ECOFRIENDLY MANAGEMENT OF MAJOR FUNGAL FOLIAR DISEASES AFFECTING YARD LONG BEAN IN POLYHOUSE

by

DEEPTHI S. NAIR

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled "**Ecofriendly management of major fungal foliar diseases affecting yard long bean in polyhouse**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani
Date:

Deepthi S. Nair
(2017-11-053)

CERTIFICATE

Certified that this thesis entitled "**Ecofriendly management of major fungal foliar diseases affecting yard long bean in polyhouse**" is a record of research work done independently by Ms. Deepthi S. Nair 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.

Vellayani
Date:

Dr. Sajeena A.
(Major Advisor, Advisory Committee)
Assistant Professor (Plant Pathology)
IFSRS, Karamana

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Deepthi S. Nair, a candidate for the degree of **Master of Science in Agriculture** with major in Plant Pathology, agree that the thesis entitled **"Ecofriendly management of major fungal foliar diseases affecting yard long bean in polyhouse"** may be submitted by Ms. Deepthi S. Nair, in partial fulfilment of the requirement for the degree.

Dr. Sajeena A.
(Chairperson, Advisory Committee)
Assistant Professor
IFSRS, Karamana
Thiruvananthapuram 695002

15/10/19

Dr. Joy M.
(Member, Advisory Committee)
Associate Professor and Head
Dept. of Plant Pathology
College of Agriculture, Vellayani
Thiruvananthapuram 695522

15/10/19

Dr. Jacob John
(Member, Advisory Committee)
Professor and Head
IFSRS, Karamana,
Thiruvananthapuram 695002

15/10/'19

Dr. S. Sarada
(Member, Advisory Committee)
Assistant Professor
Dept. of Vegetable Science
College of Agriculture, Vellayani
Thiruvananthapuram 695522

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Deepthi S. Nair

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LIST OF ABBREVIATIONS

g	Gram
et al.	And other co workers
PDI	Per cent Disease Index
DI	Disease Incidence
viz.	Namely
%	Per cent
cm	Centimetre
mM	Milli molar
^{0}C	Degree celsius
µg	Micro gram
mL	Millilitre
M	Molar
MIC	Minimum Inhibitory Concentration
mg	Milligram
PDCI	Per cent Disease Control Index
spp.	Species (several)
sp.	Species (single)
ppm	Parts per million
IDM	Integrated Disease Management
mm	Millimetre
AMF	Arbuscular Mycorrhizal Fungi
P	Phosphorus
N	Nitrogen
t ha^{-1}	Tonnes per hectare
CRD	Completely Randomized Design
kg	Kilogram
PDA	Potato Dextrose Agar
ITS	Internal Transcribed Spacer
psi	Pound-force per square inch
WG	Water Dispersible Granule
DAP	Days after planting
var.	Variety
Fig.	Figure
DAI	Days After Inoculation
DAG	Days After Growth
NCBI	National Center for Biotechnology Information
KAU	Kerala Agricultural University
IFSRS	Integrated Farming System Research Station
LSU	Large subunit ribosomal rRNA

q ha^{-1}	Quintals per hectare
T	Treatments
NS	Non-significant
CD	Critical Difference
SEm	Standard Error of the mean
SD	Standard Deviation
cfu	Colony Forming Units

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Introduction

1. INTRODUCTION

Vegetables and fruits are rich in minerals, vitamins, fibres, micronutrients and bioactive compounds including antioxidants which help to maintain the health of human beings (Rao, 2013). Worldwide, India is ranked second in vegetable production (Kumar *et al.*, 2017) but the production is not sufficient to meet the balanced diet requirements of the growing population. The daily requirement of vegetables per person is accounted to be 300 g as against the actual availability of 245 g (Mohanty *et al.*, 2013). In India, vegetable cultivation is largely undertaken in open field conditions where the crop yield is adversely affected by biotic and abiotic stress. Polyhouse cultivation, an emerging technology, increases the quality and production of vegetable crops (Singh, 1998). Though profitable, cultivation in polyhouses has the possibility of severe crop loss, once a disease incidence is initiated. The common diseases which drastically affect the quality and quantity of vegetable produce under polyhouse conditions include powdery mildew, downy mildew, wilts, root rots, leaf spots etc. (Daughtrey and Horst, 1990). The high humidity condition in polyhouses favours pathogen development and alters the physiology of host-pathogen interactions (Coakley *et al.*, 1999).

Among the vegetables, yard long bean is the most common crop cultivated by farmers in Kerala (FIB, 2018). The crop is adversely affected by several diseases, resulting in drastic reduction of quality and yield (Quin, 1997). Anthracnose, fusarium wilt, powdery mildew, *Cercospora* leaf spot, web blight and collar rot were reported to be the major diseases of yard long bean in Thiruvananthapuram district under open field conditions (Sreeja, 2014). Yield reduction of 25- 50 per cent was reported in cowpea due to powdery mildew disease (Amazue and Adewale, 2016). Powdery mildew was reported to considerably reduce the yield of yard long bean cultivated in polyhouse conditions (Beevi, 2018).

Chemical fungicides are the most preferred tool for disease management among the farmers. However, most of the diseases of yard long bean are intensified

after flowering and the crop has to be harvested very frequently, which limits the use of fungicides in the crop. Besides, the consumer preference is to reduce the usage of chemical fungicides. Thus, eco-friendly management strategies are the need of the hour (Butt *et al.*, 2001). Utilization of cost effective, eco-friendly, indigenous organic preparations, botanicals and non-hazardous chemicals could be an alternate strategy to chemical fungicides for the management of diseases in yard long bean (Karthika *et al.*, 2017).

Thus, the present study aims for the eco-friendly management of the most important foliar disease of yard long bean identified under polyhouse conditions with the following objectives.

- Survey of polyhouses having cultivation of yard long bean in Thiruvananthapuram district
- Identification of major fungal foliar diseases of yard long bean in polyhouse
- Screening of yard long bean varieties in polyhouse against major fungal diseases
- Eco-friendly management of the major fungal foliar diseases of yard long bean identified in polyhouse

Review of Literature

2. REVIEW OF LITERATURE

Yard long bean is a common vegetable crop cultivated throughout Kerala (FIB, 2018). The crop has been listed as one among the major and profitable vegetable crops cultivated in polyhouses of Kerala (Kutty *et al.*, 2014). The crop is adversely affected by several diseases, drastically reducing its quality and yield (Quin, 1997). Many diseases affect the crop at flowering, the stage at which the use of fungicides has to be limited since the crop has to be harvested in every 2-3 days. Hence, the present study entitled "Eco-friendly management of major fungal foliar diseases affecting yard long bean in polyhouse" was undertaken with the objective to identify the most important fungal foliar diseases of yard long bean in polyhouse and to develop an eco-friendly strategy for the disease management. The relevant information regarding the important fungal foliar diseases affecting yard long bean, etiology, symptomatology and their eco-friendly management strategies have been reviewed in this chapter.

2.1. POLYHOUSE CULTIVATION

Polyhouse cultivation is an emerging technology to increase the quality and quantity of crop produce (Singh, 1998). It is a form of protected cultivation which modifies the natural conditions of temperature, relative humidity, water, light, soil and nutrients to maximize crop yield, improve quality and ensure year round availability of crop produce (Sabir and Singh, 2013). It also ensures efficient use of land and limits the wastage of crop resources such as water, nutrients etc.

Vegetables are one among the most common crops cultivated in polyhouse. However, polyhouse cultivation of vegetables has the major disadvantage of disease incidence including powdery mildew, downy mildew, wilts, root rots, leaf spots etc. which drastically affect the yield and other qualitative attributes of the crop produce (Daughtrey and Horst, 1990).

Pathogen development in poly house was studied to be due to the changes in the physiology of host-pathogen interactions and as a result of the modification of host resistance (Coakley *et al.*, 1999). High humidity was created inside the polyhouse due to restricted air exchange (El-Mougy *et al.*, 2011). The controlled conditions inside polyhouse led to the outbreak of several pests resulting in complete crop loss (Abdel-kader *et al.*, 2013; Thamilarasi, 2016).

The major diseases of greenhouse grown vegetables included gray mould, powdery mildew, damping off, *Fusarium* crown and root rot (Shipp *et al.*, 1991). Anthracnose, powdery mildew, *Cercospora* leaf spot, brown rust, *Septoria* leaf spot were reported in cowpea (Emechebe and Florini, 1997). Soni *et al.* (2017) studied the effect of weather parameters on the development of early blight of tomato caused by *Alternaria solani* in polyhouse and field conditions. The disease was favoured by low temperature and high relative humidity. The percent disease index (PDI) was comparatively high in polyhouse condition (PDI - 84.8) than open field condition (PDI - 82.2).

Nisar *et al.* (2006) observed 50 per cent yield loss due to the powdery mildew fungus *Erysiphe pisi* in pea (*Pisum sativum* L. sub sp. *hortense*). Rongai *et al.* (2009) revealed a leaf area infection of 56 per cent in sugar beet caused by *Erysiphe betae*. Amazue and Adewale (2016) reported that powdery mildew disease due to *Erysiphe polygoni* resulted in a yield loss of 40 per cent in mung bean and 45 per cent in sesamum. Thirty per cent disease incidence of powdery mildew caused by *E. polygoni* was recorded in black gram (Jayasekhar and Ebenezar, 2016) and 40 per cent in broad bean and pea (Nongmaithem *et al.*, 2017). Teshome and Tegegn (2017) reported a disease severity of 41.98 per cent in field pea caused by *E. polygoni*.

2.2. SCREENING OF YARD LONG BEAN VARIETIES

2.2.1. Biometric and yield attributes of varieties

Vidya *et al.* (2002) screened a total of fifty yard long bean cultivars including 48 local cultivars and two pure line selections. The study revealed significant variation among the varieties for all the characters studied. Pod yield per plant exhibited the highest coefficient of phenotypic and genotypic variations followed by pod number per plant and pod weight, which indicated the scope of improvement of these yield attributes through crop selection.

Varghese (2015) screened 30 accessions of yard long bean in polyhouse and reported that the maximum yield per plant was recorded in the variety, Gitika followed by Hari Rani and NS 621. Lakshmi (2016) evaluated eight yard long bean accessions and 28 hybrids which were compared to two checks including Vellayani Jyothika (KAU variety) and NS 634 (a commercial yard long bean hybrid) to study heterosis and combining ability in yard long bean. The cross combination of VS 34 (Gitika) and VS 50 (Kakkamoola Local) revealed the highest standard heterosis value, whereas VS 54 (Thirupuram Local) x VS 26 (Vellayani Jyothika), VS 50 x VS 16 (Pattom Local) and VS 34 x VS 13 (Neyyattinkara Local) recorded the highest heterosis value for pod length, pod weight and pods per plant respectively. The author also identified that Githika, Kakkamoola Local, Vellayani Jyothika and Neyyattinkara Local were the best parents to maximize yield and yield related attributes in yard long bean.

Varghese (2017) screened seven yard long bean accessions, ten hybrids and a commercial check both under rain shelter and open field conditions to compare their performance in terms of biometric and yield attributes. The study revealed that the maximum pod yield per plant was obtained from the cross combination between Gitika and Kakkamoola Local under both the conditions.

2.2.2. Disease incidence and severity

Sowmya (2011) screened 308 green gram genotypes to study their reaction to powdery mildew disease. Among them, 76 were resistant, 108 were moderately

resistant, 98 were moderately susceptible and 16 were found to be promising for cultivation under field conditions. Powdery mildew adversely affected the growth and yield attributes of the crop including pods per plant, pod weight, pod length and seeds per pod.

Pea genotypes (701 numbers) were screened to compare their reaction to natural incidence of powdery mildew caused by *Erysiphe pisi*. The pea accessions *viz*., EC598655, EC598878, EC598704, IC278261, and IC218988 were identified to be resistant which were suggested to be used as parents in disease resistance breeding (Rana *et al*. 2013).

Nag *et al*. (2018) reported that Pant Vegetable Pea recorded the maximum powdery mildew disease severity (78.57 %) and the minimum severity (2.47%) was recorded in the entry 2011/PMPM-1. Bitter gourd powdery mildew caused by *Podosphaera xanthii* was an important disease resulting in drastic yield loss up to 70 per cent in the crop. Powdery mildew disease incidence of 0 to 100 per cent was observed in various varieties of bitter gourd under conditions of natural incidence and artificial inoculation (Prasanth *et al*., 2019).

2.2.3. Characterization of *Diaporthe* sp.

Diaporthe spp. (syn. *Phomopsis*) included pathogenic, endophytic as well as saprophytic fungi belonging to the kingdom fungi, phylum ascomycota, subphylum pezizomycotina, class sordariomycetes, order diaporthales, family diaporthaceae and genus *Diaporthe* containing several species affecting many economically important crops (Maharachchikumbura *et al*. 2015; Dissanayake *et al*. 2017). Dissanayake *et al*. (2017) listed the current status of different *Diaporthe* spp. identified from different crops on molecular basis.

Kanematsu *et al*. (1999) reported several isolates of *Phomopsis* from peach, pear and apple in Japan. They also studied the conidial morphology, culture characteristics and pathogenicity of the fungus. Several species of the fungus were

identified till date and hence, identification at species level was of utmost importance. Seed decay of soyabean was reported to be caused by a complex of *Diaporthe* fungi including *D. longicola, D. sojae, D. caulivora, and D. aspalathi* (Li, 2011). *Phomopsis / Diaporthe* sp. was reported to cause diseases in brinjal (leaf spot and fruit rot) as well as in soybean. Mahadevakumar and Janardhana (2016) reported that *D. vexans* caused leaf blight and fruit rot disease in brinjal, which was a serious constraint to brinjal production in southwest regions.

Petrovic *et al.* (2015) for the first time identified the role of *D. eres* in inciting soybean seed decay, besides other species of *Diaporthe* in Serbia. *Diaporthe tulliensis* has been reported in lotus (Shivas *et al.*, 2015), kiwi fruit (Bai *et al.*, 2016) and in cocoa (Chen and Kirschner, 2018).

2.3 *IN VITRO* INHIBITION OF MYCELIAL GROWTH

2.3.1. Effect of bicarbonates on fungal pathogens

The mycelial growth of *Rhizopus* sp. was completely inhibited by 0.5 per cent of sodium bicarbonate. The bicarbonate also resulted in the suppression of the mycelial growth of *Alternaria alternata*, *Fusarium* spp. and *Rhizoctonia stolonifer* (Aharoni *et al.*, 1997). A colony diameter of 0.38 cm of *Botrytis cinerea* was observed in PDA amended with 50 mM sodium bicarbonate (Palmer *et al.*, 1997).

Erper *et al.* (2011) revealed that 750 and 100 mM potassium bicarbonate completely inhibited the mycelial growth of *R. solani* (AG 4HG-1) and *Sclerotinia sclerotiorum* respectively. Potassium bicarbonate (50 mM) was effective in inhibiting the sclerotial development and germination of *Sclerotinia cepivorum* by 100 and 86 percentage respectively (Ortega-Aguilar *et al.*, 2011). Yildirim (2014) reported that 600 µg mL^{-1} sodium bicarbonate resulted in 74.70 per cent inhibition of the mycelial growth of *Phomopsis viticola*. Zaker (2014) recorded 95.35 and 100 per cent inhibition of the mycelial proliferation and spore germination respectively of

Fusarium oxysporum (potato dry rot fungus), *Alternaria alternata* (tomato leaf spot fungus) and *Botrytis cinerea* (grape grey mould fungus) in 200 mM potassium bicarbonate. Jabnoun-Khiareddine (2016) observed complete inhibition of the mycelial growth of *Pythium aphanidermatum* and *S. sclerotiorum* by 0.1 M potassium bicarbonate.

Shekhar *et al.* (2009) observed complete suppression of the mycelial growth of *Aspergillus flavus* by 30 mM potassium and sodium bicarbonates. Sodium bicarbonate at 0.25 per cent completely inhibited mycelial growth of *Geotrichum candidum* (Talibi *et al.*, 2011). Sodium bicarbonate could effectively inhibit 80.66 per cent mycelial growth of *Fusarium oxysporum* f. sp. *cepae* as reported by Turkkan and Erper (2014). Ammonium carbonate, ammonium bicarbonate, sodium carbonate, sodium bicarbonate, potassium carbonate and potassium bicarbonate at 10, 25, 25, 50, 50 and 75 mM completely inhibited the mycelial growth (100%) of *Botrytis cinerea* (Turkkan *et al.*, 2017) proving the antifungal potential of carbonate and bicarbonates.

