2%	47.66	11.00^{f}	31.67^{de}
T5- *Trichoderma viride* -2%	49.33	75.67^{a}	56.67^{a}
T6- Trichoderma viride LF-0.5%	47.00	69.00^{b}	58.33^{a}
T7 - *Pseudomonas fluorescens* - 2%	46.00	35.67^{d}	46.33^{b}
T8-Neem oil-2%	46.33	23.67^{e}	37.33^{c}
T9 control	46.00	47.00^{c}	48.00^{b}
CV	3.69	7.55	5.18
CD	NS	4.17	3.69

*Mean of three replications; DAS-Days after spraying; LF –Liquid formulation

Fig. 5. Effect of treatments on phylloplane fungi in polyhouse

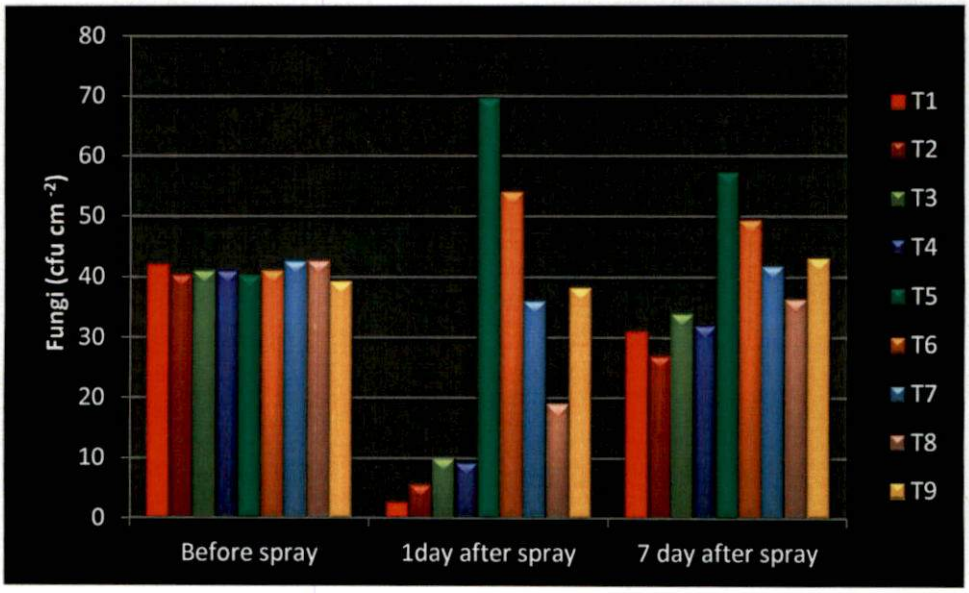
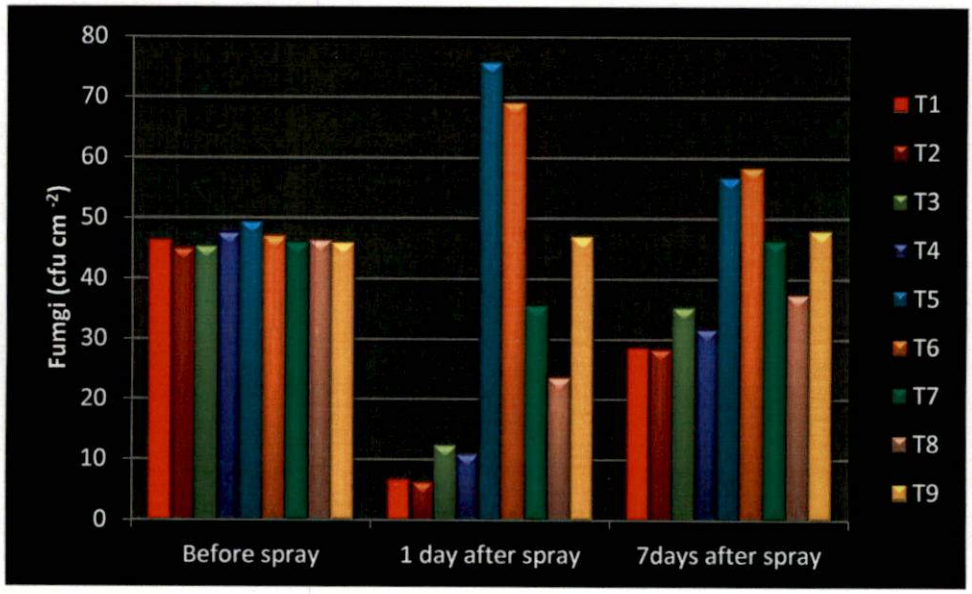


Fig. 6. Effect of treatments on phylloplane fungi in rain shelter



T1- Difenoconazole 0.05%

T2- Tebuconazole 0.1%

T3-Wettable sulphur- 0.1%

T4- Wettable sulphur- 0.2%

T5- *Trichoderma viride* 2%

T6- *Trichoderma viride* (Liquid formulation)-0.5%

T7- *Pseudomonas fluorescens* 2%

T8- Neem oil 2%

T9- Control

The foliar application of *Trichoderma* significantly increased the fungal population to 75.67 cfu cm^{-2} in T5 and 69 cfu cm^{-2} in T6. However, the population got reduced to 56.67 and 58.33 cfu cm^{-2} after seven days. Though spraying *Pseudomonas* on the foliage caused a slight reduction in fungal population, rapid revival of population to initial level was evidenced after seven days of spraying.

The botanical treatment neem oil (T7) also reduced the fungal population on the phylloplane to 23.67 cfu cm^{-2}, but the reduction was not severe as caused by chemical treatments. Moreover, the population increased to 37.33 cfu cm^{-2} after seven days.Throughout the experiment, the population of fungi in control do not varied much (Fig. 6).

4.6.3 Population of Phylloplane Bacteria in Polyhouse

The change in phylloplane bacterial population in polyhouse due to foliar application of different treatments are depicted in Table 21. On the phylloplane, the population of bacteria was less compared to that of fungi. Before spraying, the bacterial population on the crop inside the polyhouse was more or less uniform and ranged from 18 to 20.67 cfu cm^{-2}. However, a steep reduction was observed in the population of bacterial flora in all the chemical treatments. Highest reduction (3.33 cfu cm^{-2}) was recorded in T2 (tebuconazole) followed by T1 (difenoconazole) (3.67 cfu cm^{-2}). On statistical analysis, it was found that these treatments were on par. A gradual increase in population to 9.00 and 12.67 cfu cm^{-2} was observed after seven days of spraying in these treatments *viz.*, T2 and T1 respectively. The foliar application of wettable sulphur also reduced the bacterial population to 6.67 in T3 and 4.33 in T4. After seven days of spraying the population gradually got built up to 12.33 and 11.67 cfu cm^{-2} in T3 and T4 respectively. Though the chemicals were composed mainly of fungicides, the reduction in bacterial population was evident and this clearly indicates the toxicity and non-specificity of fungicides.

Foliar application of bacterial biocontrol agent *Pseudomonas fluorescens* (T7) caused a sudden increase in bacterial population on the phylloplane. The population surged from 18.33 to 45 cfu cm^{-2} immediately after spraying. However,

the population got reduced to 33.33 cfu cm^{-2} after seven days of spraying. Though treatments T5 and T6 where *T. viride* was sprayed on the foliage also caused increase in bacterial population, it was only nominal and after seven days, the population reached the initial level. Neem oil (T8) reduced the population of bacteria from 18 to 10.33 cfu cm^{-2}, but the reduction was less compared to chemical treatments and the population increased to initial level by seven days of spraying (Fig.7).