2.3.2. Effect of weed extracts on fungal pathogens

The hexane fractions of extracts of the leaf and inflorescence of *Ageratum conyzoides* resulted in 84 per cent reduction in mycelial growth of *Fusarium solani* of brinjal (Javed and Uzma, 2012). Pal *et al.* (2013) revealed that the chloroform extract of *A. conyzoides* resulted in 80 per cent inhibition of the mycelial growth of *Alternaria* spp. which was followed by the methanol extract of *A. conyzoides* (52.85%) and *Parthenium hysterophorus* (52.85%), thereby suggesting an avenue for the development of botanical based fungicide formulations. The water and methanol leaf extracts of the weed, *P. hysterophorus* (250 mg mL^{-1}) revealed 54 and 100 per cent mycelial inhibition of *Sclerotium rolfsii* respectively, besides inhibiting *F. oxysporum* (Devkota and Sahu, 2017).

The leaf extracts of the invasive plants *viz.*, *Cestrum laevigatum*, *Nicotiana glauca, Solanum mauritianum, Lantana camara, Datura stramonium, Ricinus*

communis and *Campuloclinium macrocephalum* inhibited the growth of *Aspergillus niger*, *Penicillium expansum* and *R. solani in vitro*, with the minimum inhibitory concentration (MIC) values of 0.81, 0.83 and 0.84 mg mL^{-1} respectively (Mdee *et al.*, 2009). The maximum inhibition of *Colletotrichum gloeosporioides* was exhibited by the leaf extracts of *C. macrocephalum* which had the maximum antifungal potential against *C. gloeosporioides* even at 0.05 mg mL^{-1} MIC (Mdee *et al.*, 2009). Singh *et al.* (2017) observed that the water extracts of the leaves of *D. stramonium* exhibited significant *in vitro* suppression of *Alternaria solani* (80.78 %) followed by *Parthenium* sp. (76.48%), *Achyranthus* sp. (69.66), *Salix* sp. (65.14) and *Physallis minima* (49.53).

Qasem and Abu-Blan (1996) reported that among the 64 weed species tested for their antifungal potential against *A. solani, Helminthosporium sativum* and *R. solani*, the aqueous shoot extract of *Ranunculus asiaticus* completely inhibited the mycelial growth and spore formation of all the three fungi.

Srivastava and Singh (2011) reported that the maximum per cent inhibition of *Alternaria* sp. was exhibited by 20 mg mL^{-1} of *Lantana camara* (59.5%). Sanguri *et al.* (2012) reported the antifungal potential of the alcohol and methanol leaf extracts of *Quisqualis indica* and *Achyranthes aspera* against *Aspergillus oryzae Alternaria porri, Penicillium chrysogenum, Aspergillus flavus and Aspergillus niger.* The acetone leaf fraction of the invasive weed *viz.*, *Pseudognaphalium luteoalbum* resulted in the maximum mycelial growth inhibition of *Phytophthora nicotiana* and *Fusarium oxysporum* (20 and 160 µg ml^{-1} MIC respectively) (Aderogba *et al.*, 2014).

Complete inhibition of spore germination of *F. oxysporum* was recorded by the flavanoid component of *Capparis decidua* as well as the sterol and flavanoid components of *Tridax procumbens* (Sharma and Kumar, 2009). The methanol and hexane extracts of the aerial parts of *Amaranthus spinosus* and *Vigna unguiculata* at 0.5 mg ml^{-1} resulted in the maximum inhibition *of Fusarium verticillioides* and *Fusarium proliferatum* (Thembo *et al.*, 2010).

2.4. *IN VIVO* MANAGEMENT STUDIES

2.4.1. Effect of bicarbonates on yield improvement and disease suppression

Reuveni *et al.* (1996) recorded 96.5 per cent reduction of powdery mildew disease (*Sphaerotheca fuliginea*) in cucumber plants treated with sodium bicarbonate compared to control plants. Foliar spray of potassium and sodium bicarbonates resulted in significant reduction of powdery mildew (*Leveilula taurica*) disease severity in pepper plants (10 and 12% PDI) compared to untreated control (60% PDI) (Fallik *et al.*,1997). Potassium and sodium bicarbonates could influence different plant growth parameters positively. Sodium, ammonium and potassium bicarbonates at 0.5 per cent resulted in significant values of per cent disease control index (PDCI) (63.44, 68.05 and 58.68 respectively) of powdery mildew caused by *Uncinula nicator* in table grapes while the control registered a PDCI value of zero (Sawant and Sawant, 2008).

The antifungal potential of Armicarb®, a formulated product of potassium bicarbonate was tested against powdery mildew disease of several crops including tomato, cucumber, rosemary, sage and corn salad (Koller, 2011). The study revealed that 0.5 percentage of the formulated product resulted in significant reduction of the disease in all the crops studied (PDI of 0.1, 0.1, 1.7, 0.2 and 0.03 in cucumber, corn salad, rose mary, sage and pansy respectively).

Zaki *et al.* (2011) recorded an increase of fruit yield in squash plants sprayed with potassium bicarbonate (2%). The sprayed plants revealed a yield of 8.9 ton compared to 7.2 ton in the case of untreated control plants. An increase of plant height (19.55%) and root weight (27.94%) was observed in tomato plants sprayed with 50 mM potassium bicarbonate (Jabnoun-Khiareddine, 2016).

Jabnoun-Khiareddine (2016) reported that foliar spray of 0.4 M potassium bicarbonate led to a reduction of 60.86 per cent of wilt by *Verticillium dahliae*, 12.5 per cent reduction in wilt caused by *Fusarium,* 10.19 per cent reduction in grey

mould disease caused by *Botrytis cinerea* and 12.83 per cent reduction of *Rhizoctonia* rot in tomato. Turkkan *et al.* (2017) observed that there was a reduction of grey mould (*B. cinerea*) infection in kiwi fruits (lesion area of 3.02 cm^2) compared to untreated fruits (lesion area of 5.21 cm^2).

Zaki *et al.* (2011) observed that foliar sprays of sodium bicarbonate at 2 per cent at 30 and 60 days after sowing resulted in a reduction of disease severity and colony number of powdery mildew fungus of squash (variety Karishma) grown under green house conditions by 80.0 and 93.1 per cent respectively.

Turkkan *et al.* (2018) observed that spraying of sodium bicarbonate (1.5%) followed by potassium bicarbonate (4.5%) significantly reduced the incidence of powdery mildew (*Phyllactinia guttata* and *Erysiphe corylacearum*) of hazelnuts (PDI of 1.80 and 1.53 respectively) indicating that the salts could be used as biocompatible fungicides for the management of powdery mildew disease.

2.4.2. Effect of weed extracts on yield improvement and disease suppression

Plant extracts have been reported to promote plant growth as well as to improve the various yield parameters of crop plants. The application of the aqueous methanol extract of the invasive weed *viz*., *Inula graveolens* (1000 ppm) in cucumber plants inoculated with *Fusarium* spp. revealed the highest shoot height (59.4 cm) compared to uninoculated, untreated control plants (43.1 cm). The treatment with the weed extract at 500 ppm also resulted in the highest dry root weight (1.2 g) compared to the untreated control plants (0.75 g) (Abu-Irmaileh *et al.,* 2017). Karthika (2017) reported that foliar application of fermented *Setaria barbata* (weed) extract on 45, 60, 75 and 90 days after sowing in rice (variety Uma) resulted in the maximum plant height (96.70 cm), tillers per plant (11.40), productive tillers per plant (11.40) as well as grain yield (15.98 g).

Patel *et al.* (2006) screened leaf extracts (water and ether-water extracts) of several plants against *A. niger* and *Aspergillus awamori*, the two important fungi

affecting the seeds of crop plants. Both types of extracts of the weed, *Lantana camara* completely inhibited the mycelial growth of the fungi on the seeds. The water extract of *L. camara* accelerated the growth of the germinated seeds upto a maximum of 11 cm whereas the ether extract treated seeds failed to germinate. This was followed by ether-water extract of *D. stramonium* which could result in the complete suppression of the mycelial growth of the fungus and also resulted in the maximum growth of the germinated seed (18 cm) compared to the untreated control seeds (7 cm). Nair (2011) concluded that fruit rot of brinjal caused by *Phomopsis vexans* could be managed by an integrated disease management (IDM) strategy which included foliar application of *Pseudomonas fluorescens* (2%) along with the spray of the plant extract *viz*., *Ocimum sanctum* at 20 per cent as a major component of the IDM.

Abu-Irmaileh *et al.* (2017) studied the antifungal potential of the aqueous methanol extract of the invasive weed *viz*., *Inula graveolens* (Stinkwort weed) which inhibited the growth of *Alternaria* sp., *Fusarium* spp. and *Rhizoctonia* sp. *in vitro*. The length of browning at the crown region of *Fusarium* treated cucumber plants was the least (0.4 mm) for the plants sprayed with the aqueous methanol extract of *Inula graveolens* (1000 ppm) compared to the fungicide (hymexazol) treatment (11.2 cm). Karthika (2017) revealed that fermented *S. barbata* extract completely inhibited the mycelial growth of *R. solani*, the rice sheath blight fungus *in vitro* proving its antifungal potential.

2.4.3. Effect of AMF on yield improvement

Cely *et al.* (2016) tested the infectivity of the inoculum of the arbuscular mycorrhizal fungus (AMF) *viz*., *Rhizophagus clarus* and its effectiveness as an alternative for nutrient supply in soybean (*Glycine max* L.) and cotton (*Gossypium hirsutum* L.) and compared its effect with conventional chemical fertilization under field conditions. No difference was observed in plant height between the control and fertilizer applied or AMF inoculated treatments. The data on plant biomass and

nutrients (N and P) uptake revealed that *R. clarus* (AMF) inoculation had the same effect as that of the conventional fertilization; and *R. clarus* inoculation was as effective as that of half the dose of fertilizer application. This combination resulted in increased uptake of N and P to 24 per cent, when compared to soybean plants which were only fertilized.

Bona *et al.* (2016) reported that the presence of AMF alone increased (27%) the collar diameter (2.13 cm) of tomato plants compared to the uninoculated plants (1.70 cm). Moreover, mycorrhizal inoculated plants showed the highest number of inflorescence per branch (7.73), even higher than those produced by the uninoculated plants with traditional fertilization (6.06). The most important effect induced by AMF was an improvement of citric acid (263.52 mg / 100g fresh weight) in tomato fruits compared to un-inoculated plants (223.78 mg / 100g fresh weight).

El-Wakeil and El-Sebai (2007) observed a significant positive effect of the combination of rhizobia strains with AMF as evident from the fresh (193.3 g over 146.3 g of NPK) and dry weights (27.3 g against 26.1 g of NPK) of leaves and stems, root / shoot ratio (0.5 against 0.4 of NPK), pods / flowers ratio (0.9 against 0.6 of NPK) as well as the number and weight of nodules compared to the NPK fertilizer plots of Faba bean. The highest number of pods was recorded in the treatment of rhizobia mixed with pseudomonads (320.7) or mycorrhiza (313). The number of seeds was higher in plants inoculated with *Rhizobia* and AMF (905) compared to un-inoculated plants (739).

Do-Rego *et al.* (2015) proposed that inoculation of cowpea with the *Rhizobium* LCM Nieara alone and in mixture with mycorrhizal fungi improved significantly the seed production of cowpea with an increase of 92.01 per cent and 62.85 per cent respectively, compared to the control plants.

Marjorie (2016) opined that AMF might improve cowpea production by enhancing the uptake of nutrients, particularly P and N. AMF inoculation in cowpea

had a positive effect on different growth parameters including nodule number, nodule dry weight as well as root and shoot dry weights.

Silva *et al.* (2018) found that AMF inoculation resulted in the maximum production of dry mass of shoot compared to non inoculated control plants, with an average increase of 511 per cent. The grain yield was also influenced by the fungal treatments.

Raklami *et al.* (2019) revealed that inoculation of the consortium of PGPR-rhizobia-AMF (PRM) improved the growth parameters (dry weight of shoots and roots) of faba bean and wheat. An improvement of 130, 200, and 78 per cent was observed in *Vicia faba* shoot dry weight, root dry weight and the number of leaves respectively. Similarly, the shoot and root dry weight and number of leaves of *Triticum durum* were enhanced by 293, 258, and 87 per cent respectively. The beneficial microbial consortium inoculation improved the productivity of the studied plants as observed by the increase in the number and weight of bean pods (270×10^4 ha^{-1} and 30.74 t ha^{-1} respectively) and wheat spikes (440×10^4 ha^{-1} and 10.56 t ha^{-1} respectively). In addition, the mineral analyses showed that the inoculation of PGPR-rhizobia-mycorrhizae consortium improved N, P, Ca, K, and Na contents in shoots, as well as the contents of sugar and proteins.

Wahid *et al.* (2019) studied in detail on the effect of inoculation of AMF on phosphorus (P) transfer from composted dung of cattle with a diet supplemented with powdered rock phosphate (RP) and their successive uptake by mung bean plants in alkaline soil. The results revealed that the association of AMF with composted powdered rock phosphate (RP) fed dung had a positive effect on mung bean shoot (3.04 g) and root (2.62 g) biomass, chlorophyll content, carotenoid content as well as on the N (58.38 mg $plant^{-1}$) and P (4.61 mg $plant^{-1}$) uptakes. Similarly, the per cent root colonization (56%) and nodulation of mung bean plant roots and their post-harvest soil properties were also improved by the inoculation of AMF together with the application of composted RP fed dung.

The perusal of literature indicated that leaf spot caused by *Diaporthe tulliensis* was the first report of the disease in yard long bean worldwide and no detailed studies were undertaken on the characterization of the fungus and its management using sodium bicarbonate (0.5%) or fermented *S. barbata* extract (10%).

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Ecofriendly management of major fungal foliar diseases affecting yard long bean in polyhouse" was undertaken during 2017-19 at College of Agriculture, Vellayani and Integrated Farming System Research Station (IFSRS), Karamana, Thiruvananthapuram. A survey was conducted in selected polyhouses in Thiruvananthapuram district to identify the most important fungal foliar diseases affecting yard long bean. Five varieties of yard long bean were screened to identify the major fungal foliar diseases affecting them in the polyhouse at IFSRS, Karamana. Laboratory and pot culture studies were undertaken for the management of the most important fungal foliar disease identified during the study. The materials used and the methodology followed are detailed in this chapter.

3.1. SURVEY LOCATIONS, VARIETIES, AND INCIDENCE & SEVERITY OF MAJOR FUNGAL FOLIAR DISEASES OF YARD LONG BEAN

3.1.1. Location of survey

A survey was conducted in 15 polyhouses located in different panchayats of Thiruvananthapuram district having the cultivation of yard long bean to identify the major fungal foliar diseases affecting the crop. The panchayats where the polyhouses are located with their corresponding blocks in parenthesis are depicted in Plate 1.

3.1.2. Variety, diseases and pests of yard long bean

The details of the variety as well as the major diseases and pests of yard long bean cultivated in the 15 polyhouses were collected during the survey.

3.1.3. Disease incidence and severity

The incidence and severity of the major fungal foliar diseases observed in different varieties of yard long bean cultivated in the 15 polyhouses were assessed during the survey. In each polyhouse, 100 - 200 plants were selected to assess the

1. Thirupuram panchayat (Parassala block)
2. Vellanadu panchayat (Kattakada block)
3. Kuttichal panchayat (Kattakada block)
4. Tholicode panchayat (Kattakada block)
5. Malayinkeezhu panchayat (Pallichal block)
6. Vilavoorkal panchayat (Pallichal block)
7. Thiruvananthapuram corporation (Pallichal block)
8. Thiruvananthapuram corporation (Pallichal block)
9. Thiruvananthapuram corporation (Pallichal block)
10. Vembayam panchayat (Nedumangad block)
11. Nedumangad panchayat (Nedumangad block)
12. Pothencode panchayat (Kazhakoottam block)
13. Nanniyode panchayat (Vamanapuram block)
14. Manickal panchayat (Vamanapuram block)

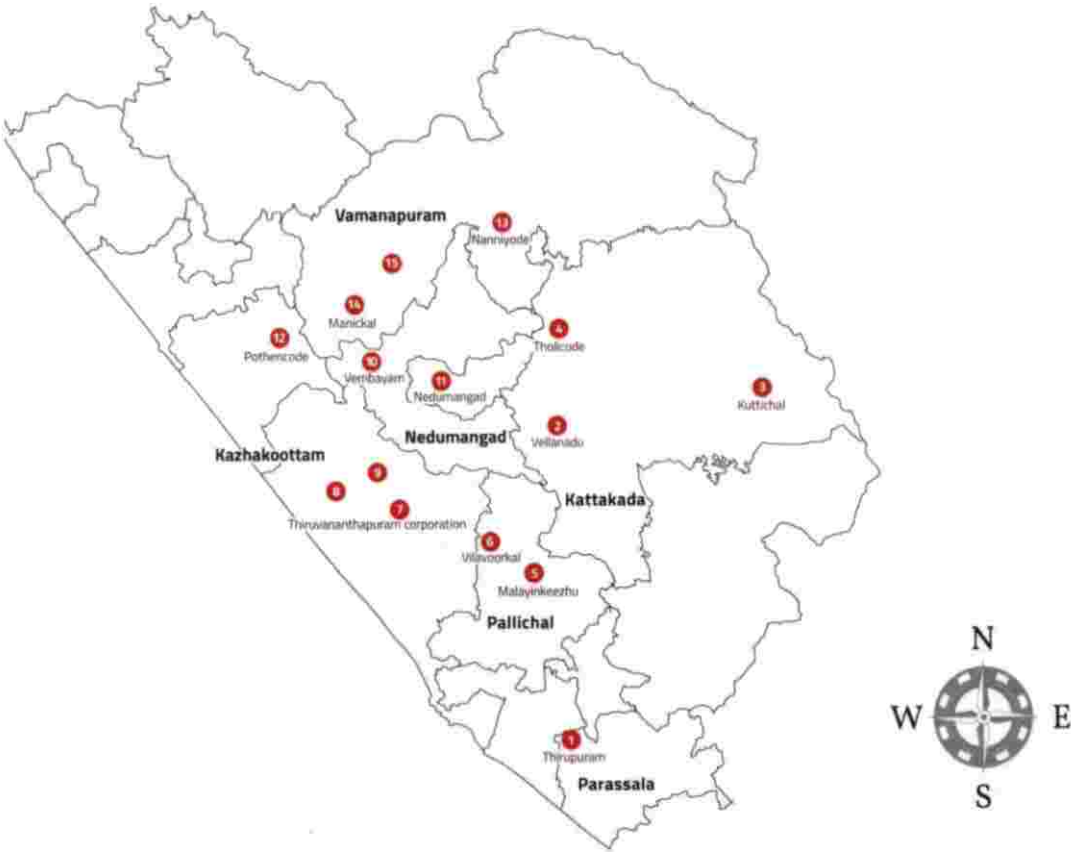


Plate 1. Location map of polyhouses surveyed to assess different diseases of yard long bean in Thiruvananthapuram district

incidence of the major fungal foliar diseases. The disease incidence (DI) was calculated using the formula developed by Singh (2002) as follows,

$$\text{Disease incidence (DI)} = \frac{\text{No. of infected plants}}{\text{Total number of plants}} \times 100$$

The disease severity was assessed by determining the per cent disease index (PDI) using standard score charts for each disease. Ten plants were selected at random in each polyhouse and ten leaves were selected at random in each selected plant. The standard disease severity score charts developed by Mayee and Dattar (1986) were used for the assessment of the diseases. The picture representation of powdery mildew and *Diaporthe* leaf spot are presented in Plates 2 and 3 respectively.

Score chart to assess the severity of powdery mildew disease in yard long bean

Grade	Description
0	No symptoms
1	Powdery flecks covering less than 1 per cent of leaf area
3	Powdery lesions covering 1 to 10 per cent of leaf area
5	Irregular, enlarged powdery lesions covering 11 to 25 per cent of leaf area
7	Irregular, white to grey powdery lesions covering 26 to 50 per cent of leaf area
9	Powdery lesions covering 50 per cent or more of leaf area with symptoms on pods and flowers as well

0

1

3

5

7

9

Plate 2. Score chart for the assessment of severity of powdery mildew in yard long bean caused by *Erysiphe polygoni*

0

1

3

5

7

9

Plate 3. Score chart for the assessment of severity of *Diaporthe* leaf spot in yard long bean caused *D. tulliensis*

Score chart to assess the severity of *D. tulliensis* leaf spot in yard long bean

Grade	Description
0	No symptoms
1	Small, circular to irregular, off white lesions with brown margin covering 1 per cent or less of leaf area
3	Irregular, off white lesions covering 1-10 per cent of leaf area
5	Lesions with brown margin covering 11-25 per cent of leaf area with drying of leaves starting from the leaf tip
7	Lesions covering 26-50 per cent of leaf area with prominent blighting and drying of leaves
9	Lesions covering more than 50 per cent of leaf area, with prominent blighting and drying of leaves followed by defoliation

Score chart to assess the severity of *Cercospora* leaf spot, rust and anthracnose

Grade	Description
0	Healthy
1	Less than one per cent of leaf area infected
3	1 to 10 per cent of leaf area infected
5	11-25 per cent of leaf area infected
7	26-50 per cent of leaf area infected
9	More than 50 per cent of leaf area infected

Based on the above score charts, disease severity of each disease was calculated using the formula developed by Wheeler (1969) as follows,

$$PDI = \frac{\text{Sum of individual ratings} \times 100}{\text{No. of leaves observed} \times \text{maximum grade}}$$

3.2. SCREENING OF YARD LONG BEAN VARIETIES

Five varieties of yard long bean *viz*., Lola, Vellayani Jyothika, Gitika (KAU varieties), VS 50 (KAU pre-release culture) and NS 621 (Namdhari Seeds Private Limited) were screened in the polyhouse located at IFSRS, Karamana to identify the major fungal foliar diseases affecting the varieties and the most susceptible variety to the major foliar fungal disease. The study was undertaken in completely randomized design (CRD) using the five varieties with four replications during Kharif, 2018. The seeds were sown in portrays containing the potting mixture of coirpith and vermicompost in the ratio of 1:1. Two weeks old seedlings were transplanted into UV stabilized 600 gauge, 150 μ growbags (40 cm x 35 cm x 32 cm) containing sand, soil and cowdung mixed in the ratio 1:1:1. Fertigation was provided as per KAU *ad hoc* recommendation for precision farming (KAU, 2011).

3.2.1. Biometric and yield attributes of varieties

3.2.1.1. Plant height (cm)

The height of the plant was recorded from the soil level to the tip of the top most leaf during final harvest.

3.2.2.2. Number of leaves

Total number of leaves per plant was recorded during final crop harvest.

3.2.2.3. Internodal length (cm)

Length from one node to the next node was measured during final crop harvest and was expressed in cm.

3.2.2.4. Number of lateral branches

Number of lateral branches from the main vine of the crop was recorded during final harvest.

3.2.2.5. Length of lateral branches (cm)

Length of lateral branches of each plant was recorded during final harvest and expressed in cm.

3.2.2.6. Pods per plant

Total number of pods per plant was recorded throughout the crop period.

3.2.2.7. Pod length

Ten pods per plant were selected at random during peak harvest stage. Length of pods was measured beginning from the base to apex of pods and expressed in cm.

3.2.2.8. Pod weight (g)

Weight of ten pods selected at random from each plant at peak harvest stage was measured and expressed in grams.

3.2.2.9. Seeds per pod

Seeds from ten pods selected at random were collected at peak harvest stage and the number was recorded.

3.2.2.10. Yield per plant (kg)

Weight of the total pods harvested from each plant throughout its crop period was measured and expressed in kilo gram.

3.2.2.11. Days to harvest

The duration between sowing to first harvest was recorded for each variety.

3.2.2.12. Number of harvests

Total number of harvests from each plant was recorded throughout its crop period.

3.2.2.13. Yield per harvest (kg)

Yield of pods during each harvest was recorded and expressed in kilo gram.

3.2.2. Disease incidence and severity

The natural incidence and severity of major fungal foliar diseases were determined in the five varieties of yard long bean which were screened in the polyhouse at IFSRS, Karamana. The DI was calculated as follows (Singh, 2002),

$$\text{Disease incidence (DI)} = \frac{\text{No. of infected plants}}{\text{Total number of plants}} \times 100$$

Ten plants (ten leaves per plant) of each variety were used to assess the disease severity. The standard score charts developed for powdery mildew and *Diaporthe* leaf spot diseases (Mayee and Dattar, 1986) were adopted. Based on the score charts, the disease severity was calculated as follows (Wheeler, 1969),

$$\text{PDI} = \frac{\text{Sum of individual ratings} \times 100}{\text{No. of leaves observed} \times \text{maximum grade}}$$

3.2.3. Isolation of *Diaporthe* leaf spot pathogen

3.2.3.1. Isolation of the pathogen

The leaf spot pathogen was isolated in potato dextrose agar (PDA) medium following the standard procedures developed by Rangaswami (1958). Infected leaf samples with typical leaf spot symptoms were washed in running water, cut into small bits (0.2 x 0.2 cm^2) and surface sterilized using 0.1 per cent mercuric chloride for one min followed by three washes in sterile distilled water.

The leaf bits were placed in petri plates (9 cm) containing solidified PDA medium and were incubated at room temperature (28 ± 2°C). The mycelial growth of the pathogen was observed and was further sub cultured to PDA slants to obtain the pure culture of the pathogen (Aneja, 2003; Rangaswami and Mahadevan, 2006).

3.2.3.2. Pathogenicity studies

The pathogenicity studies were conducted in two week old seedlings of the yard long bean var. NS 621. The leaves were gently pin pricked using sterilised needle and the mycelial bits (5 mm) of fifteen day old culture of the fungal pathogen was placed on the pricked leaf surface and was covered with moistened cotton. The seedlings inoculated with PDA discs alone served as the control. All the inoculated seedlings were enclosed using perforated poly propylene covers to maintain congenial relative humidity for the development of characteristic leaf spot symptoms (Tourville *et al.*, 1988; Jocić *et al.*, 2004).

3.2.4. Characterization and identification of *Diaporthe* leaf spot pathogen

3.2.4.1. Morphological and cultural identification

The morphology of the pathogen including mycelial and conidial characters was observed using lactophenol cotton blue stain under compound light microscope at a magnification of 1000X. The fungus was identified based on the morphological characters including mycelium, fruiting body and conidium. Cultural characteristics of the fungus were studied in PDA medium at room temperature. The growth pattern as well as the development of fruiting bodies of the fungus were observed in the growth medium.

3.2.4.2. Molecular identification

The genomic DNA of the fungus was isolated using NucleoSpin® Plant II Kit (Macherey-Nagel). Polymerase chain reaction was performed to amplify the internal transcribed spacer (ITS) region using specific primers (ITS 1

/ ITS4). Sequencing was performed using BigDye Terminator v3.1. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems) (White *et al.*, 1990). The obtained sequence was used for performing BLAST in NCBI database.

3.2.4.3. Phylogenetic analysis

Phylogenetic analysis was conducted in MEGA 7 software (Kumar *et al.*, 2016) to analyse the evolutionary identity of this Indian isolate of *Diaporthe tulliensis* isolated from yard long bean with that of the other exiting isolates throughout the world.

3.3. *IN VITRO* INHIBITION OF DIAPORTHE TULLIENSIS

In vitro efficacy of selected organic preparations, botanicals and non hazardous compounds (Table 1) were tested for their potential to inhibit the mycelial growth of *D. tulliensis* by poisoned food technique (Nene and Thapliyal, 1979) at IFSRS, Karamana.

3.3.1. Organic preparations, botanicals and non hazardous compounds used for *in vitro* inhibition assay

3.3.1.1. Fermented egg-lemon juice extract (FEE)

Hen eggs (12 nos.), sieved lemon juice (to keep the eggs dipped) and jaggery powder (500 g) were required for the preparation. The eggs were dipped in lemon juice for ten days in a container and covered with muslin clothe to avoid gas formation. After ten days, 500g of jaggery powder was added and the entire contents were stirred thoroughly and maintained for ten more days. The extract was ready after 21 days of preparation which was used at the rate of 10 per cent (Sajeena *et al.*, 2016).

3.3.1.2. Fermented weed extract (FWE)

Two hundred and fifty gram of the weed *viz.*, *Setaria barbata* (East Indian bristle grass / Corn grass / Mary grass) was washed and cut into small pieces in a container. Two grams of salt powder, jaggery powder, tamarind (pulp) and 250 ml

Table 1. Treatments selected for *in vitro* inhibition of *Diaporthe tulliensis*

Treatments	Description
T_1	Fermented egg-lemon juice extract 10% (FEE)
T_2	Fermented weed (*Setaria barbata*) extract 10% (FEW)
T_3	Potassium silicate 1% (PS)
T_4	FEE + FWE (1:1)
T_5	FEE + PS (1:1)
T_6	PS + FWE (1:1)
T_7	FEE+FWE+PS (1:1:1)
T_8	Sunflower oil (25 ml) + baking soda (10 g) + bar soap (10 g) + water (5 l)
T_9	Sodium bicarbonate 0.5%
T_{10}	Nimbecidine 0.5%
T_{11}	*Pseudomonas fluorescens* (KAU formulation) ($(1x10^8$ cfu $ml^{-1})$) (treated control)
T_{12}	Tebuconazole 50% + Trifloxystrobin 25% WG (@ 0.04% (treated control)
T_{13}	Untreated control

of water were added into the container (100 per cent stock solution). The preparation was kept for 21 days with daily stirring. The fermented extract after filtration through muslin cloth was used for further studies (Sajeena *et al.*, 2015).

3.3.1.3. Potassium silicate (PS) (1%)

Commercial formulation of potassium silicate (silica 25 % and potassium 7 %) at the rate of one per cent was used to screen its *in vitro* inhibition potential against the fungus.

3.3.1.4. FEE + FWE (1:1)

Fermented egg lemon juice extract (10 %) and fermented weed extract (10 %) were combined at 1:1 ratio by volume and used for the *in vitro* assay.

3.3.1.5. FEE + PS (1:1)

Fermented egg lemon juice extract (10 %) and potassium silicate (1 %) were combined at 1:1 ratio by volume and used for the inhibition study.

3.3.1.6. FWE + PS (1:1)

The combination of fermented weed extract (10 %) and potassium silicate (1 %) at 1:1 ratio by volume was used for the inhibition study.

3.3.1.7. FEE+ FWE + PS (1:1:1)

The combination of fermented egg lemon juice extract (10 %), fermented weed extract (10 %) and potassium silicate (1 %) at 1:1:1 ratio by volume was used for the inhibition assay.

3.3.1.8. Sunflower oil + baking soda + bar soap + water

Sunflower oil (25 ml), baking soda (10 g), bar soap (10 g) and water (5 L) were mixed together and used for the inhibition assay (Peethambaran and Reghunath, 2014).

3.3.1.9. Sodium bicarbonate

The commercially available sodium bicarbonate was used at the rate of 0.5 per cent to screen its potential for *in vitro* mycelial growth inhibition of the fungus.

3.3.1.10. Nimbecidine

The commercial formulation of nimbecidine was used at the rate of 0.5 per cent for the *in vitro* inhibition study.

3.3.1.11. Treated controls

The biocontrol agent *viz*., *Pseudomonas fluorescens* (KAU isolate) and the combination fungicide *viz*., tebuconazole (50%) + trifloxystrobin (25%) WG (0.04%) were maintained as the treated controls.

3.3.2. *In vitro* evaluation of organic preparations, botanicals and non hazardous compounds against *D. tulliensis*

Four hundred grams of potato, forty grams each of dextrose and agar as well as one litre of water were used for the preparation of double strength potato dextrose agar (PDA) medium. The media was sterilized at 121°C and 15 psi pressure for 20 min. The organic preparations, botanicals and their combinations except potassium silicate, sodium bicarbonate, nimbecidine and tebuconazole (50%) + trifloxystrobin (25%) WG were filtered through muslin cloth and Whatman No. 1 filter paper. Subsequently, the filtrates were passed through bacterial filters aseptically in laminar air flow chamber. The treatments as well as the treated control *viz*., tebuconazole (50%) + trifloxystrobin (25%) WG were mixed with the required quantity of sterile, distilled water and amended to the double strength PDA medium. Fifteen mL of the amended medium was poured in sterilized petri plates (9 cm diameter). After solidification of the media, culture discs (5 mm) of seven days old *D. tulliensis* was placed at the centre of medium amended plates and incubated at room temperature (28 ± 2^{0}C). The efficacy of *P. fluorescens* (treated control) to inhibit the mycelial growth of *D. tulliensis* was

tested by dual culture method (Dennis and Webster, 1971). The petri plates containing unamended double strength PDA media was maintained as untreated control for the inhibition assay.