Table 21 . Effect of different treatments on phylloplane bacteria in polyhouse

Treatments	Bacteria (cfu cm^{-2})*		
	Before spraying	1 DAS	7 DAS
T1- Difenoconazole -0.05%	18.67	3.67^{e}	12.67^{d}
T2- Tebuconazole- 0.1%	20.00	3.33^{e}	9.00^{e}
T3- Wettable sulphur 0.1%	20.67	6.67^{d}	12.33^{d}
T4- Wettable sulphur -0.2%	20.00	4.33^{de}	11.67^{d}
T5- *Trichoderma viride* -2%	19.00	19.67^{b}	20.33^{c}
T6- *Trichoderma viride* LF-0.5%	19.00	21.00^{b}	19.33^{c}
T7 - *Pseudomonas fluorescens* - 2%	18.33	45.00^{a}	33.33^{a}
T8- Neem oil-2%	18.00	10.33^{c}	19.33^{c}
T9- control	19.67	21.33^{b}	26.33^{b}
CV	7.9	9.23	7.65
CD	NS	2.40	2.42

*Mean of three replications; DAS-Days after spraying; LF –Liquid formulation

4.6.4 Population of Phylloplane Bacteria in Rain Shelter

Table 22 depicts the variation in phylloplane bacterial population brought about by the foliar application of different treatments in rain shelter. As in polyhouse, the population before spraying do not varied significantly. Compared to polyhouse, population of phylloplane bacteria was slightly higher in rain shelter and was in the range of 22.33 to 24.44 cfu cm^{-2}. The leaf surfaces on which chemical

treatments were applied was found to harbour less number of bacteria. The reduction was more prominent in case of systemic fungicides, T1 (difenoconazole) and T2 (tebuconazole) in which bacterial population was only 4 and 5.67 cfu cm^{-2} respectively after one day of spraying. Though the population increased to 19.33 and 18.67 cfu cm^{-2} respectively after seven days of spraying, the population was lower than initial level.

Table 22. Effect of different treatments on phylloplane bacteria in rain shelter

Treatment	Bacteria (cfu cm^{-2})*		
	Before spraying	1 DAS	7 DAS
T1- Difenoconazole -0.05%	24.33	4.00^{d}	19.33^{e}
T2- Tebuconazole- 0.1%	23.67	5.67^{d}	18.67^{e}
T3- Wettable sulphur 0.1%	25.00	7.00^{cd}	23.33^{bcd}
T4- Wettable sulphur -0.2%	23.00	4.67^{d}	20.33^{de}
T5- *Trichoderma viride* -2%	24.33	26.00^{b}	20.00^{e}
T6- *Trichoderma viride* LF-0.5%	23.33	26.00^{b}	20.67^{cde}
T7 - *Pseudomonas fluorescens* - 2%	23.33	53.33^{a}	43.00^{a}
T8- Neem oil-2%	23.67	10.67^{c}	23.67^{bc}
T9- control	22.33	23.67^{b}	25.33^{b}
CV	10.80	14.55	7.77
CD	NS	4.51	3.20

*Mean of three replications; DAS-Days after spraying; LF –Liquid formulation

Enormous increase in bacterial population from 23.33 to 53.33 cfu cm^{-2} was recorded in T7 where *Pseudomonas fluorescens* was sprayed on leaves. However, this increase was not stable and after seven days, the population got reduced to 43cfu cm^{-2}. In T5 and T6 where *Trichoderma* was sprayed on leaves, slight increase in bacterial population was observed. However, the population got reduced below the initial level after seven days. In case of neem oil, bacterial population was

Fig. 7. Effect of treatments on phylloplane bacteria in polyhouse

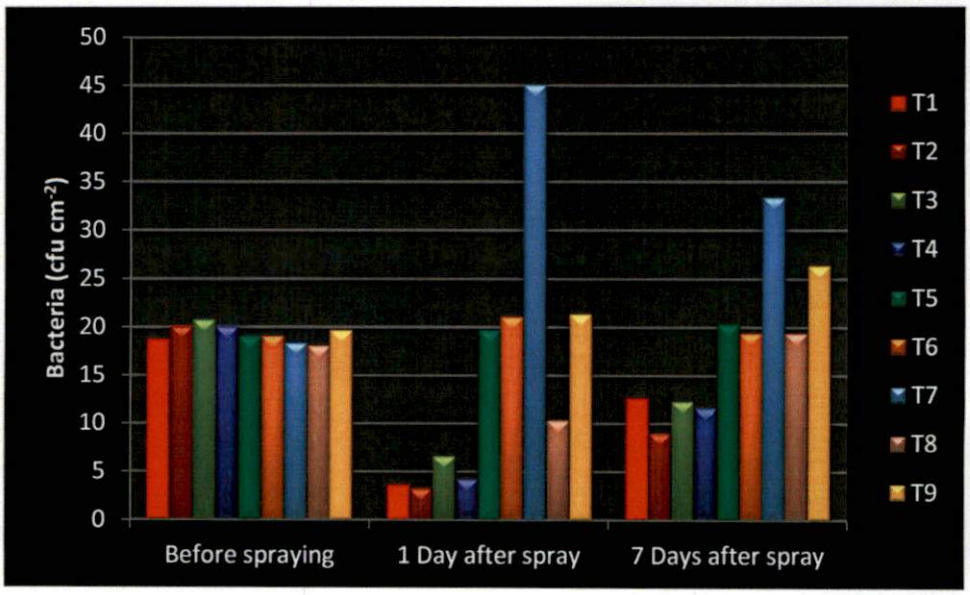
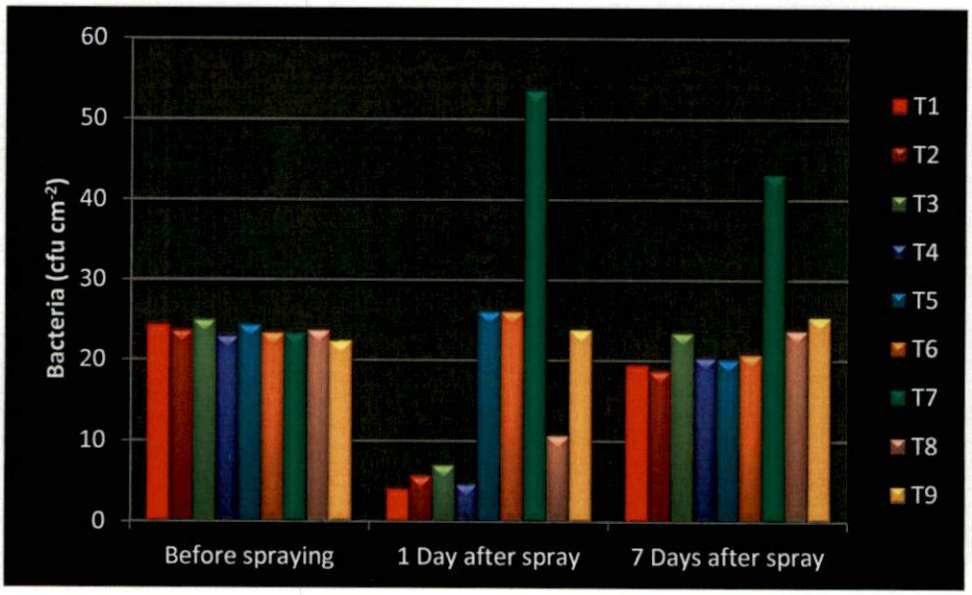


Fig. 8. Effect of treatments on phylloplane bacteria in rain shelter



T1- Difenoconazole 0.05%

T2- Tebuconazole 0.1%

T3-Wettable sulphur- 0.1%

T4- Wettable sulphur- 0.2%

T5- *Trichoderma viride* 2%

T6- *Trichoderma viride* (Liquid formulation)-0.5%

T7- *Pseudomonas fluorescens* 2%

T8- Neem oil 2%

T9- Control

reduced to 10.67 cfu cm^{-2} immediately after spraying. However the population got built up to the initial level after seven days. The population of bacteria in control was more or less stable throughout the experimental period (Fig 8).