When the mycelial growth of the fungus in untreated control plate was completed (9 cm), the mycelial growth in diameter (cm) and per cent mycelial growth inhibition of *D. tulliensis* in each treatment amended medium were recorded. The per cent mycelial growth inhibition was calculated according to the formula developed by Vincent (1947) as follows,

$$I = \frac{C-T}{C} \times 100$$

I - per cent inhibition of mycelial growth of the fungus

C - growth of the fungus (diameter in cm) in unamended media

T - growth of the fungus (diameter in cm) in treatment amended media

3.3.3. Selection of best treatments

The two best treatments which had the potential to inhibit the mycelial growth of the fungus to the maximum extend during the *in vitro* inhibition assay were selected for *in vivo* management studies.

3.4 *IN VIVO* MANAGEMENT STUDIES ON *DIAPORTHE* LEAF SPOT AND POWDERY MILDEW DISEASES OF YARD LONG BEAN IN POLYHOUSE

A pot culture experiment was conducted in the polyhouse at IFSRS, Karamana during Rabi, 2018 to test the efficacy of selected treatments in reducing the incidence and severity of the two most important fungal foliar diseases of yard long bean (*Diaporthe* leaf spot and powdery mildew) identified from the survey and varietal screening. The study was conducted in the yard long bean var. NS 621 using eight treatments with three replications in CRD. The first two treatments of the trial were the foliar application (at 20, 40 and 60 days after planting (DAP)) of the two best effective treatments selected from the *in vitro* trial and the third treatment was the soil application of arbuscular mycorrhizal fungi

(AMF) (KAU formulation) (5g plant $^{-1}$) at the time of planting. The fourth and fifth treatments included the soil application of AMF and foliar application (20, 40 and 60 DAP) of the first and second best effective treatments respectively. The sixth and seventh treatments were the foliar application (20, 40 and 60 DAP) of tebuconazole (50%) + trifloxystrobin (25%) WG (0.04%) and *P. fluorescens* (2%) ($1x10^8$ cfu g^{-1}) (treated controls) respectively. The plants sprayed with water at 20, 40 and 60 DAP (untreated control) served as the eighth treatment.

3.4.1. Biometric and yield attributes

Observations as that in **3.2.1.** were recorded.

3.4.2. Disease incidence and severity

The efficacy of the selected treatments on the natural incidence and severity of *Diaporthe* leaf spot and powdery mildew diseases were determined in var. NS 621 of yard long bean in the polyhouse at IFSRS, Karamana. The disease incidence (DI) was calculated using the formula by Singh (2002) as follows,

$$\text{Disease incidence (DI)} = \frac{\text{No. of infected plants}}{\text{Total number of plants}} \times 100$$

Ten plants from each treatment at the rate of ten leaves per plant were randomly selected to assess the disease severity. The standard score charts developed by Mayee and Dattar (1986) were adopted as in the case of the varietal screening trial. Based on the score charts, disease severity for the two diseases was calculated using the formula (Wheeler, 1969) as follows,

$$\text{PDI} = \frac{\text{Sum of individual ratings} \times 100}{\text{No. of leaves observed} \times \text{maximum grade}}$$

3.5 STATISTICAL ANALYSIS

The data were statistically analyzed using analysis of variance (ANOVA). The data recorded on PDI and per cent mycelial inhibition were transformed using arc sine transformation. The data on mycelial growth in diameter were transformed using square root transformation. The Duncans Multiple Range Test (DMRT) (Steel and Torrie, 1960) was used in all the data derived from the various experiments conducted as part of this study.

Results

4. RESULTS

The study entitled "Ecofriendly management of major fungal foliar diseases affecting yard long bean in polyhouse" was conducted at College of Agriculture, Vellayani and Integrated Farming System Research Station (IFSRS), Karamana during 2017-2019 with an objective to determine the major fungal foliar diseases affecting yard long bean grown in polyhouse and their management using natural resources and ecofriendly methods. The collected experimental data were statistically analysed. The results obtained are detailed in this chapter.

4.1. SURVEY LOCATIONS, VARIETIES AND INCIDENCE & SEVERITY OF MAJOR FUNGAL FOLIAR DISEASES OF YARD LONG BEAN

4.1.1. Location of survey

The survey was conducted in 15 polyhouses located in Thirupuram panchayat (Parassala block), Vellanadu, Kuttichal and Tholicode panchayats (Kattakada block), Malayinkeezhu panchayat, Vilavoorkal panchayat and Thiruvananthapuram Corporation (3 sites) (Pallichal block), Vembayam and Nedumangad panchayats (Nedumangad block), Pothencode panchayat (Kazhakoottam block) as well as in Manickal (2 sites) and Nanniyode panchayats (Vamanapuram block) of Thiruvananthapuram district, where yard long bean cultivation was undertaken (Table 2).

4.1.2. Variety, diseases and pests of yard long bean

Among the 15 polyhouses surveyed, NS 621 was the major variety of yard long bean cultivated in ten polyhouses (Thirupuram, Kuttichal, Tholicode, Vilavoorkal, Thiruvananthapuram Corporation (1 site), Vembayam, Nedumangad, Manickal (2 sites) and Nanniyode). The other varieties of yard long bean cultivated were Haari (Vellanadu), Vellayani Jyothika (Malayinkeezhu), Gitika (Thiruvananthapuram Corporation) (1 site) and Babli (Thiruvananthapuram

Table 2. Locations of polyhouses surveyed to assess different diseases of yard long bean in Thiruvananthapuram district

Sl. No.	Blocks	Panchayat	No. of polyhouses
1	Parassala	Thirupuram	1
2	Kattakada	Vellanadu, Kuttichal,Tholicode	3
3	Pallichal	Malayinkeezhu, Vilavoorkal, Thiruvananthapuram corporation (3)	5
4	Nedumangad	Vembayam , Nedumangad	2
5	Kazhakoottam	Pothencode	1
6	Vamanapuram	Nanniyode, Manickal (2)	3
		Total polyhouses surveyed	15

Corporation) (1 site) and Pothencode). Powdery mildew, *Diaporthe* leaf spot, *Cercospora* leaf spot, anthracnose and rust were the major fungal diseases observed in these polyhouses (Plate 4). The major pests observed included leaf miner, red spider mite, thrips, aphids, mealy bug and leaf eating caterpillar (Table 3).

4.1.3. Disease incidence and severity

Among the diseases observed in yard long bean cultivated in the various polyhouses *viz*., powdery mildew, *Diaporthe* leaf spot, *Cercospora* leaf spot, anthracnose and rust, the maximum incidence and severity were observed for powdery mildew and *Diaporthe* leaf spot diseases. The maximum incidence (100 %) and severity (60.70 %) of powdery mildew was observed in the variety NS 621, cultivated at Thirupuram which was at its final harvest. The variety NS 621 in the polyhouse located at Manickal revealed the maximum incidence (80 %) and severity (45.77 %) of *Diaporthe* leaf spot at its final harvest (Table 4).

4.2. SCREENING OF YARD LONG BEAN VARIEITES

4.2.1. Biometric and yield attributes of the varieties

The five varieties were screened in the polyhouse and their various biometric attributes *viz*., plant height, number of leaves, internodal length, number of lateral branches and length of lateral branches were compared during the final harvest. There was no significant difference between the varieties with regard to the number of leaves, internodal length and length of lateral branches. The maximum plant height was observed in NS 621 (508.47 cm) followed by Vellayani Jyothika (481.18 cm). The varieties, Gitika and VS 50 had the maximum number of lateral branches (5.75 and 5.63 respectively) which were on par with each other (Table 5).

There was significant difference between the varieties in terms of all their yield attributes. The yield parameters *viz*., pod length, pod weight and seeds per pod were recorded at peak harvest. The varieties, VS 50 and NS 621 recorded the

Powdery mildew (*Erysiphe polygoni)*

Diaporthe leaf spot (*D. tulliensis*)

Rust (*Uromyces* sp.)

Anthracnose (*Colletotrichum lindemuthianum*)

Cercospora leaf spot (*C. canescens*)

Plate 4. Major fungal foliar diseases of yard long bean cultivated in polyhouses of Thiruvananthapuram district

Table 3. Variety, crop stage and major diseases and pests of yard long bean cultivated in polyhouses of Thiruvananthapuram (TVPM) district

Sl. No.	Location of polyhouse	Area (sq. m.)	Variety	Crop stage	Fungal diseases observed	Pests observed
1	Thirupuram (Parassala block)	400	NS 621	Towards final harvest	Powdery mildew	Red spider mite
2	Vellanadu (Kattakada block)	400	Haari	Pod formation	Powdery mildew, *Diaporthe* leaf spot	Red spider mite, Thrips
3	Kuttichal (Kattakkada block)	864	NS 621	Flowering	*Diaporthe* leaf spot	Aphid, Red spider mite
4	Tholicode (Kattakkada block)	1600	NS 621	Flowering	Diseases not observed	Leaf miner
5	Malayinkeezhu (Pallichal block)	372	Vellayani Jyothika	Pod formation	Powdery mildew	Leaf miner
6	Vilavoorkal (Pallichal block)	420	NS 621	Pod formation	Powdery mildew, *Cercospora* leaf spot	Pests not observed
7	TVPM corporation (Pallichal block)	450	NS 621	Pod formation	Powdery mildew, Rust, Anthracnose	Pests not observed

Table 3. Variety, crop stage and major diseases and pests of yard long bean cultivated in polyhouses of Thiruvananthapuram district (Continued)

Sl. No.	Location of polyhouse	Area (sq. m.)	Variety	Crop stage	Fungal diseases observed	Pests observed
8	TVPM corporation (Pallichal block)	400	Gitika	Towards final harvest	Powdery mildew	Red spider mite
9	TVPM corporation (Pallichal block)	350	Babli	Flowering	*Diaporthe* leaf spot	Red spider mite
10	Vembayam (Nedumangad block)	400	NS 621	Pod formation	Powdery mildew, *Cercospora* leaf spot	Red spider mite, Mealy bug
11	Nedumangad (Nedumangad block)	1200	NS 621	Pod formation	*Diaporthe* leaf spot	Leaf miner
12	Pothencode (Kazhakoottam block)	400	Babli	Pod formation	Powdery mildew, *Diaporthe* leaf spot, Rust, Anthracnose	Pests not observed
13	Nanniyode (Vamanapuram block)	400	NS 621	Towards final harvest	Powdery mildew, *Diaporthe* leaf spot	Red spider mite, Mealy bug, Leaf eating caterpillar
14	Manickal (Vamanapuram block)	400	NS 621	Towards final harvest	Powdery mildew, *Diaporthe* leaf spot,	Red spider mite
15	Manickal (Vamanapuram block)	134	NS 621	Vegetative	Anthracnose	Red spider mite, Thrips

Table 4. Incidence and severity of major diseases of yard long bean cultivated in polyhouses of Thiruvananthapuram district

Sl. No.	Location of polyhouse	Variety	Crop stage	Powdery mildew		*Diaporthe* leaf spot		*Cercospora* leaf spot		Rust		Anthracnose	
				DI (%)	PDI	DI (%)	PDI	DI (%)	PDI	DI (%)	PDI	DI (%)	PDI
1	Thirupuram (Parassala)	NS621	Towards final harvest	100.00	60.70	0	0	0	0	0	0	0	0
2	Vellanadu (Kattakada)	Haari	Pod formation	76.00	41.33	34.40	16.41	0	0	0	0	0	0
3	Kuttichal (Kattakkada)	NS 621	Flowering	0	0	33.33	13.69	0	0	0	0	0	0
4	Tholicode (Kattakkada)	NS 621	Flowering	0	0	0	0	0	0	0	0	0	0
5	Malayinkeezhu (Pallichal)	Vellayani Jyothika	Pod formation	65.60	33.56	0	0	0	0	0	0	0	0
6	Vilavoorkal (Pallichal)	NS 621	Pod formation	70.00	50.70	0	0	14.40	5.88	0	0	0	0
7	TVPM corporation (Pallichal)	NS 621	Pod formation	40.00	23.82	0	0	0	0	10.40	5.33	30.40	22.88
8	TVPM corporation (Pallichal)	Gitika	Towards final harvest	73.60	51.06	0	0	0	0	0	0	0	0

Table 4. Disease incidence and severity of major diseases of yard long bean cultivated in polyhouses of Thiruvananthapuram district (Continued)

Sl. No.	Location of polyhouse	Variety	Crop stage	Powdery mildew		*Diaporthe* leaf spot		*Cercospora* leaf spot		Rust		Anthracnose	
				DI (%)	PDI	DI (%)	PDI	DI (%)	PDI	DI (%)	PDI	DI (%)	PDI
9	TVPM corporation (Pallichal)	Babli	Flowering	0	0	28.80	13.69	0	0	0	0	0	0
10	Vembayam (Nedumangad)	NS 621	Pod formation	93.33	59.10	0	0	9.60	3.55	0	0	0	0
11	Nedumangad (Nedumangad)	NS 621	Pod formation	0	0	44.80	23.99	0	0	0	0	0	0
12	Pothencode (Kazhakoottam)	Babli	Pod formation	52.80	13.45	41.60	21.84	0	0	8.66	3.33	40.00	25.10
13	Nanniyode (Vamanapuram)	NS 621	Towards final harvest	81.60	51.56	66.67	42.18	0	0	0	0	0	0
14	Manickal (Vamanapuram)	NS 621	Vegetative	0	0	0	0	0	0	0	0	25.60	21.66
15	Manickal (Vamanapuram)	NS 621	Towards final harvest	58.40	37.74	80.00	45.77	0	0	0	0	0	0

Table 5. Performance of different varieties of yard long bean based on biometric characters at final harvest in the polyhouse

Sl. No.	Varieties	Plant height (cm)	No. of leaves	Internodal length (cm)	Number of lateral branches	Length of lateral branches (cm)
1	Lola	467.43 ± 2.16^{c}	50.88 ± 1.20	33.64 ± 1.04	5.25 ± 0.28^{ab}	165.74 ± 5.97
2	Vellayani Jyothika	481.18 ± 2.56^{b}	49.44 ± 1.29	34.77 ± 0.83	4.81 ± 0.33^{bc}	172.54 ± 8.78
3	Gitika	464.54 ± 2.09^{c}	48.50 ± 1.09	34.15 ± 0.97	5.75 ± 0.25^{a}	164.49 ± 7.15
4	VS 50	409.98 ± 5.87^{d}	50.50 ± 1.51	34.36 ± 0.97	5.63 ± 0.27^{a}	172.70 ± 5.63
5	NS 621	508.47 ± 8.26^{a}	50.81 ± 1.54	33.81 ± 0.60	4.25 ± 0.21^{c}	169.84 ± 6.41
	CD (0.05)	13.733	NS	NS	0.770	NS
	SEm±	4.865	1.34	0.895	0.273	6.88

*Mean ± SD of sixteen replications

Values followed by similar superscripts are not significantly different at 5% level

maximum pod length (61.39 cm and 60.89 cm respectively) which were on par with each other. The maximum pod weight was observed in NS 621 (25.95 g). The next highest pod weight was observed in two varieties *viz*., Gitika (25.41 g) and VS 50 (24.94 g) which were on par with each other. NS 621 had the maximum number of seeds per pod (21.63) followed by VS 50 (20.75) (Table 6).

The performance of the varieties in terms of other major yield attributes *viz*., pods per plant, yield per plant, days to harvest, number of harvests as well as yield per harvest throughout the crop period was also compared. The two varieties *viz*., NS 621 and Gitika revealed the maximum number of pods per plant (49.25 and 46.13 respectively) and were on par with each other. The highest pod yield per plant was recorded in NS 621 (1.30 kg) followed by Gitika (1.12 kg). The variety, NS 621 took the minimum days to first harvest (42.06), followed by VS 50 (43.19). The maximum number of harvests was recorded in NS 621 (16.13) and Gitika (16.06) which were on par with each other, followed by Vellayani Jyothika (15.06). The maximum yield per harvest per plant was observed in NS 621 (0.081 kg) followed by Gitika (0.070 kg) (Table 7). Thus, it was observed that among the five varieties, NS 621 was superior to others in terms of plant height and all the yield attributes.