4.7 SURVIVAL OF BIOCONTROL AGENTS ON THE PHYLLOPLANE OF YARD LONG BEAN UNDER PROTECTED CONDITION

The survival of biocontrol agents sprayed on leaves was assessed by re-isolation and enumeration using serial dilution plating on suitable selective media at periodic intervals (Plate 16).

4.7.1 Survival of *Trichoderma viride* on the Phylloplane of Yard Long Bean

Before treatment application, the naturally occurring resident *Trichoderma* population was only 2.67 cfu cm^{-2} in T5 and 1.33 cfu cm^{-2} in T6 (Table 23). After spraying, the population shot up to 34.67 cfu cm^{-2} in T5 with 12 fold increase from initial level (Fig 9). However, the population gradually reduced to 12.33 cfu cm^{-2} after seven days of spraying. Despite the reduction in population , the population was sufficiently high compared to initial level. In T6 where liquid formulation was applied, it was found that the population was much lower (26.33 cfu cm^{-2}) than T5 and declined to 9.33 cfu cm^{-2} after seven days.

Table 23. Survival of biocontrol agents on the phylloplane of yard long bean

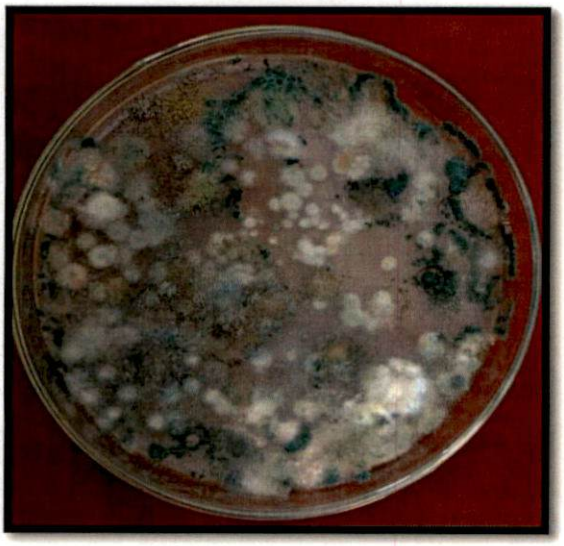
Treatments	Polyhouse (cfu cm^{-2})			Rain shelter (cfu cm^{-2})		
	Before spray	1 day after spray	7 days after spray	Before spray	1 day after spray	7 days after spray
***T.viride* 2%**	2.67	34.67	12.33	4.33	36.67	14.00
***T.viride* LF 0.5%**	1.33	26.33	9.33	2.33	32.33	11.67
***P.fluorescens* 2%**	4	16	5.67	3.33	23	6.33

LF- Liquid Formulation

In rain shelter also, survival of *Trichoderma* exhibited same trend as that of polyhouse. The initial population was slightly higher compared to polyhouse.

Plate 16. Biocontrol agents isolated from phylloplane of yard long bean

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



Trichoderma viride

Pseudomonas fluorescens

Fig.9. Survival of of biocontrol agent *T.viride* on the phylloplane of yard long bean in polyhouse

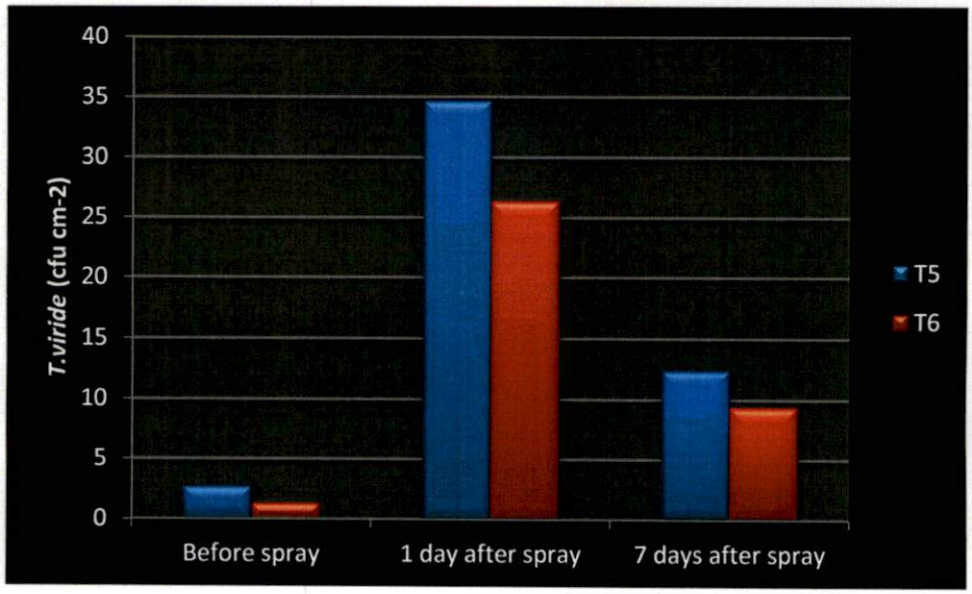
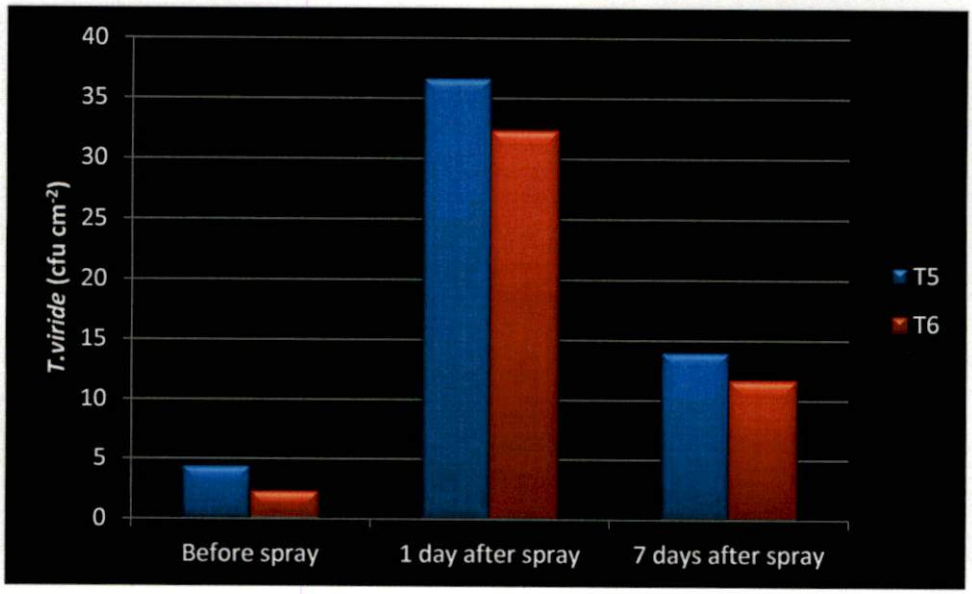


Fig. 10. Survival of of biocontrol agent *T.viride* on the phylloplane of yard long bean in rain shelter



T5- *Trichoderma viride* 2%

T6- *Trichoderma viride* (Liquid formulation)-0.5%

Fig.11. Survival of of biocontrol agent *P. fluorescens* on the phylloplane of yard long bean in polyhouse

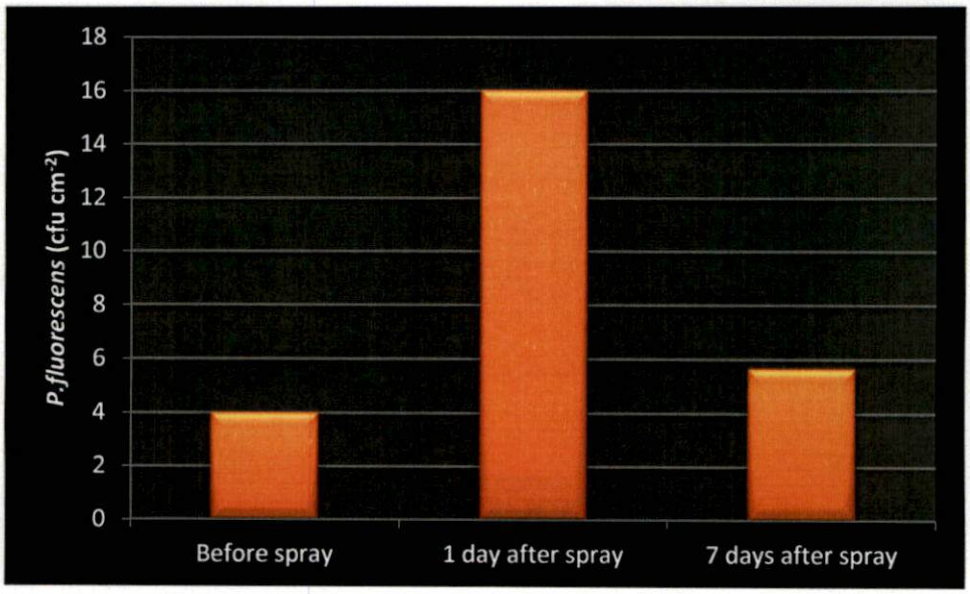
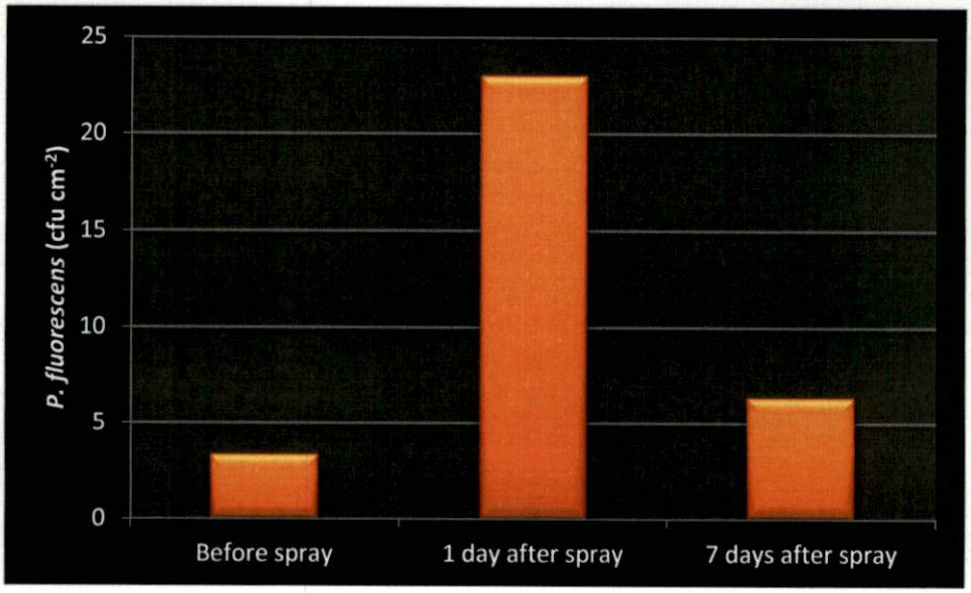


Fig. 12 .Survival of of biocontrol agent *P. fluorescens* on the phylloplane of yard long bean in rain shelter



Before spraying, the population was 4.33 in T5 and 2.33 in T6 whereas a tremendous increase in population of *Trichoderma* was observed after spraying and was 36.67 in T5 and 32.33 in T6. Thereafter the population gradually decreased to 14 and 11.67 after seven days of spraying (Fig.10).

4.7.2 Survival of *Pseudomonas fluorescens* on the Phylloplane of Yard Long Bean Under Protected Cultivation

Natural population of *Pseudomonas* sp. was present on yard long bean phylloplane and when isolated using King's B medium, 4 and 3.33 cfu cm^{-2} were obtained in polyhouse and rain shelter respectively (Table 23). However after the foliar application of *Pseudomonas fluorescens*, the population surged to 16 and 23 cfu cm^{-2} in polyhouse and rain shelter respectively. The population of introduced *Pseudomonas* sp. declined to 5.67 and 6.33 cfu cm^{-2} in polyhouse and rain shelter after seven days of spraying (Fig.11,12). The results clearly depicts that the bacterial antagonist successfully survived up to 7 days in polyhouse and rain shelter.

4.8 Persistence and Dissipation of Difenoconazole on Yard Long Bean

Residue analysis of pods collected from plots sprayed with difenoconazole was carried out at pesticide residue research and analytical laboratory of the All India Network Project on Pesticide Residue, KAU Centre, College of Agriculture, Vellayani, Thiruvananthapuram. The results (Table 24) revealed that even after seven days, the chemical residue was not completely dissipated.

Residue level of difenoconazole 0.05 per cent in polyhouse showed an initial deposit of 0.21mg kg^{-1} dissipated to 0.14 mg kg^{-1} with a reduction of 33.33 per cent one day after spraying. On the third day, residue was reduced to 0.13 mg kg^{-1} and the dissipation percentage increased to 38.09. During subsequent days also the residue dissipated gradually reaching 0.09 mg kg^{-1} on seventh day with a dissipation percentage of 57.14. However, complete degradation of chemical do not occurred even on the seventh day of spraying. The residue in rain shelter after seven days of spraying was 0.19 per cent, which is almost double the value of residue quantified in polyhouse after seven days of spraying.

Table 24. Persistence and degradation pattern of difenoconazole on yard long bean pods

Days after spraying	Residue of difenoconazole (ppm)	Dissipation percentage (%)
Polyhouse		
2 h after spraying	0.21	-
1	0.14	33.33
3	0.13	38.09
5	0.10	52.38
7	0.09	57.14
Rain shelter		
7	0.19	9.5

Discussion

5. DISCUSSION

Yard long bean is an important vegetable crop grown throughout Kerala because of its high protein content and nutritive value. Due to high market demand of the crop, during the last decade, the crop has been cultivated widely under protected condition which has led to increased productivity (Varghese and Celine, 2015). However, the congenial microclimatic conditions inside these structures predispose the crop to various fungal diseases. Among them, powdery mildew is reported to have devastating effect on the yield of yard long bean.

A perusal of the literature revealed that no attempts have been made so far for characterisation and management of powdery mildew of yard long bean in Kerala. Hence, it is pertinent to have a detailed investigation on the characters of the pathogen inciting powdery mildew of yard long bean and to develop an efficient management strategy.