4.2.2. Disease incidence and severity

The natural incidence and severity of various fungal foliar diseases were assessed in the five varieties of yard long bean cultivated in the polyhouse at IFSRS, Karamana. *Diaporthe* leaf spot was the most important disease observed in all the varieties during the trial followed by powdery mildew disease (Plate 5). The incidence and severity of the two diseases were assessed during 30, 60 and 90 days after planting (DAP) in the five varieties. Among the varieties screened, the maximum incidence and severity of *Diaporthe* leaf spot at 30 DAP (50 % and 16.11 %), 60 DAP (75 % and 32.67 %) and 90 DAP (100 % and 58.66 % respectively were

Table 6. Performance of different varieties of yard long bean on yield characters at peak harvest in polyhouse

Sl. No.	Varieties	Pod length (cm)	Pod weight (g)	Seeds per pod
1	Lola	52.48 ± 0.95^{b}	20.85 ± 0.37^{c}	20.00 ± 0.37^{b}
2	Vellayani Jyothika	55.29 ± 1.60^{b}	23.77 ± 0.74^{b}	20.31 ± 0.34^{b}
3	Gitika	46.61 ± 0.95^{c}	25.41 ± 0.65^{ab}	19.81 ± 0.44^{b}
4	VS 50	61.39 ± 1.11^{a}	24.94 ± 0.53^{ab}	20.75 ± 0.54^{ab}
5	NS 621	60.89 ± 1.17^{a}	25.95 ± 0.79^{a}	21.63 ± 0.33^{a}
	CD (0.05)	3.329	1.787	1.154
	SEm±	1.179	0.633	0.409

* Mean ± SD of sixteen replications

Values followed by similar superscripts are not significantly different at 5% level

Table 7. Performance of different varieties of yard long bean on yield characters in polyhouse

Sl. No.	Varieties	Pods per plant	Yield per plant (kg)	Days to harvest	Number of harvests	Yield per harvest (kg)
1	Lola	39.56 ± 1.04^{b}	0.84 ± 0.01^{d}	44.88 ± 0.46^{a}	14.19 ± 0.33^{c}	0.060 ± 0.002^{d}
2	Vellayani Jyothika	40.88 ± 0.95^{b}	0.97 ± 0.03^{c}	44.56 ± 0.35^{ab}	15.06 ± 0.28^{b}	0.065 ± 0.002^{c}
3	Gitika	46.13 ± 1.81^{a}	1.12 ± 0.04^{b}	43.44 ± 0.35^{bc}	16.06 ± 0.31^{a}	0.070 ± 0.002^{b}
4	VS 50	32.44 ± 0.66^{c}	0.82 ± 0.02^{d}	43.19 ± 0.38^{cd}	15.00 ± 0.26^{bc}	0.055 ± 0.002^{e}
5	NS 621	49.25 ± 1.08^{a}	1.30 ± 0.05^{a}	42.06 ± 0.50^{d}	16.13 ± 0.26^{a}	0.081 ± 0.004^{a}
	CD (0.05)	3.311	0.090	1.172	0.815	0.007
	SEm±	1.173	0.032	0.415	0.289	0.002

*Mean ± SD of sixteen replications

Values followed by similar superscripts are not significantly different at 5% level

Plate 5. Severity of powdery mildew (left) and *Diaporthe* leaf spot (right) in yard long bean var. NS 621 in the polyhouse

observed in NS 621. The minimum incidence and severity of the disease were observed in VS 50 at 90 DAP (75 % and 34.66 %) (Table 8; Fig. 1 and 2).

The maximum incidence and severity of powdery mildew disease was observed in NS 621 (Plate 5) at 30 DAP (37.50 % and 23.44 %), 60 DAP (50 % and 29.11 %) and 90 DAP (62.50 % and 50.89 %). VS 50 revealed the minimum incidence and severity of powdery mildew at 90 DAP (37.50 % and 11.11 %) (Table 9; Fig. 3 and 4).

The results from the survey of polyhouses of Thiruvananthapuram district as well as the varietal screening revealed the presence of a new fungal disease *viz.*, *Diaporthe* leaf spot in yard long bean. The leaf spot disease was manifested as small, circular to irregular, off white lesions with characteristic brown margins which later coalesced to result in leaf blighting. Characteristic drying of the leaves were observed starting from leaf tip and proceeding downwards leading to drying and defoliation.

4.2.3. Isolation of *Diaporthe* leaf spot pathogen

4.2.3.1. Isolation of the pathogen

The fungus was isolated from the disease affected leaf samples of yard long bean on PDA medium using standard protocols.

4.2.3.2. Pathogenicity studies

Characteristic circular to irregular, off white lesions with brown margins were observed on fifteenth day after inoculation (DAI) on the inoculated leaves. Blighting and drying of the leaves were observed which later resulted in defoliation (Plate 6).

4.2.4. Identification of *Diaporthe* leaf spot pathogen

Table 8. Natural incidence and severity leaf spot disease in different varieties of yard long bean caused by *D. tulliensis* in polyhouse at 30, 60 and 90 DAP

Sl. No.	Variety	30 DAP		60 DAP		90 DAP	
		DI (%)	PDI	DI (%)	PDI	DI (%)	PDI
1	Lola	37.50	7.66 (12.43)^{b}	62.50	18.88 (23.96)^{b}	100	47.44 (43.49)^{b}
2	Vellayani Jyothika	31.25	7.00 (11.71)^{b}	56.25	16.11 (20.73)^{b}	100	48.10 (43.87)^{b}
3	Gitika	31.25	5.22 (9.17)^{b}	56.25	15.77 (20.76)^{b}	81.25	43.88 (41.36)^{b}
4	VS 50	31.25	2.44 (5.64)^{b}	35.25	9.77 (15.19)^{b}	75.00	34.66 (35.98)^{c}
5	NS 621	50	16.11 (21.18)^{a}	75.00	32.67 (34.53)^{a}	100	58.66 (50.07)^{a}
	CD (0.05)	-	9.349	-	9.857	-	4.808
	SEm±	-	3.271	-	3.449	-	1.682

*Mean ± SD of sixteen replications

Values followed by similar superscripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

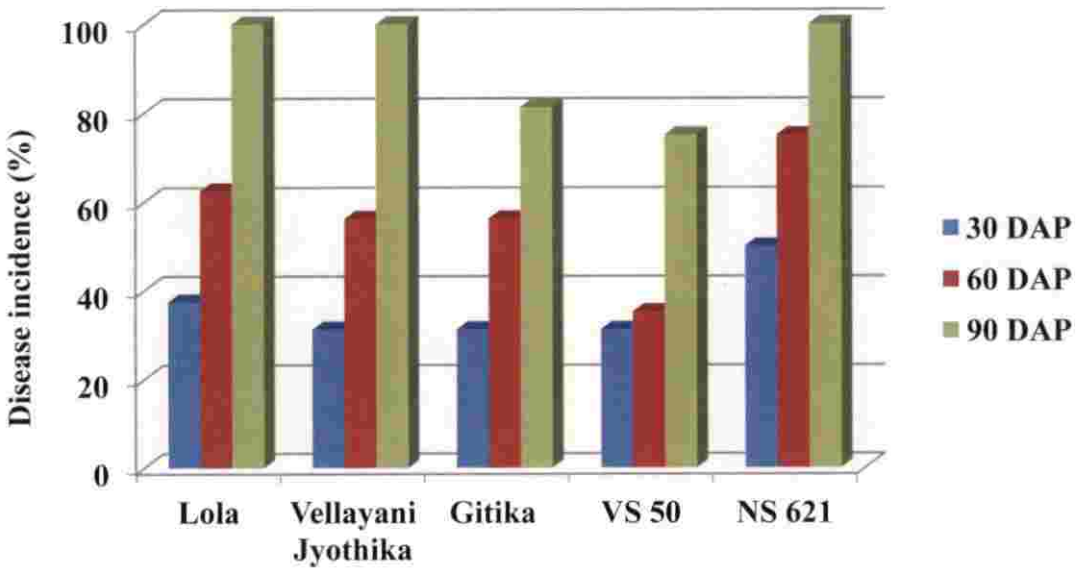


Fig. 1. Incidence of leaf spot disease of yard long bean caused by *D. tulliensis* in different varieties in the polyhouse at 30, 60 and 90 DAP

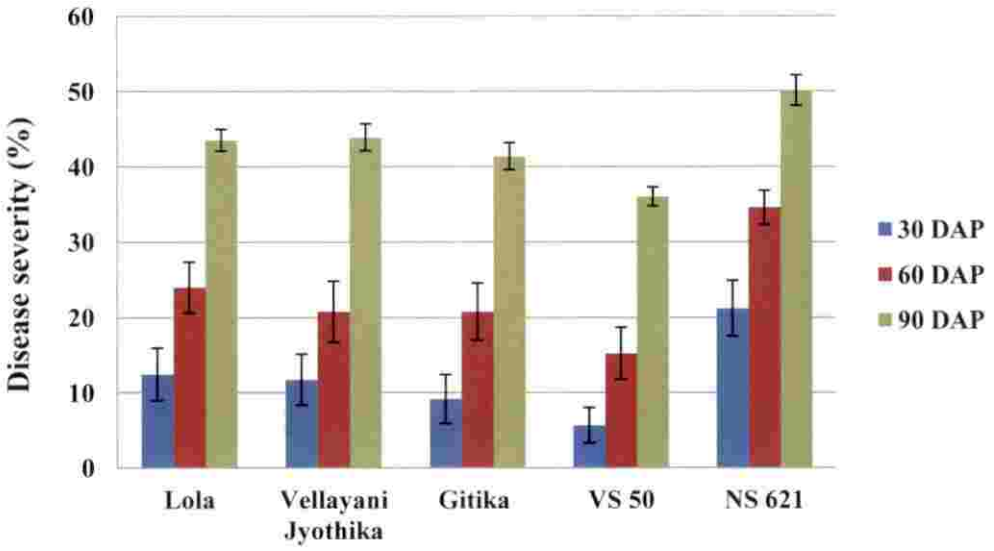


Fig. 2 Severity of leaf spot disease of yard long bean caused by *D. tulliensis* in different varieties in the polyhouse at 30, 60 and 90 DAP

Table 9. Natural incidence and severity of powdery mildew disease in different varieties of yard long bean caused by *E. polygoni* in polyhouse at 30, 60 and 90 DAP

Sl. No.	Variety	30 DAP		60 DAP		90 DAP	
		DI (%)	PDI	DI (%)	PDI	DI (%)	PDI
1	Lola	25.00	8.22 (11.76)^{ab}	37.50	14.44 (17.52)^{b}	43.75	34.66 (35.98)^{b}
2	Vellayani Jyothika	25.00	7.44 (11.00)^{b}	31.25	8.44 (12.02)^{b}	43.75	23.11 (25.71)^{c}
3	Gitika	25.00	6.66 (10.34)^{b}	31.25	12.55 (16.19)^{b}	37.50	18.22 (21.23)^{cd}
4	VS 50	18.75	2.78 (6.04)^{b}	25.00	6.55 (9.50)^{b}	37.50	11.11 (16.15)^{d}
5	NS 621	37.50	23.44(24.57)^{a}	50.00	29.11 (30.83)^{a}	62.50	50.89 (45.48)^{a}
	CD (0.05)	-	(11.716)	-	(12.386)	-	(10.45)
	SEm±	-	(4.1)	-	(4.334)	-	(3.66)

*Mean ± SD of sixteen replications

Values followed by similar superscripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

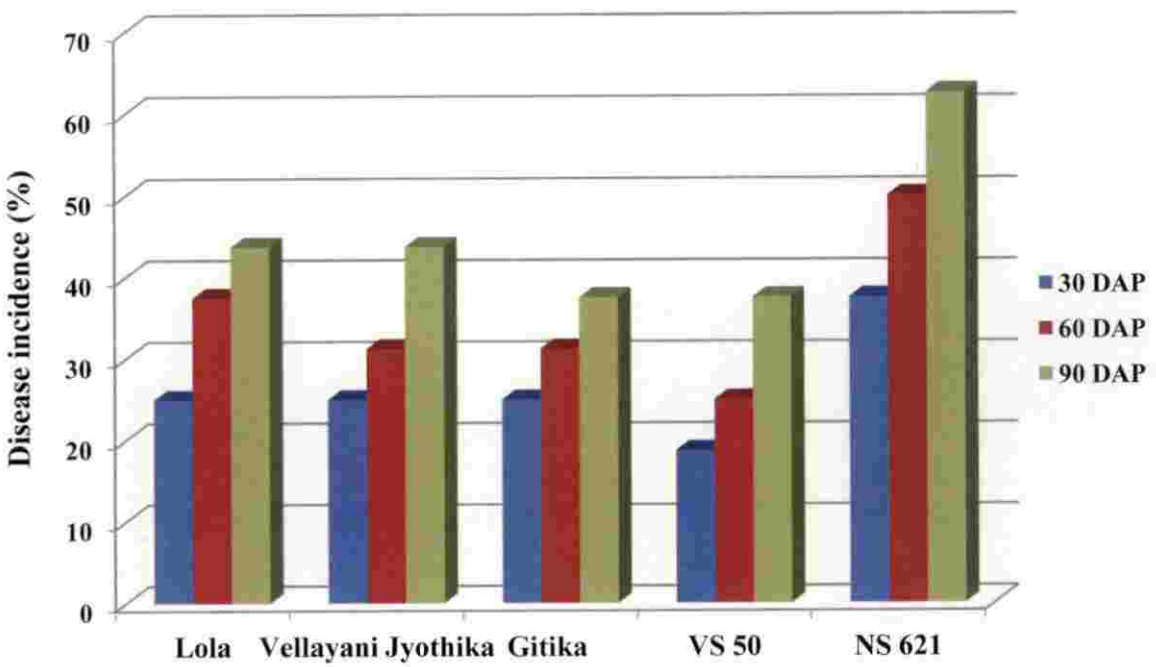


Fig. 3. Incidence of powdery mildew disease of yard long bean caused by *Erysiphe polygoni* in different varieties in the polyhouse at 30, 60 and 90 DAP

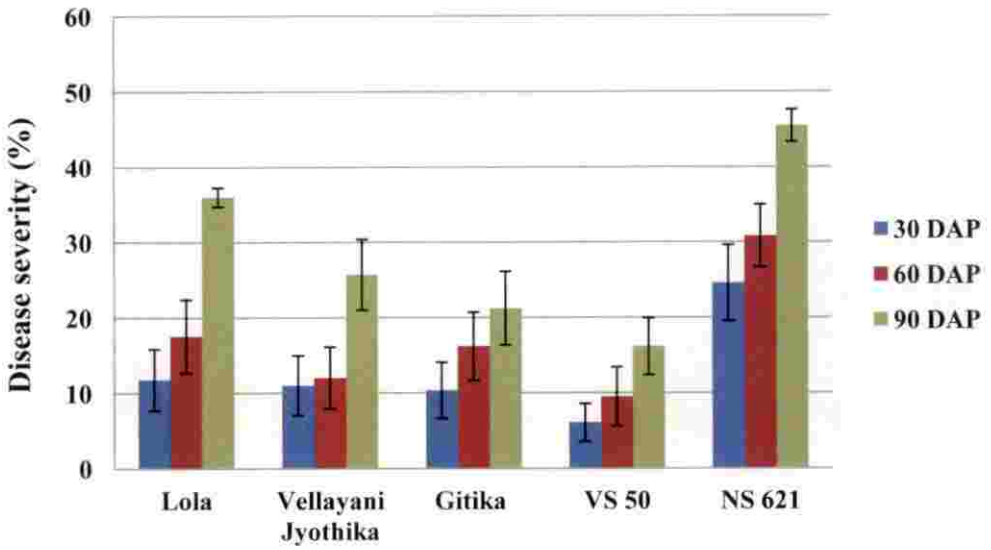
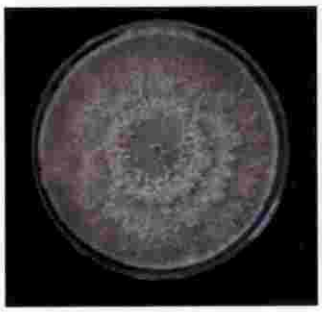


Fig. 4. Severity of powdery mildew disease of yard long bean caused by *Erysiphe polygoni* in different varieties in the polyhouse at 30, 60 and 90 DAP

Isolation of the fungus



Diaporthe leaf spot

Isolated *D. tulliensis* in PDA

Artificial inoculation on yard long bean

Re-isolation

Re-isolated *D. tulliensis* in PDA

Symptom expression after inoculation

Plate 6. Pathogenicity test of *D. tulliensis* causing leaf spot in yard long bean

4.2.4.1. Morphological and cultural identification

The growth of the fungus appeared as whitish mycelia turning to greyish white in colour on PDA medium. The growth was characterised by the presence of aerial mycelia and concentric grey coloured zonations on top view. The reverse view appeared as off white growth with characteristic grey zonations (Plate 7). The fungus took four days to complete its growth in petri plates (9 cm) containing PDA medium.