5.1 SURVEY FOR ASSESSMENT OF SEVERITY OF POWDERY MILDEW

The severity of powdery mildew varies with changes in temperature, relative humidity, cultivar, age of the crop, location and cultural practices followed like monocropping and crop sanitation. Therefore, purposive sampling surveys were conducted in Thrissur district during September 2017 to January 2018 and it was found that the disease severity varied from location to location but highest disease severity of 67.33 per cent was recorded at Elanadu followed by 54.33 per cent at Koratty. The high intensity of disease at these locations may be attributed to the repeated cultivation of crop throughout the year, which ensured the continuous availability of the host for the pathogen. The susceptibility of the local cultivar and the favourable environmental conditions prevailed during crop growth may also have contributed to the high severity. The severity was found to be high during the months of November and December when the temperature and relative humidity was low. This observation parallels with Xu and Butt (1998) and Ashtaputre *et al.* (2007). The results of the survey also indicated that severity of powdery mildew was high during pod bearing and harvesting stages. It is

documented that powdery mildew assumes devastating proportions during the late stages of the crop like pod formation and harvesting (Channaveeresh and Kulkarni, 2017). The conducive microclimate due to heavy crop canopy during these stages also might have helped in pathogen multiplication. However, the low severity (3.67%) at harvesting stage recorded at Chelakkara even during December may be attributed to the crop rotation with non-leguminous crops which interrupted the survival of inoculum.

5.2 SYMPTOMATOLOGY AND CHARACTERISATION OF THE PATHOGEN

5.2.1 Symptomatology of Powdery Mildew of Yard Long Bean

The powdery mildew of yard long bean initiated as small white powdery lesions on upper surface of older leaves. Later, profused mycelial growth along with conidia led to the development of conspicuous white powdery spots on the leaves covering the entire leaf surface. With the advancement of disease, white color changed to grey and then to black. Under very severe conditions, the leaves especially the lower leaves turned yellow and defoliated. The symptoms observed were similar to that described by Soylu *et al.* (2004) and Chavan *et al.* (2014). In the present study, at Koratty, the powdery growth was found to be restricted along the veins of the leaves. This may be due to the peculiarity of the local cultivar grown in the location. Another variability noticed was between the symptoms on young plants and mature plants. The powdery lesions formed on leaves of young plants dried up after three to four days and no further spread of disease was noticed as in the case of hyper sensitive reaction. Such response in case of young plants may be due to the fact that defence mechanism is very active in young tissues compared to older ones.

5.2.2 Characterization of Powdery Mildew Pathogen

Powdery mildews being obligate pathogens which cannot be cultured on artificial media, characterization was done mainly based on the microscopic observation of morphological characters like hypha, conidia and conidiophore present on the leaf. Variability was observed regarding conidia and conidiophore

characters of powdery mildew collected from different locations during the survey and based on which the isolates were grouped into two *viz*., PM 1 and PM 2.

PM1 type obtained from all the survey locations except Vellanikkara was characterised by hyaline, cylindrical conidia without fibrosin bodies, which germinated apically producing short germ tubes. These were consistent with the characters of *Erysiphe polygoni*, the widely reported pathogen causing powdery mildew of legumes. Similar description was given by researchers while working with *E.polygoni* on various leguminous crops (Upadheyay and Singh 1994; Malani *et al*., 2000; Jamaluddin *et al*., 2004). Moreover, the measurements of conidia in the present study fell in the range described for *E.polygoni* in the CMI descriptions of pathogenic fungi and bacteria (CMI,1976).

PM 2 type was obtained only from Vellanikkara and was characterised by ellipsoid to oval conidia with crenate edges borne as chains on straight unbranched conidiophores and contained rod shaped refractive fibrosin bodies, which was the characteristic feature of *Podosphaera* sp. The characters of PM 2 were consistent with that of *Podosphaera xanthii* (Braun and Cook, 2012). Since *Podosphaera* sp. is rarely found on legumes, molecular characterization using ITS sequence of rDNA was carried out to confirm the identity and the isolate showed 100 per cent homology with *P. xanthii*. The sequence obtained was deposited in NCBI Genbank nucleotide database with accession number MH645799. Thus the pathogen causing powdery mildew of yard long bean at Vellanikkara was identified as *Podosphaera xanthii.* Perusal of literature revealed that this is the first report of powdery mildew of yard long bean incited by *Podosphaera xanthii*.

5.3 *IN VITRO* EVALUATION OF FUNGICIDES, BIO AGENTS AND BOTANICALS

Despite extensive research on their pathogenesis, epidemiology and control, powdery mildew infections remain as one of the most important plant pathological problems worldwide. Powdery mildew develops very quickly under favourable condition because the time length between infection and symptom development is

only three to seven days. In yard long bean, powdery mildew continues as a destructive disease crippling the production especially under polyhouse. So adoption of timely management measures is important in limiting the loss inflicted by the disease (Chavan *et al*., 2014). In spite of the negative impact of chemical fungicides on the environment and human health, fungicides still constitute the predominant part of control measures adopted against powdery mildew. *In vitro* experiments were conducted prior to the field experiments to evaluate efficacy of fungicides, bioagents and botanicals on powdery mildew since researches have shown that the results have medium to high predictability in the field. Lower as well as recommended dose of each treatment was tested.

Initially, ideal conditions for maximum conidial germination was standardised following the technique suggested by Manojkumar *et al*. (2008) in which five per cent sucrose solution was used for suspending the conidia whereas in the present study, collapse of conidia was observed in sucrose solution. However, germination of spores was observed in sterile water. The conidia used for spore germination assay were those of *Podosphaera xanthii* and collapse of may be due to higher sensitivity of conidia of *Podosphaera* to osmotic stress.

However, sterile water which was reported to be inimical to most of the powdery mildew spores (Yarwood., 1957) was found suitable for *P. xanthii* spore germination at 20°C. Hence, evaluation of various treatments was carried out using conidial suspension in sterile water. The results revealed that all the treatments recorded cent per cent inhibition of conidial germination.

For taking forward eight promising treatments to the field experiments out of the 17 treatments, they were tested *in vitro* on detached leaves. All the treatments were found to be significantly superior over control. Even though, the fungicidal treatments showed lowest per cent leaf area infection, it was ideal to select the promising treatments from all the four categories *viz*., systemic fungicides, contact fungicides, biocontrol agents and botanicals. Thus two treatments from systemic fungicides, two from contact fungicides, fungal biocontrol agent in two

formulations, one bacterial biocontrol agent and one botanical were selected for field evaluation.

5.4 FIELD EXPERIMENT FOR MANAGEMENT OF POWDERY MILDEW OF YARD LONG BEAN UNDER POLYHOUSE AND RAIN SHELTER CONDITION

Yard long bean cultivation in protected structures is often encountered with the problem of powdery mildew. The foremost objective of the study was to assess the severity of powdery mildew of yard long bean under protected condition and to formulate an effective management strategy against the disease. Hence, it was imperative to conduct a field experiment to evaluate different compounds to manage the disease. In this context, a field experiment was undertaken under polyhouse and rain shelter in the Department of Plant Pathology, College of Horticulture, Vellanikkara during October 2017 to March 2018. The treatments included systemic fungicides, contact fungicides, biocontrol agents and botanicals.

5.4.1 Effect of Different Treatments on Powdery Mildew of Yard Long Bean Under Protected Condition

Incidence of powdery mildew was noticed 55 days after sowing (DAS) in poyhouse. After two sprays, lowest severity was recorded in systemic fungicides T1 (difenoconazole) and T2 (tebuconazole) and per cent reduction was found to be 91.1 and 84.25 per cent respectively. These are triazole fungicides which are also known as demethylaition inhibitors (DMI). The superior performance of triazole fungicides is attributed to their ability to eradicate established fungal infections and suppressing subsequent disease development (Loganathan *et al.*, 2011). These fungicides inhibit C-14 demethylase activity preventing the demethylation of sterols and make them saturated. They are reported to possess preventive, curative and anti-sporulant action against powdery mildews. They interfere with biosynthesis of ergosterol which is an essential component of fungal cell wall and its absence causes irreparable damage to the cell wall leading to death of fungus. They also inhibit conidia and haustoria formation of the fungus. These changes in

the sterol content and saturation of polar fatty acids leads to alterations in membrane fluidity and thereby causing change in behaviour of membrane bound enzymes (Nene and Thapliyal, 1993). Similar results were obtained by Amaresh *et al* (2013), who showed the superior performance of difenoconazole in controlling powdery mildew disease compared to 12 other systemic fungicides. The results of the present study confirm the findings of Jarial *et al*. (2015), who recorded a minimum disease severity of 17.01 per cent in treatment with difenoconazole over the untreated control.

The lower concentration (0.1%) as well as higher concentration (0.2%) of wettable sulphur were statistically on par in their efficacy to control powdery mildew. The first chemical used to control powdery mildew was sulphur. Because powdery mildew grows on surface leaves, wettable sulphur can come in direct contact with mycelium of the fungus and supress the disease. Moreover, due to vaporisation, the compound get redistributed over the applied surface to some extent. However, the compound is usually not recommended in polyhouse as the vapours of sulphur are reported to cause damage to the structure.

Biological control is an emerging avenue in the management of foliar pathogens. Biocontrol agents suppress the foliar pathogens employing the same mechanisms as they do in case of soil borne pathogens. The best disease control among biocontrol agents in polyhouse was given by T5 *Trichoderma viride* 2% with 41.10% reduction in disease severity compared to control. T5 was followed by T6 (*Trichoderma viride* 0.5% liquid formulation) and T7 (*Pseudomonas fluorescens* 2%) where the reduction was 39.04 per cent and 36.99 per cent respectively. The management of powdery mildew using *Trichoderma* sp. under commercial glass house conditions were demonstrated by Elad (2000). The mechanisms involved in the biocontrol of foliar pathogens by *Trichoderma* sp. include mycoparasitism, antibiotic production, competition for space and nutrients, extracellular enzyme production, induction of host plant defence. Among these mechanisms, mycoparasitism is most common. Various stages of mycoparasitism involve coiling of the antagonist around the hyphae of pathogen, penetration of

pathogen hyphae and finally lysis. Lysis of fungal cell wall is facilitated by the release of series of enzymes including chitinase and 1,3-gluconase (Sawant., 2014). The major problem encountered when using biocontrol agents as foliar treatment is the reduced survival on leaf surface. However, in powdery mildew, since the mycelium is ectophytic in nature, it provide direct access to the biocontrol agent for parasitisation and survival. The performance of biocontrol agent should not be expected to be like that of chemical fungicide. Many biocontrol agents have been reported to be as effective as fungicides, but in most cases the results are intermediate. It seems that, a moderately efficient but consistent agent might be sufficient to establish non chemical control of foliar diseases and reduce the level of pesticide residues in the produce.

The effect of disease was reflected on yield of the crop receiving various treatments. All the treatments recorded higher yield compared to control. The reduction in yield is mainly due to the reduced photosynthetic efficacy of the leaves which were covered with the mycelium of the fungus. The pods harvested from severely affected plots were of reduced size. The highest yield was recorded in T1 (difenconazole 0.05%) in both polyhouse and rain shelter.

5.5 METEOROLOGICAL FACTORS INFLUENCING THE DISEASE

The data on meteorological parameters collected during the experiment revealed that temperature and relative humidity was higher in polyhouse compared to rain shelter. The higher temperature inside polyhouse is due to the greenhouse effect brought about by the polythene roof where, most of the reflected radiation from earth's surface is retained inside (Board., 2004). The increased transpiration rate induced by high temperature is the main reason for high RH inside polyhouse. The lower temperature and RH favourable for powdery mildew (Xu and Butt, 1998; Ashtaputre *et al*., 2007) may be the reason for high severity and early incidence of the disease in rain shelter.

As a testimonial to the above observation, correlation analysis performed between disease severity and meteorological parameters inside polyhouse and rain

shelter showed that the severity of powdery mildew was negatively correlated with temperature and relative humidity.

5.6 POPULATION OF PHYLOPLANE MICROFLORA OF YARD LONG BEAN UNDER PROTECTED CONDITIONS

The epiphytic micro-organisms residing on the aerial plant parts especially the phylloplane have prominent role in the biological control of foliar diseases (Bakker, 2004). In the present study, it was found that compared to the bacterial population, fungal flora is found to be high on the phylloplane of yard long bean. According to Blakeman (1985), bacteria colonise the leaves during the early stages of crop and filamentous fungi become abundant on the phylloplane of mature crop. This explains the reason for the lesser bacterial population obtained during the study as the phylloplane population was assessed subsequent to the disease incidence and treatment application which occurred during the late stages of crop. No population of actinomycetes was obtained from the phylloplane of yard long bean. Dickinson (1973) opined that actinomycetes being predominant soil inhabitants, they are sparsely or rarely found on leaf surfaces.

The study on effects of treatments on phylloplane microflora revealed that, there occurred a drastic reduction in the population of fungi and bacteria in polyhouse and rain shelter immediately after the application of chemical fungicides *ie.,* difenoconazole, tebuconazole and wettable sulphur. The non-revival of population to the initial level even after seven days is an indication of the persistence of chemicals for more than a week on the phylloplane, which causes permanent loss of highly sensitive organisms from the leaves. This shows the toxicity as well as non-selectivity of fungicides against non-target microflora of the phylloplane, which is known to be beneficial for plant growth in numerous ways. The effect of fungicides on the fungal and bacterial population had undergone extensive research in the past. The large reduction of microbial activity following the fungicidal application was similar to that recorded by Bainbridge and Dickinson (1972), Dickinson (1973) and Gokulapalan (1989) who studied the effect of

fungicides on the microflora of potato leaves, barley and rice respectively. Contradictory to the above reports and results of present study, Bertelson *et al.* (2001) recorded increase in the population of fungi following application of fungicide epoxyconazole on winter wheat leaves where he suggested the specificity of the chemical towards the pathogen as the reason.

The findings of present study are in confirmation with Bakker (2004) who opined that even a single foliar application of fungicides can affect the microbial population densities and species diversity on the phylloplane. Moreover, the destruction of phylloplane microflora following pesticide application can cause negative effect on the naturally occurring biological control which, in turn will make the plants more susceptible to other diseases as well (Bosshard *et al.*,1987).

The increase in fungal population following the application of *Trichoderma viride* in T5 and T6 may be attributed to the additive effect of the applied biocontrol agent as pointed out by Raj (2016). Similar is the case with bacterial population followed by the application of *Pseudomonas fluorescens*. Since it is evident from the results that biocontrol agents and neem oil cause less harm to the phylloplane microflora compared to chemical fungicides, they can be considered as more safe to the environment as well as crop health.