The asexual fruiting bodies of the fungus *viz*., the conidiomata (0.4 x 0.3 cm^2) appeared on the seventh day after growth (DAG) on PDA medium as initially white, flat mass of mycelia which later turned to white blisters with black centre and honey dew secretions on the top (Plate 8). The mycelia were septate with an average width of 3.55 µm at 1000X magnification. Alpha conidia (4.41- 5.11 µm × 1.57- 2.24 µm) were single celled, hyaline, ovoid in shape with a submedian constriction (Plate 9). Beta conidia were not observed.

4.2.4.2. Molecular identification

The molecular identification of the fungal pathogen was performed based on Internal Transcribed Spacer (ITS) regions, specifically with ITS 1F/4R primers. The amplicon was sequenced and the sequence similarity was assessed. The fungus revealed 99.77 per cent identity with three strains *viz*., TS-129, KFRD-49 and K.L.Chen L133 with accession numbers *viz*., MG832549.1, KX866889.1 and KT821499.1 respectively of *Diaporthe tulliensis* on BLAST analysis in National Center for Biotechnology Information (NCBI) database (Table 10). Thus, the fungus isolated from the leaf spot symptoms was confirmed to be *Diaporthe tulliensis*, which is the first report of the fungus in yard long bean in India. The sequence of *D. tulliensis* was deposited in NCBI and the accession number assigned by NCBI was MN267826.

Upper side view

2nd day | 4th day | 7th day | 10th day

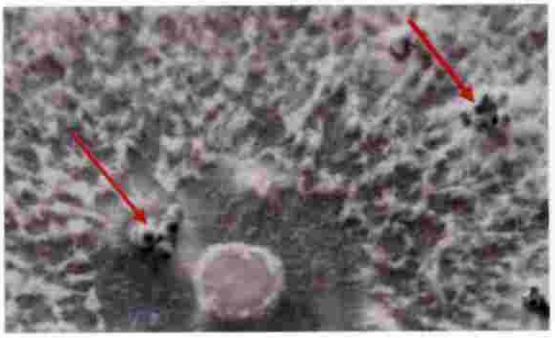
Rear side view

2nd day | 4th day | 7th day | 10th day

Plate 7. Growth pattern of *D. tulliensis* from 2 to 10 days after growth (DAG) on potato dextrose agar (PDA) medium at room temperature

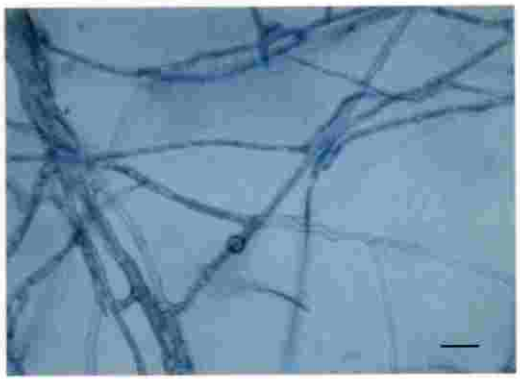
15th day

30th day

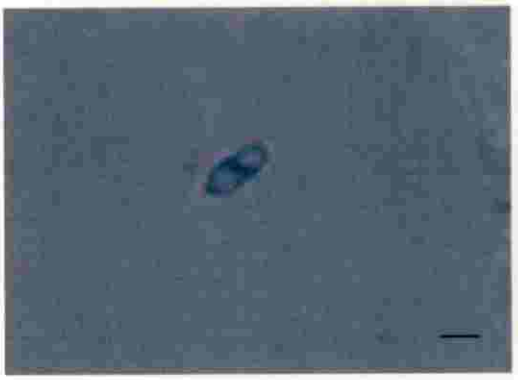


Conidiomata

Plate 8. Development of Conidiomata of *D. tulliensis* on PDA medium at 15th and 30th DAG



Mycelia of *D. tulliensis* at 1000X



Alpha conidium of *D. tulliensis* at 1000X

Plate 9. Morphological characters of *D. tulliensis* causing leaf spot disease in yard long bean (Scale bar – 10 µm)

Table 10. Molecular identification of *D. tulliensis* based on ITS region and BLAST at NCBI

Gene Bank Accession No.	Description	Maximum score	Total score	Query coverage	E value	Identity (%)
MG832549.1	*Diaporthe tulliensis* strain TS-129	798	798	100 %	0.0	99.77 %
KX866889.1	*Diaporthe tulliensis* strain KFRD-49	798	798	100 %	0.0	99.77%
KT821499.1	*Diaporthe tulliensis* strain K. L. Chen L133	798	798	100 %	0.0	99.77%
KX688170.1	*Diaporthe tulliensis* strain HL31	793	793	100%	0.0	99.54%
KX457967.1	*Diaporthe tulliensis* isolate LP-1	793	793	100%	0.0	99.54%
MF480336.1	*Diaporthe tulliensis* isolate CJMR99	784	784	100%	0.0	99.08%
NR_147574.1	*Diaporthe tulliensis* BRIP 62248a ITS region; from TYPE material	765	765	100%	0.0	98.39%

4.2.4.3. Phylogenetic analysis

The phylogenetic relationship of the Indian isolate of *D. tulliensis* identified from yard long bean during the present study was compared in NCBI with the existing isolates of *D. tulliensis* throughout the world to study its evolutionary history. The phylogenetic tree was constructed using the neighbour-joining method in MEGA 7 soft ware programme. The analysis revealed that the isolate of *D. tulliensis* identified from yard long bean which has been designated as "Indian" isolate in NCBI was having more close relationship with the Taiwan isolate of *D. tulliensis* (strain K.L. Chen L133) from lotus and the China isolate 2 of *D. tulliensis* (strain KFRD-49) from kiwi fruit. The isolate was also found to be distantly related to the Indian isolate of *D. tulliensis* from jute (isolate CJMR99) and China isolate 1 (strain TS-129) from kiwi fruit (Fig. 5).

4.3. *IN VITRO* INHIBITION OF *D. tulliensis*

Selected organic preparations, botanicals and non hazardous compounds were screened for their potential to inhibit the mycelial growth of *D. tulliensis* in PDA medium using poisoned food technique. Among the treatments, fermented *S. barbata* extract (10 %) (11.9 pH), sodium bicarbonate (0.5 %) (8.45 pH), combination of fermented egg lemon juice extract (10 %) and fermented *S. barbata* extract (10 %) (5.13 pH) as well as the treated control *viz*., tebuconazole (50 %) + trifloxystrobin (25 %) WG (0.04%) resulted in 100 per cent mycelial inhibition of the fungus. Fermented egg lemon juice extract (10 %) (4.5 pH), potassium silicate (1 %) (10.8 pH) as well as *Pseudomonas fluorescens* (KAU isolate) had no inhibitory effect on the mycelial growth of the fungus (Table 11; Plate 10).

4.4 *IN VIVO* MANAGEMENT STUDIES ON POWDERY MILDEW AND *DIAPORTHE* LEAF SPOT DISEASES

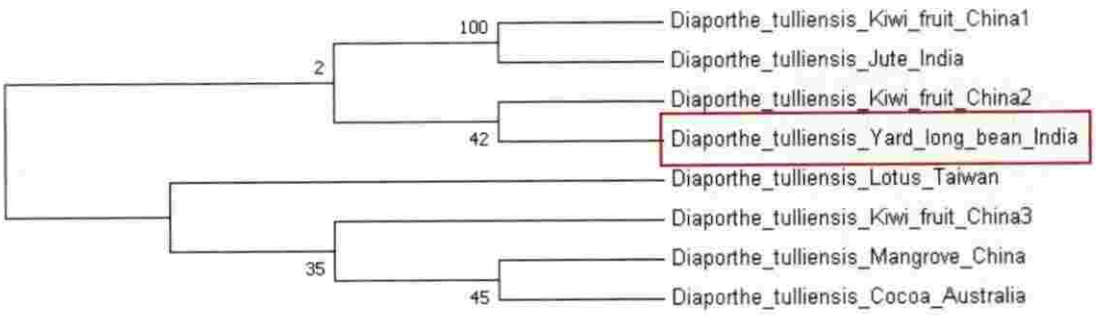


Fig. 5. Phylogenetic analysis of *D. tulliensis* isolated from yard long bean with its different isolates

Table 11. Effect of organic preparations, botanicals and non hazardous compounds on inhibition of *D. tulliensis* in PDA medium

Sl. No.	Treatments	Mycelial growth in diameter (cm) on 4th DAG **	Percentage inhibition of mycelial growth*
1	FEE (10%)	9.00 (3.16 ± 0.00)^{a}	0.00 ± 0.00^{d}
2	FWE (10%)	0.00 (1.00 ± 0.00)^{d}	100 (90.00 ± 0.00)^{a}
3	PS (1%)	9.00 (3.16 ± 0.00)^{a}	0.00 ± 0.00^{d}
4	FEE + FWE (1:1)	0.00 (1.00 ± 0.00)^{d}	100 (90.00 ± 0.00)^{a}
5	FEE + PS (1:1)	9.00 (3.16 ± 0.00)^{a}	0.00 ± 0.00^{d}
6	PS + FWE (1:1)	9.00 (3.16 ± 0.00)^{a}	0.00 ± 0.00^{d}
7	FEE+FWE+PS (1:1:1)	9.00 (3.16 ± 0.00)^{a}	0.00 ± 0.00^{d}
8	Sunflower oil (25 ml) + baking soda (10 g) +bar soap (10 g) + water (5 l)	6.77 (2.79 ± 0.0.02)^{b}	24.72 (29.78 ± 0.76)^{c}
9	Sodium bicarbonate (0.5%)	0.00 (1.00 ± 0.00)^{d}	100 (90.00 ± 0.00)a
10	Nimbecidine 0.5%	4.10 (2.26 ± 0.03)^{c}	54.44 (47.53 ± 0.83)^{b}
11	*Pseudomonas fluorescens*	9.00 (3.16 ± 0.00)^{a}	0.000 ± 0.00^{d}
12	Tebuconazole 50% + Trifloxystrobin 25% WG (@ 0.04%)	0.00 (1.00 ± 0.00)^{d}	100 (90.00 ± 0.00)^{a}
13	Untreated control	9.00 (3.16 ± 0.00)^{a}	0.00 ± 0.00^{d}
	CD (0.05)	0.027	0.892
	SEm±	0.009	0.310

Mean ± SD of four replications

Values followed by similar superscripts are not significantly different at 5% level

* Values in parenthesis are arcsine transformed values

** Values in parenthesis are square root transformed values

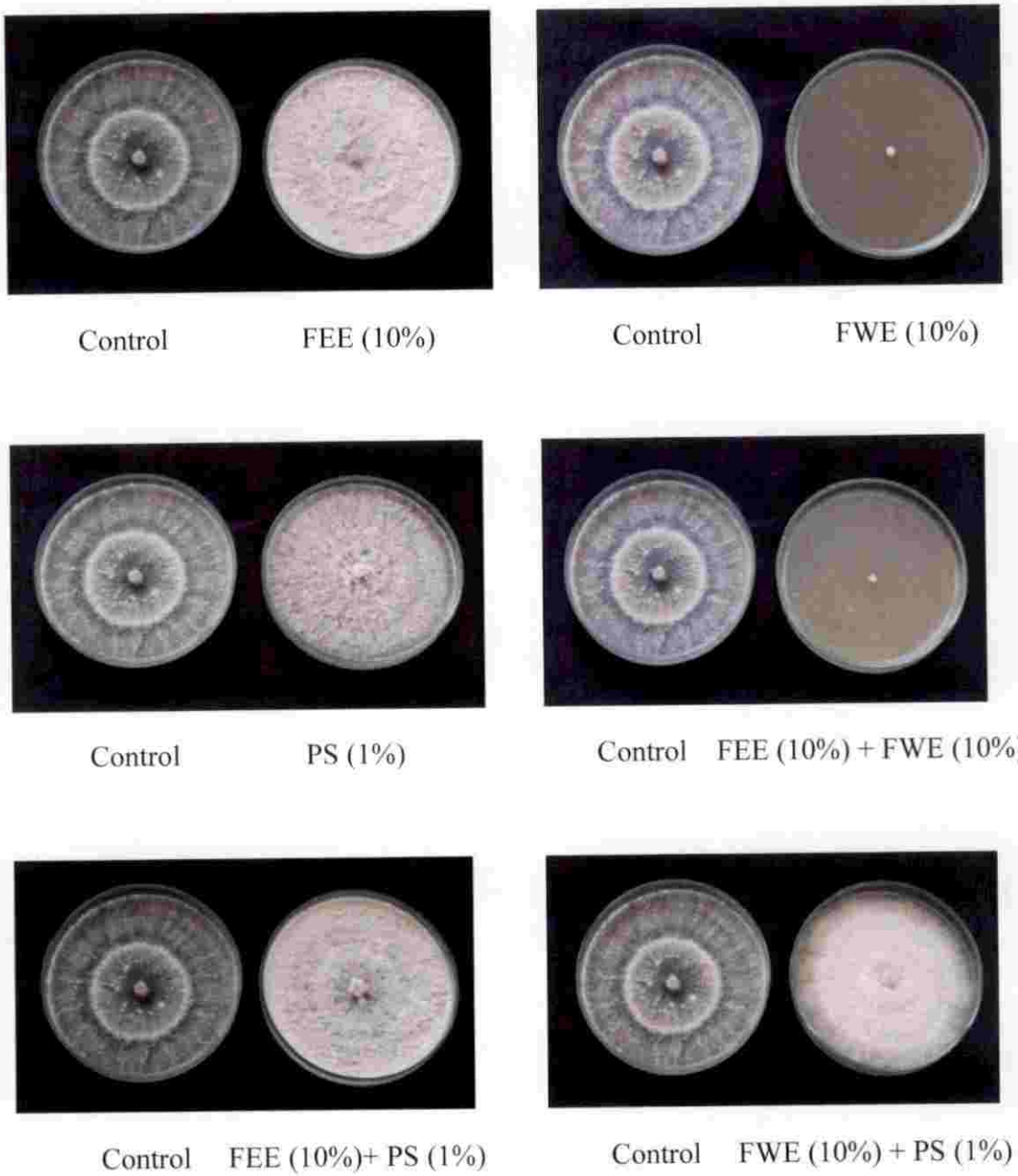
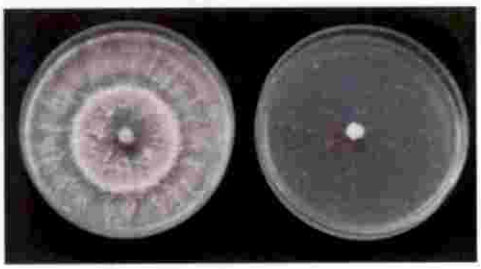


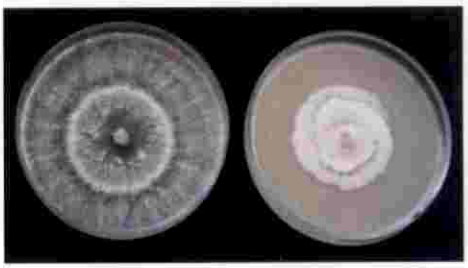
Plate 10. Effect of organic preparations, botanicals and non hazardous compounds on inhibition of *D. tulliensis* in PDA medium

Control FEE (10%)+FWE (10%)+PS(1%)

Control Sunflower oil + baking soda



Control NaHCO3 (0.5%)



Control Nimbecidine (0.5%)

Dual culture-*Pseudomonas fluorescens*

Control Tebuconazole (50%) + Trifloxystrobin (25%)

Plate 10. Effect of organic preparations, botanicals and non hazardous compounds on inhibition of *D. tulliensis* in PDA medium (Continued)

The treatments used for the pot culture experiment in yard long bean var. NS 621 conducted in polyhouse at IFSRS, Karamana are detailed in Table 12. The influence of each treatment on the biometric and yield attributes as well as on the incidence and severity of *Diaporthe* leaf spot and powdery mildew diseases of yard long bean (NS 621) were recorded and analyzed.

4.4.1. Biometric and yield attributes

The treatments did not have any significant difference on the biometric attributes *viz*., internodal length and length of lateral branches of the variety NS 621. However, there was significant difference among the treatments in terms of plant height, number of leaves and number of lateral branches. The soil application of AMF (5g plant $^{-1}$) at planting and foliar spray (20, 40 and 60 DAP) of fermented *S. barbata* extract (10%) resulted in the maximum plant height (561.98 cm), number of leaves (65.07) and number of lateral branches (8.07). The foliar spray of fermented *S. barbata* extract (10 %) was on par with the above treatment in terms of plant height (553.12 cm) and number of lateral branches (7.60). The soil application of AMF at planting was also on par with the above treatments in terms of number of lateral branches (7.53) (Table 13). Thus, with regards to the biometric attributes of yard long bean, soil application of AMF (5g plant $^{-1}$) at planting and foliar spray of fermented *S. barbata* extract (10 %) was the most superior treatment.

The treatments did not significantly differ among each other in terms of the yield attributes such as pod length, pod weight, seeds per pod, days to harvest and yield per harvest per plant (Table 14). The maximum number of pods per plant (56.60), pod yield per plant (1.49 kg) and number of harvests (17.93) were recorded in the plants treated with AMF in soil at the time of planting along with the foliar spray of sodium bicarbonate (0.5 %). Soil application of AMF along with the foliar spray of fermented *S. barbata* extract (10 %) was on par with the above treatment in terms of yield per plant (1.47 kg) and was the next best treatment in terms of pods per

Table 12. Treatments selected for *in vivo* disease management studies in yard long bean var. NS 621 cultivated in polyhouse

Treatments	Description
T_1	Sodium bicarbonate (0.5%)
T_2	Fermented *Setaria barbata* extract (10%)
T_3	Arbuscular Mycorrhizal Fungi (AMF) (5 g plant^{-1})
T_4	AMF (5 g plant^{-1}) + Sodium bicarbonate (0.5%)
T_5	AMF (5 g plant^{-1}) + Fermented *Setaria barbata* extract (10%)
T_6	Tebuconazole (50%) + Trifloxystrobin (25%) WG (0.04%)
T_7	*Pseudomonas fluorescens* (KAU talc formulation) (2%)
T_8	Untreated control

Table 13. Effect of fermented *S. barbata*, sodium bicarbonate, biocontrol agents and their combinations on biometric characters of yard long bean var. NS 621 in the polyhouse at final harvest

Sl. No.	Treatments	Plant height (cm)	Number of leaves	Internodal length (cm)	Number of lateral branches	Length of lateral branches (cm)
1	Sodium bicarbonate (0.5%)	514.43 ± 15.44^{bc}	53.27 ± 1.73^{c}	34.41 ± 0.65	5.13 ± 0.30^{bc}	173.70 ± 7.35
2	Fermented extract of *Setaria barbata* (10%)	553.12 ± 12.17^{a}	59.33 ± 1.79^{b}	34.93 ± 0.98	7.60 ± 0.48^{a}	171.10 ± 7.03
3	AMF (5 g/plant)	534.55 ± 10.56^{ab}	57.40 ± 2.14^{bc}	35.96 ± 1.00	7.53 ± 0.59^{a}	171.53 ± 7.10
4	AMF (5g) + sodium bicarbonate (0.50%)	535.53 ± 11.97^{ab}	53.27 ± 0.98^{c}	34.73 ± 0.78	5.53 ± 0.413^{b}	173.85 ± 6.41
5	AMF + Weed extract (*Setaria barbata*) (10%)	561.98 ± 6.28^{a}	65.07 ± 1.80^{a}	34.34 ± 1.08	8.07 ± 0.37^{a}	172.94 ± 8.17
6	Tebuconazole + Trifloxystrobin (0.04%)	542.19 ± 12.36^{ab}	60.20 ± 2.32^{ab}	34.69 ± 0.71	6.00 ± 0.34^{b}	173.35 ± 7.64
7	*Pseudomonas fluorescens* (2%)	534.91 ± 14.17^{ab}	55.40 ± 2.10^{bc}	34.23 ± 0.79	5.60 ± 0.52^{b}	174.85 ± 11.23
8	Untreated control	496.05 ± 8.48^{c}	45.27 ± 1.35^{d}	34.19 ± 1.06	4.13 ± 0.19^{c}	170.15 ± 5.36
	CD (0.05)	32.998	5.112	NS	1.176	NS
	SEm±	11.76	1.822	0.895	0.419	7.704

*Mean ± SD of fifteen replications

Values followed by similar superscripts are not significantly different at 5% level

Table 14. Effect of fermented *S. barbata*, sodium bicarbonate, biocontrol agents and their combinations on yield characters characters of yard long bean var. NS 621 in the polyhouse at peak harvest

Sl. No.	Treatments	Pod length (cm)	Pod weight (g)	Seeds per pod
1	Sodium bicarbonate (0.5%)	61.15 ± 1.165	25.69 ± 0.76	20.73 ± 0.33
2	FWE (10%)	63.74 ± 1.26	26.67 ± 0.96	20.80 ± 0.30
3	AMF (5 g plant^{-1})	62.01 ± 1.51	26.10 ± 0.89	20.33 ± 0.49
4	AMF (5 g plant^{-1}) + Sodium bicarbonate (0.5%)	62.72 ± 1.26	26.47 ± 0.60	21.07 ± 0.42
5	AMF (5 g plant^{-1}) + FWE (10%)	63.44 ± 0.85	26.21 ± 1.10	20.80 ± 0.43
6	Tebuconazole + Trifloxystrobin (0.04%)	63.42 ± 1.25	25.43 ± 0.82	20.93 ± 0.33
7	*Pseudomonas fluorescens* (2%)	60.79 ± 1.45	25.46 ± 0.85	20.73 ± 0.38
8	Untreated control	60.52 ± 1.55	25.14 ± 0.74	20.73 ± 0.44
	CD (0.05)	NS	NS	NS
	SEm±	1.304	0.849	0.396

Mean ± SD of fifteen replications

plant (55.60). Both the treatments were significantly superior to the untreated control in terms of yield per plant (1.10 kg) and pods per plant (47.40) (Table 15).

4.4.2. Disease incidence and severity

The disease incidence and severity of the two most important diseases of yard long bean (variety NS 621) identified during the study *viz*., *Diaporthe* leaf spot and powdery mildew were assessed and analysed.

The plants sprayed with tebuconazole (50 %) + trifloxystrobin (25 %) WG (0.04 %) at 20, 40 and 60 DAP resulted in the maximum control of *Diaporthe* leaf spot (PDI- 4.89) and powdery mildew (PDI-1.22) compared to the untreated control (PDI - 60.40 and 50.17 respectively for *Diaporthe* leaf spot and powdery mildew diseases). Among the treatments, soil application of AMF at planting and foliar spray of fermented *S. barbata* extract (10 %) resulted in the maximum control of *Diaporthe* leaf spot disease (PDI – 11.35) (Fig. 6) whereas, the soil application of AMF at planting and the foliar spray of sodium bicarbonate (0. 5%) resulted in the maximum control of powdery mildew disease (PDI – 11.55) (Table 16; Fig. 7).

Thus, from the present study, it can be concluded that soil application of AMF at planting (5 g plant $^{-1}$) and the foliar spray of sodium bicarbonate (0.5 %) at 20, 40 and 60 DAP was the best treatment for the management of powdery mildew disease of yard long bean along with improvement of most of the yield attributes including per plant yield of the crop. Conversely, soil application of AMF at planting and foliar spray of fermented *S. barbata* extract (10 %) at 20, 40 and 60 DAP was the most effective treatment to manage *Diaporthe* leaf spot, the disease which was identified for the first time in yard long bean as part of this study as well as to improve the per plant yield and most of the biometric attributes of the crop.

Three foliar sprays of the treated control *viz*., tebuconazole (50 %) + trifloxystrobin (25 %) WG (0.04 %) at 20, 40 and 60 DAP resulted in the maximum control of both the major diseases of yard long bean identified in polyhouse

Table 15. Effect of fermented *S. barbata*, sodium bicarbonate, biocontrol agents and their combinations on yield characters of yard long bean var. NS 621 in the polyhouse

Sl. No.	Treatments	Pods per plant	Yield per plant (kg)	Days to harvest	Number of harvests	Yield per harvest (g)
1	Sodium bicarbonate (0.5%)	51.80 ± 2.25^{bc}	1.37 ± 0.07^{ab}	42.00 ± 0.45	17.53 ± 0.34^{ab}	0.078 ± 0.004
2	Weed extract (*S. barbata*) (10%)	51.33 ± 1.44^{bc}	1.36 ± 0.05^{ab}	42.27 ± 0.53	17.40 ± 0.24^{abc}	0.079 ± 0.003
3	AMF (5 g / plant)	52.73 ± 2.20^{ab}	1.36 ± 0.06^{ab}	41.53 ± 0.44	16.93 ± 0.37^{bcd}	0.080 ± 0.004
4	AMF (5g) + sodium bicarbonate (0.50%)	56.60 ± 2.06^{a}	1.49 ± 0.06^{a}	42.00 ± 0.47	17.93 ± 0.36^{a}	0.084 ± 0.004
5	AMF + Weed extract (*S. barbata*) (10%)	55.60 ± 1.03^{ab}	1.47 ± 0.05^{a}	42.07 ± 0.47	16.73 ± 0.45^{bcd}	0.081 ± 0.004
6	Tebuconazole + Trifloxystrobin (0.04%)	56.87 ± 1.44^{a}	1.50 ± 0.06^{a}	41.80 ± 0.43	18.07 ± 0.28^{a}	0.083 ± 0.003
7	*Pseudomonas fluorescens* (2%)	48.00 ± 0.78^{c}	1.27 ± 0.06^{b}	41.87 ± 0.58	16.60 ± 0.25^{cd}	0.077 ± 0.004
8	Untreated control	47.40 ± 0.63^{c}	1.10 ± 0.07^{c}	41.93 ± 0.43	16.33 ± 0.25^{d}	0.077 ± 0.005
	CD (0.05)	4.479	0.17	NS	0.913	NS
	SEm±	1.596	0.061	0.476	0.326	0.004

*Mean ± SD of fifteen replications

Values followed by similar superscripts are not significantly different at 5% level

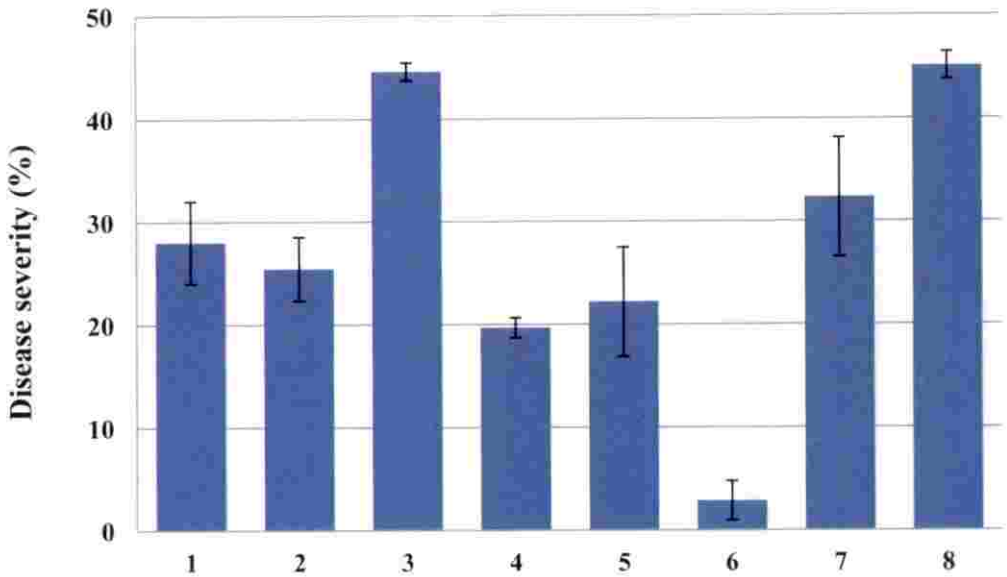


Fig. 6. Effect of fermented *S. barbata*, sodium bicarbonate, biocontrol agents and their combinations on severity of powdery mildew in yard long bean var. NS 621 in the polyhouse at 90 DAP

T_1 - Sodium bicarbonate (0.5%)
T_2 – Fermented *S. barbata* extract (10%)
T_3 - AMF (5 g plant^{-1})
T_4 - AMF + Sodium bicarbonate (0.5%)
T_5 - AMF + Fermented *S. barbata* extract (10%)
T_6 - Tebuconazole + Trifloxystrobin (0.04%)
T_7 - *Pseudomonas fluorescens* (2%)
T_8 - Untreated control

Table 16. Effect of fermented *S. barbata*, sodium bicarbonate, biocontrol agents and their combinations on natural disease incidence and severity of powdery mildew and *Diaporthe* leaf spot diseases of yard long bean var. NS 621 in the polyhouse at 90 DAP

Sl. No.	Treatments	*Diaporthe* leaf spot		Powdery mildew	
		DI (%)	PDI	DI (%)	PDI
1	Sodium bicarbonate (0.5%)	46.67	23.78(26.02)^{c}	33.33	20.44 (25.43)^{cd}
2	Fermented *S. barbata* extract (10%)	40.00	21.78(25.97)^{c}	40.00	24.89 (28.02)^{bc}
3	AMF (5 g plant^{-1})	86.67	35.22 (36.32)^{b}	93.33	49.32 (44.59)^{a}
4	AMF (5 g plant^{-1}) + sodium bicarbonate (0.50%)	33.33	18.33 (22.59)^{cd}	20.00	11.55 (19.68)^{de}
5	AMF (5 g plant^{-1}) + Fermented *S. barbata* extract (10%)	33.33	11.35 (17.59)^{de}	26.67	19.89 (22.19)^{cd}
6	Tebuconazole + Trifloxystrobin (0.04%)	13.33	4.89 (8.75)^{e}	13.33	1.22 (2.86)^{e}
7	*Pseudomonas fluorescens* (2%)	53.33	35.21 (36.22)^{b}	66.67	33.88 (32.33)^{b}
8	Untreated control	100	60.49 (51.12)^{a}	100	50.17 (45.08)^{a}
	CD (0.05)	-	(6.909)	-	(8.564)
	Sem±	-	(2.446)	-	(3.032)

*Mean ± SD of fifteen replications

Values followed by similar superscripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

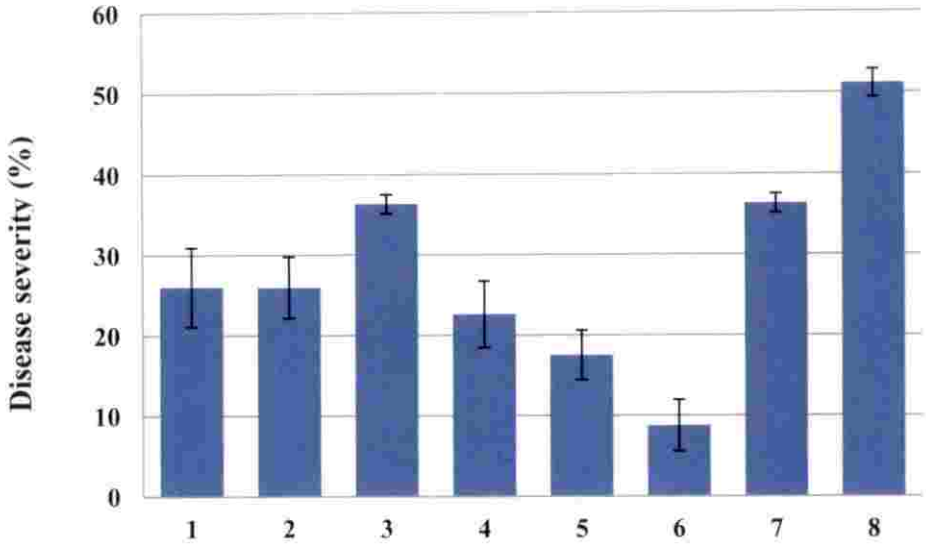


Fig. 7. Effect of fermented *S. barbata*, sodium bicarbonate, biocontrol agents and their combinations on severity of *Diaporthe* leaf spot in yard long bean var. NS 621 in the polyhouse at 90 DAP

T_1 - Sodium bicarbonate (0.5%)
T_2 – Fermented *S. barbata* extract (10%)
T_3 - AMF (5 g plant^{-1})
T_4 - AMF + Sodium bicarbonate (0.5%)
T_5 - AMF + Fermented *S. barbata* extract (10%)
T_6 - Tebuconazole + Trifloxystrobin (0.04%)
T_7 - *Pseudomonas fluorescens* (2%)
T_8 - Untreated control

cultivation. The fungicide spray was also the most effective treatment in improving the number of pods per plant (56.87), yield per plant (1.50) and the number of harvest (18.07) which equalled the most effective treatment in terms of yield attributes. However, in depth studies are required to identify the possible threats (residues) involved in the application of a fungicide combination (thrice) in a vegetable crop like yard long bean, which is harvested very frequently.