5.7 SURVIVAL OF BIOCOTROL AGENTS ON THE PHYLLOPLANE OF YARD LONG BEAN UNDER PROTECETD CONDITION

In addition to the antagonistic activity, a biocontrol agent in order to be successful should possess the ability to survive and establish an active population on the applied surface. Besides, the knowledge on period of survival of biocontrol agents is critical in deciding the interval between foliar sprays. So far only few biocontrol agents had been commercialised for the management of foliar diseases. Unlike soil, leaf surface provide sparse nutrients for which, the introduced biocontrol agent will have to compete with the resident epiphytes. The survival ability of *Trichoderma viride* and *Pseudomonas fluorescens* on yard long bean leaves was assessed in the study. From the results, it was clear that biocontrol agents

sprayed on the leaves, showed survival up to seven days or more. Longer the period of survival of biocontrol agent, better will be the disease control efficiency.

The fungal biocontrol agent *Trichoderma viride* parasitize and survive on the hyphae of pathogen. Since powdery mildew grows epiphytically on leaf surface, the hyphae is readily available for *Trichoderma* for colonisation. The results are in conformity with the findings of Kurian *et al.* (2004) who observed active *Trichoderma* population on bittergourd phylloplane, three days after spraying. Successful biological control of powdery mildew of strawberry in commercial glass house had been demonstrated by Pertot *et al.* (2008) where *Trichoderma harzianum* T39 survived up to ten days on the phylloplane.

Pseudomonas fluorescens, the bacterial biocontrol agent survive on the leaf wetness brought about by leaf exudates or water film (Hirano and Upper, 2000). The results were in agreement with the findings of Anand *et al.* (2010) who reported that an active population of *P.fluorescens* sufficient enough to give satisfactory control of powdery mildew of chilly survived up to seven days. Gal *et al.* (2003) suggested that the success of *P.fluorescens* on plant surface may be due to the high anti-microbial activity, nutrient acquisition efficiency and competitive colonisation.

5.8 PERSISTENCE AND DISSIPATION OF FUNGICIDES ON YARD LONG BEAN

From the results of field experiment, it was evident that difenoconazole 0.05 per cent was the most effective chemical treatment in managing powdery mildew of yard long bean. Besides, the chemical caused decline of non-target phylloplane microfloral population. This may be perceived as an indication of toxicity of the chemical as well as its persistence on the crop. Since tender pods of yard long bean harvested at every two or three days interval are used as vegetable, the persistence of fungicide residues in the harvested produce and their dissipation rates are a limiting factor in the production of high quality residue free safe pods. Hence it was pertinent to evaluate the dissipation kinetics prior to the recommendation for field use.

Difenoconazole when sprayed on yard long bean resulted in an initial deposit of 0.21 mg kg^{-1} which got gradually dissipated to 0.09 mg kg^{-1} on the seventh day. Compared to polyhouse, residue of difenoconazole was almost double in rain shelter on the seventh day. These results clearly depicts that the degradation of the fungicides is rapid in polyhouse, which may be due to the higher temperature. Despite the residue in both rain shelter and polyhouse was well below the MRL of difenoconazole for cowpea prescribed by Codex alimentarius *ie.,* 0.7mg kg^{-1}, it is better to observe a waiting period of seven days before harvest of pods.

Evaluating the data on management of powdery mildew of yard long bean, it was found that even though chemical fungicides provided best disease control, considering their toxic effect on beneficial non-target microflora on the phylloplane and the residue left on edible pods, biocontrol agents which exhibit consistent performance with moderate reduction of disease severity and sufficient survival will be ideal to manage the disease if the disease severity is low. Moreover, if systemic fungicides are applied continuously, there are chances of pathogen attaining resistance against these compounds. Hence, if the disease severity is very high, they can be recommended as alternative sprays with contact fungicides and biocontrol agents.

Summary

6. SUMMARY

Protected cultivation is a contemporary approach to raise horticultural crops during all the seasons by manipulating the environment. It makes use of recent advances in technology to control the environment for better productivity and quality of produce. However, in Kerala, naturally ventilated polyhouses are also popular where complete manipulation of environmental is not possible. Yard long bean being one of the most preferred vegetables in Kerala, it is widely cultivated in such protected structures like polyhouses and rain shelters. However, the performance of the crop is usually affected by various diseases among which powdery mildew is found to be the most devastating one. Farmers usually relies upon chemical control to manage the disease which has far reaching undesirable effects on human health and environment. Hence it is pertinent to formulate an efficient management strategy against the disease with emphasis on biological cntrol. So the study entitled "Characterization and management of powdery mildew of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) under protected cultivation" was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2017-18.

1. Survey was conducted in seven locations of Thrissur district namely Koratty, Elanadu, Chelakkara, Punnayur, Mannuthy, Vellanikkara and Vadanappally to assess the severity of powdery mildew of yard long bean. Per cent disease severity varied from 1.67 to 67.33. Highest disease severity was recorded at Elanadu (67.33%) followed by Koratty (54.33%).
2. From the survey conducted, it was clear that disease severity was high during months of November and December, when temperature and RH are low. Moreover, the severity varied with age of the crop and was high during the late stages of crop such as pod bearing and harvesting.
3. Leaves with symptoms of powdery mildew were collected during the survey and the fungal growth present on the surface of leaves was subjected to microscopic examination for characterization of the pathogen. Two distinct morphotypes of the pathogen was identified and were grouped into PM 1

and PM 2. The isolates coming under PM1 produced hyaline, branched, straight or flexuous and septate hyphae. Conidiophores were erect and unbranched with conidia borne in chains. The hyaline cylindrical conidia showed apical germination. Based on these characters, the pathogen was identified as *Erysiphe polygoni*.

4. PM 2, the Vellanikkara isolate showed marked difference in case of conidiophores and conidia from all other isolates. The conidiophores showed prominent constriction at the junction of the cells. The conidia contained well defined rod shaped fibrosin bodies and germinated laterally. These were the characters of *Podosphaera* sp. Since this pathogen was rarely reported on cowpea and not reported so far on yard long bean, molecular characterization was done using ITS rDNA sequencing and was confirmed as *Podosphaera xanthii*. This is the first report of powdery mildew incited by *Podosphaera xanthii* on yard long bean.
5. *In vitro* evaluation of fungicides, bioagents and botanicals using spore germination method revealed cent per cent inhibition of conidial germination by all treatments.
6. The treatments were further evaluated for their efficacy to control powdery mildew on detached leaves. Based on the per cent leaf area infected, best eight treatments were selected for evaluation under polyhouse and rain shelter condition. The treatments included two systemic fungicides, one contact fungicide in two concentrations, fungal biocontrol agent in two formulations, one bacterial biocontrol agent and one botanical.
7. In polyhouse, foliar spray with difenoconazole (0.05%) (T1) and tebuconazole (0.1%) (T2) recorded comparatively low disease severity with 91.10 and 84.25 per cent reduction over control respectively. Similar results were obtained in rain shelter also. Superior performance was exhibited by the same treatments T1 and T2 with 85.98 and 80.49 per cent reduction over control respectively.
8. Biocontrol agents *T.viride*, *P. fluorescens* and botanical neem oil also provided considerable reduction in disease severity in both polyhouse and

rain shelter and can be used effectively in protected conditions as they will be subjected to less environmental variability.

9. Highest yield was recorded in treatment T1 (difenoconzole 0.05%) in both structures. However, yield comparable to this was obtained in case of treatments with biocontrol agents *ie.*, T5 (*T. viride* 2%) and T7 (*P. fluorescens* 2%). This results points towards the impact of biocontrol agents on growth promotion and yield.
10. Economic analysis of the field experiments showed that T1 (difenconazole 0.05%) was most economic treatment with highest B:C ratio in polyhouse and rain shelter. However, if non chemical treatments could fetch 20 per cent premium price, T5 (*T. viride* 2%) and T7 (*P.fluorescens*) will be most remunerative in polyhouse and rain shelter respectively.
11. Correlation analysis of meteorological parameters and PDS revealed that powdery mildew severity is negatively correlated with temperature and relative humidity
12. In order to study the effect of foliar treatments on non-target phylloplane microflora, they were enumerated using serial dilution plating of leaf washings. The results revealed that the chemical treatments caused drastic reduction in population of fungi and bacteria. The increase in population following the spray of biocontrol agents was mainly due to the additive effect. The results indicates that chemical fungicide cause the loss of valuable microorganisms on the phylloplane which usually play a crucial role natural biocontrol of pathogens.
13. Survival of biocontrol agents on the phylloplane was also studied and it was found that both *T.viride* and *P.fluorescens* survived upto seven days after foliar application.
14. Difenoconazole was the treatment which recorded highest reduction in disease severity. However the treatment resulted in drastic reduction of phylloplane microflora which can be considered as an indication of residual toxicity of the chemical. Hence, persistence of difenoconazole on yard long bean was studied. The results showed that, residue of the chemical was

present on the pods upto seven days of spraying. On comparison of dissipation of the chemical in polyhouse and rain shelter, it was found that the degradation was faster in polyhouse. This might be due to the higher temperature prevailed inside the polyhouse.

15. Considering the results of various experiments in the present study, it was clear that, chemicals especially the systemic fungicides were superior over biocontol agents in reducing the disease severity. However, the reduction in population of beneficial phylloplane microflora induced by these chemicals will negatively affect the naturally occurring biological control and make the plant more susceptible to the pathogen in the long run. The results of residue analysis also indicate the persistence of chemical for seven days on the pods. Since tender pods of yard long bean harvested at an interval of two- three days is used as vegetable, it is better to restrict the application of systemic fungicides during the pod bearing stage. From the study, it is clear that timely application of biocontrol agents could give satisfactory control of the disease with good yield and no deleterious effect on environment or human health. Hence foliar application of biocontrol agents like *Trichoderma viride* (2%) ($2x10^6$cfu/g) and *Pseudomonas fluorescens* (2%) ($1x10^8$cfu/g) can be recommended at fortnightly interval to manage powdery mildew of yard long bean.

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Appendices

APPENDIX

1. Martin's Rose Bengal Streptomycin 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 aseptically)
Distilled water	: 1000 ml

2. Nutrrient Agar medium (NA)

Peptone	: 5.0 g
Beef extract	: 1.0 g
NaCl	: 5.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml
pH	: 6.5 to 7

3. Ken Knight's Agar medium

Dextrose	: 1.0 g
KH_2PO_4	: 0.1 g
$NaNO_3$	: 0.1 g
KCl	: 0.1 g
MgSO4	: 0.1 g
Agar	: 20.0 g
Distilled water	: 1000 ml

pH	: 7.0

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 to 7.4

5. *Trichoderma* Selective Agar medium

$MgSO_4$	: 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

CHARACTERIZATION AND MANAGEMENT OF POWDERY MILDEW OF YARD LONG BEAN (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) UNDER PROTECTED CULTIVATION

By

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ABSTRACT OF A THESIS

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ABSTRACT

Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.) is believed to be selected and developed from cowpea (*Vigna unguiculata* (L.) Walp.) for its long, succulent pods which are used as a vegetable. In Kerala, it is one of the most preferred vegetables having very high amount of protein, iron, calcium, vitamin A, Vitamin C and dietary fibre. It is considered as a remunerative crop under protected condition owing to its high market demand. However, incidence of diseases is a major setback hampering the production of yard long bean under protected conditions among which, powdery mildew is the most devastating one. In this background, the present study was undertaken to characterize the pathogen causing powdery mildew of yard long bean and to formulate a management strategy for the disease under protected cultivation.

Purposive sampling surveys were conducted in seven locations of Thrissur district and the disease severity varied from 1.67 to 67.33 per cent. The results of the survey indicated that the severity of disease was more during pod bearing and harvesting stage. Since powdery mildews are obligate parasites, characterization was done based on the microscopic observation of pathogen present on the leaves. The fungi produced hyaline, branched and septate hyphae. The conidiophores were erect and cylindrical on which conidia were born in chains. Variability was observed regarding conidia and conidiophore characters of powdery mildew collected from different locations, based on which the isolates were grouped into two *viz*., PM 1 and PM 2. PM1 type was observed in all locations except Vellanikkara. Based on the morphological characteristics of conidia and conidiophores, it was identified as *Erysiphe polygoni*. PM 2 type obtained only from Vellanikkara in which conidia and conidiophore characters were similar to *Podosphera* sp. which is very rarely reported on legumes. Hence, its identity was further confirmed as *Podosphaera xanthii* by molecular characterization. The rRNA-ITS sequence was deposited in NCBI Genbank database with accession number MH645799. This is the first report of powdery mildew of yard log bean incited by *Podosphaera xanthii*.

In-vitro evaluation of 17 treatments including fungicides, biocontrol agents and botanicals by spore germination technique revealed that all the treatments caused cent per cent inhibition of conidial germination. For taking forward eight promising treatments to the field experiments, they were tested *in-vitro* on detached leaves by artificial inoculation of conidia from infected leaves. Based on the per cent leaf area infected, two systemic fungicides, one contact fungicide, two biocontrol agents and one botanical were selected for field evaluation.

Field experiments were conducted simultaneously inside polyhouse and rain shelter to evaluate the performance of selected fungicides, biocontrol agents and botanicals against powdery mildew. Among the treatments, low disease severity of 4.33 per cent and 7.67 per cent was recorded in T1- difenoconazole and T2 – tebuconazole respectively in polyhouse and these treatments were statistically on par. In rain shelter also, T1- difenoconazole and T2- tebuconazole recorded low disease severity of 7.67 per cent and 10.67 per cent respectively. The performance of wettable sulphur at lower and higher concentration did not differed significantly. All the four non-chemical treatments were equally effective in managing the disease both in polyhouse and rain shelter. Correlation analysis between the meteorological parameters and disease severity revealed that per cent disease severity was negatively correlated with temperature and relative humidity both in polyhouse and rain shelter.

Analysis of population of phylloplane microflora showed that, there was a drastic reduction in the population of phylloplane fungi and bacteria after spraying chemical fungicides which is an indication of the toxicity and non-selectivity of these chemicals. Survival ability of biocontrol agents sprayed on the leaves were studied and found out that both *Trichodema viride* and *Pseudomonas fluorescens* survived on the leaves for seven days. Residue analysis of difenoconazole, the most effective chemical fungicide revealed that the compound with initial deposition of 0.21 mg kg^{-1} dissipated to 0.09 mg kg^{-1} after seven days in polyhouse whereas, the residue after seven days in rain shelter was 0.19 mg kg^{-1}. The faster degradation of

the chemical inside polyhouse may be attributed to the higher temperature prevailed during the experiment.

Evaluating the results various experiments in the present investigation, it was found that, even though chemical fungicides provided best disease control, considering their toxic effect on beneficial non target microflora on the phylloplane and the residue left on edible pods, biocontrl agents such as *Trichoderma viride* and *Pseudomonas fluorescens* which exhibited consistent performance with moderate disease control and sufficient survival on the leaf surface would be ideal to control powdery mildew of yard long bean if applied at right time. Moreover, frequent application of systemic fungicides with single site action can result in the development of resistant strains of pathogens. So such chemicals should be adopted only if the disease severity is very high and cannot be managed with biocontrol agents.