Discussion

5. DISCUSSION

Polyhouse cultivation of vegetables is an emerging technology to maximize the yield and other attributes along with its protection from biotic and abiotic adversities (Singh, 1998). However, the high humidity and congenial temperature conditions inside polyhouses can favour the development of several fungal pathogens and can alter the physiology of host-pathogen interactions (Coakley *et al.*, 1999) leading to drastic yield reduction. Thus, the present study entitled "Eco-friendly management of major fungal foliar diseases affecting yard long bean in polyhouse" was undertaken during 2017-19 at College of Agriculture, Vellayani and Integrated Farming System Research Station (IFSRS), Karamana to identify the most important fungal foliar diseases affecting yard long bean in polyhouse and to develop an eco-friendly management strategy against the major diseases identified in the crop. The results thus obtained are discussed in this chapter.

5.1. POLY HOUSE CULTIVATION AND DISEASES

In the present study, a survey was conducted in fifteen poly houses of Thiruvananthapuram district having the cultivation of yard long bean. The crop was found to be affected by several fungal foliar diseases including powdery mildew, *Diaporthe* leaf spot, *Cercospora* leaf spot, anthracnose and rust. Among the diseases, the maximum incidence and severity was recorded for powdery mildew and *Diaporthe* leaf spot diseases in majority of the polyhouses which were surveyed as part of the study.

Crop cultivation under protected conditions is a better option than open field in the present context of unpredicted occurrence of natural disasters including heavy rainfall, wind, drought, flood etc. (Kamaruddin, 2007). However, the incidence of pests under protected cultivation has been recorded to be higher than those in open field conditions due to the congenial micro climate prevailing inside the structures.

Fungal diseases including anthracnose, fusarium wilt, powdery mildew, *Cercospora* leaf spot, web blight and collar rot were observed to be the major diseases affecting yard long bean in Thiruvananthapuram district (Sreeja, 2014). Amazue and Adewale (2016) reported that powdery mildew infection due to *E. polygoni* could result in a yield loss of 25-50 per cent in cowpea.

Jyothi (2012) recorded the maximum severity of powdery mildew disease (78.08 %) in green gram caused by *E. polygoni* at Dharwad district, whereas the maximum severity of 95.20 per cent was observed in the crop at Shimoga district of southern Karnataka. Powdery mildew was reported to be the most important disease of yard long bean in poly house which under favourable conditions adversely affected the growth and yield attributes of the crop (Beevi, 2018).

Phomopsis vexans is an economically important fungal pathogen which has been reported to cause fruit rot and leaf blight in brinjal. It is a major disease resulting 20 - 30 per cent yield loss in the crop (Pandey *et al.*, 2002; Beura *et al.*, 2008). A disease incidence of 8.3 - 25.3 per cent was reported in egg plant (Mahadevakumar *et al.*, 2017) due to leaf blight disease. Till date, infection caused by *Diaporthe* sp. in yard long bean has not been reported anywhere in the world.

5.2. SCREENING OF YARD LONG BEAN VARIETIES IN POLYHOUSE

5.2.1. Biometric and yield attributes of varieties

Five varieties *viz.,* Lola, Vellayani Jyothika, Gitika, VS 50 and NS 621 were screened to compare their performance in terms of biometric and yield attributes as well as on the incidence and severity of fungal foliar diseases. Among the varieties screened, NS 621 was found to be superior in terms of pod yield which was followed by Gitika. Varghese (2015) observed that the maximum yield per plant was recorded in Gitika followed by Hari rani and NS 621. Lakshmi (2016) identified that Githika, VS 50, Vellayani Jyothika and VS 13 were the best parents to maximize yield and

related attributes. Varghese (2017) revealed that the maximum pod yield per plant was obtained from the cross combination between Gitika and VS 50 which was on par with the cross combination of VS 50 and Vellayani Jyothika under rain shelter conditions.

5.2.2. Disease incidence and severity

The natural incidence and severity of various fungal foliar diseases observed in the varieties during the trial were recorded to compare their resistance or susceptibility to different diseases. NS 621, the variety which recorded the maximum yield per plant was found to be the most susceptible variety compared to other yard long bean varieties to both powdery mildew and *Diaporthe* leaf spot diseases, the two important fungal foliar diseases observed during the trial.

Srikishan (2010) recorded powdery mildew disease incidence in the range of 50.50 to 76.67 with its severity ranging from 50.52 to 81.65 in cowpea. The author also reported that among the 40 cowpea (*V. unguiculata* L. Walp) varieties screened, none of them were found to be resistant to the natural incidence of powdery mildew disease.

Perusal of the literature revealed that not much study has been undertaken for the screening of yard long bean (*V. unguiculata* sub sp. *sesquipedalis*) varieties for their reaction to powdery mildew caused by an obligate biotroph. Screening for resistance to the disease has been undertaken in other vegetable crops including cowpea (*V. unguiculata* sub sp. *unguiculata*), pea, green gram, bitter gourd etc.

Diaporthe leaf spot disease which was identified in yard long bean as part of the survey and varietal screening is the first report of this disease in the crop. Hence, detailed studies on the incidence and severity of the disease in yard long bean as well as the disease reaction of yard long bean accessions to the disease are not available.

5.2.3. Characterisation of *D. tulliensis*

D. tulliensis which was identified and characterized during the present study is the first report of the fungus in yard long bean. The only other crops from which *D. tulliensis* was isolated, identified and characterized included lotus (Shivas *et al.*, 2015), kiwi fruit (Bai *et al.*, 2016) and cocoa (Chen and Kirschner, 2018).

Alpha conidia of *D. tulliensis* causing pod rot of cocoa was identified to have hyaline, oval to cylindrical shape, with a size ranging between 5–8 × 2–3 µm. The fungus produces few beta conidia. Colonies on PDA appeared flat with no aerial mycelium. The mycelial growth on PDA medium appeared white on top view and off-white as the reverse view. Sparse aerial mycelium was observed in oats agar medium (Shivas *et al.*, 2015). The colony growth of *D. tulliensis* causing stem canker, fruit rot and leaf spot in kiwi fruit (Bai *et al.*, 2016) appeared as white with yellowish green pigments in PDA medium. Mostly single celled, hyaline, fusiform and biguttulate alpha conidia (3.7-6.5 × 1.0-2.6 µm) were observed whereas the beta conidia were absent. The fungus isolated from yard long bean during the present study exhibited similar morphological characters as above and hence confirmed its morphological identity with *D. tulleinsis.*

Molecular identification based on Internal Transcribed Spacer regions (ITS) is of great significance rather than the identification based on morphological characters (Hyde *et al.,* 2011; Gomes *et al.*, 2013). Dissanayake *et al.* (2017) described 171 species of *Diaporthe* fungus which were recognized on molecular basis.

In the present study, ITS 1F and 4R were used as the forward and reverse primers for the molecular identification of the fungus in yard long bean. The identification and sequencing of pod rot fungus of cocoa using ITS (GenBank KR936130), LSU (GenBank KR936131, *tub2 (*GenBank KR936132), *tef1* (GenBank KR936133) revealed the identity with *D. tulliensis* (Shivas *et al.*, 2015). Thus the present study is of great importance as *D. tulliensis* was identified for the first time in

yard long bean which causes leaf spot disease, and its further detailed study has to be undertaken in the near future.

5.3. *IN VITRO* MYCELIAL INHIBITION

In vitro studies revealed that fermented *S. barbata* extract (10 %) and sodium bicarbonate (0.5 %) resulted in complete (100 %) inhibition of the mycelial growth of *D. tulliensis*. Weed species have been tested for their antifungal potential due to their common availability and their tolerance to fungal diseases (Iqbal *et al.*, 2001). *S. barbata* (East Indian bristle grass / mary grass / corn grass) is one of the most common weed species available in the southern parts of Kerala, especially in Thiruvananthapuram district and hence was selected for the antifungal studies (Kellog *et al.*, 2009). The fermented extract of *S. barbata* (10 %) resulted in 99.63 per cent inhibition of mycelial growth of *R. solani*, the rice sheath blight fungus (Karthika *et al.*, 2017).

Studies using *Ageratum conyzoides* revealed 70 per cent mycelial inhibition of *Phomopsis theae*, the incitant of branch canker of tea besides inhibiting *R. solani* and *A. niger in vitro*. The antifungal property of the water extracts of *A. conyzoides* had been claimed to be due to the presence of coumarin, hydrocyanic acid and an alkaloid (Lewis and Elvin-Lewis, 1977) which were found to be thermo labile (Iqbal *et al.*, 2001). Aderogba *et al.* (2014) identified two compounds *viz.*, 5,4′-dihydroxy-6-methoxy-7-O-β-glucopyranoside flavone and stigmasterol-3-O-β-glucopyranoside present in the weed, *Pseudognaphalium luteoalbum* which were responsible for the *in vitro* inhibition of *P. nicotiana* and *F. oxysporum*. The present study is the first attempt of testing the antifungal potential of *S. barbata* against *D. tulliensis*.

Sodium bicarbonate (0.5 %) was highly effective in completely (100 %) suppressing the mycelial growth of *D. tulliensis* in the present study. The concentration of sodium bicarbonate has great significance as far as the antifungal

potential is concerned. A concentration of 100 mM of potassium bicarbonate was highly effective to result in 100 per cent mycelial growth inhibition of *S. sclerotiorum and R. solani* (Erper *et al.*, 2011). Sodium bicarbonate at a MIC value of 1600 µg/ml could not result in the mycelial inhibition of *Phomopsis viticola*, the incitant of cane and leaf spot of grapes (Yildirim, 2014).

Punja and Grogan (1982) proposed that the suppression of sclerotial germination by bicarbonate salt in *S. rolfsii* was due to its fungicidal effect. However, the mode of action of sodium bicarbonate was proposed to be fungistatic and not to be fungicidal in its inhibition against *Alternaria, Fusarium* and *Rhizopus* (Aharoni *et al.* (1997). Buffering capacity (maintenance of alkaline environment) and elevation of pH were revealed to contribute mainly to the antifungal activity of bicarbonates (Palmer *et al.,* 1997). The alkaline environment thus created will compel the fungi to divert most of its energy towards conversion of the alkaline base to acidic, thereby resulting in growth suppression (Oliver *et al.,* 1998).

The bicarbonate salts adversely affected the membrane permeability and resulted in the suppression of oxidative phosphorylation (Oliver *et al.,* 1998). Fall in turgour pressure, irreversible damage of hyphae, suppression of mycelial and spore development were claimed to be some of the reasons for the antifungal potential of the salts (Jabnoun-Khiareddine *et al.,* 2016).

A toxic metabolite was reported to be produced by *D. phaseolorum* var. *caulivora* (stem canker of soybean). It was revealed that the toxin production was directly correlated with the virulence of the fungus (Burra, 1988). Thus, inhibition of toxin production was also reported as another mode of action of bicarbonates whereby the metabolic pathways of different enzymes were inhibited (Nabarawy *et al.*, 1989; Roinestad *et al.,* 1994). This mode of action of sodium bicarbonate besides their buffering action and elevation of pH has great significance in the present study as toxin production has been proved in *D. phaseolorum* var. *caulivora* (Burra, 1988).

5.4. *IN VIVO* MANAGEMENT STUDIES

5.4.1. Effect of bicarbonates on yield improvement and disease suppression

Significant increase in plant growth and vigor was observed in plants sprayed with potassium or sodium bicarbonate resulting in development of resistance to diseases or recovery from infection (Perrenoud, 1993). Significant reduction of powdery mildew disease was reported in cucurbits with foliar spray of bicarbonate solutions (Ziv and Zitter, 1992). Foliar applications of bicarbonates as sodium or potassium salts (1 or 2 %) revealed a considerable suppression of powdery mildew disease severity (12 to 21 % respectively) of euonymus plants (Ziv and Hagiladi, 1993) suggesting the importance of bicarbonates as a good alternative for fungicides for the management of powdery mildew disease. Sodium bicarbonate was reported to be highly effective for the management of powdery mildew of various crops (Homma et al., 1981; Horst *et al.,* 1992; Ziv and Zitter, 1992; Reuveni *et al.,* 1996).

The induction and activation of peroxidase enzyme was observed to be an involuntary response in a disease affected plant towards resistance (Sridhar and Ou, 1974). The increase in peroxidase activity observed was an indication of the activation of the defense pathways resulting in induced systemic resistance (Reuveni *et al.,* 1988; Irving and Kuc, 1990; Mosa, 2002). A significant increase in the activity of peroxidase was reported in squash leaves sprayed with potassium or sodium bicarbonate (Zaki *et al.,* 2011).

Several mechanisms have been reported for the antifungal effect of bicarbonates, of which the major activity proposed was their effect on the increase of pH which was maintained by their buffering capacity. The efficacy of the salts got reduced under acidic conditions due to the production of carbonic acid. The congenial pH for their efficient activity was reported to be above 8.5, at which the carbonate synthesis was found to increase. However, conidial attachment gets inhibited only at a pH of above 11 (Marloth, 1931). Increase of pH inside the conidia has been

reported in *Sphaerotheca pannosa* f. sp. *rosae* and *Alternaria brassicae* due to the effect of bicarbonates (Porter *et al.*, 1992) as well as in *Fusarium graminearum* and *Penicillium griseofulvum* after treatment with ammonium bicarbonate (DePasquale and Montville, 1990). The alkaline condition forced the pathogenic fungi to produce more acid to reduce the elevated pH, thereby adversely affecting their mycelial growth and spore development processes (Palmer *et al.*, 1997).

The increase in leaf osmotic potential is claimed to be another mechanism for the disease management capacity of bicarbonate salts (Nobecourt (1922). Bicarbonates also exhibited inhibitory activity on membrane functions. Marloth (1931) observed that carbonate salts inhibited the activity of extracellular enzymes actively engaged in the development of spore cell wall and the membrane of *Penicillium italicum*. Homma *et al.* (1981) observed a suppression of conidial development and germination in *Sclerotinia fuliginea* by bicarbonate salts. Potassium bicarbonate inhibited the development of conidia and conidiophores through their action on hyphal walls (Homma and Arimoto, 1990). Kiraly (1976) reported the synthesis of antifungal metabolites including phytoalexins, phenolic compounds and the phytohormone *viz.*, auxin in resistant plants upon bicarbonate application.

5.4.2. Effect of weed extracts on yield improvement and disease suppression

The aqueous methanol extract of the invasive weed *viz.*, *Inula graveolensis* at 100 - 250 ppm resulted in significant reduction of wilt disease of cucumber plants caused by *Fusarium oxysporum*. The foliar application caused a reduction of significant browning of the crown region which was on par with the fungicide *viz.*, hymexazol. The extract revealed a significant increase in biometric parameters including plant height, shoot and root weight as well as the pod weight of cucumber plants compared to all other treatments suggesting the plant growth promotion activity of the weed extract (Abu-Irmaileh *et al.*, 2017).

The leaf extracts of *L. camera* and *D. stromonium* were highly effective in reducing the seed infection caused by several fungi such as *A. niger* and *A. awamori*; and were effective in increasing the germination of seeds (Patel *et al.,* 2006). The leaf extract (20 %) of *Ocimum sanctum* was one of the most effective components for the integrated management of *Phomopsis* blight and fruit rot disease of brinjal caused by *Phomopsis / Diaporthe vexans* (Nair, 2011).

Ekka *et al.* (2018) reported that 10 per cent leaf extract of *Allamanda catheritica* and the combination fungicide (tebuconazole 50 % + trifloxystrobin 25 %) (0.2 %) were effective in complete mycelial inhibition of *Diaporthe vexans*, the fruit rot fungus of brinjal. Seed treatment with carbendazim + Mancozeb (Saaf) @ 0.2 per cent and two foliar applications of 10 per cent leaf extract of the botanical resulted in 31.11 PDI and 29.82 per cent disease suppression over control, with a crop yield of 21.71 t ha^{-1}. The treatment resulted an increase in yield of 1.68 q ha^{-1} over control and a B: C ratio of 1:1.05 which signifies the economics of application as well as the effect of the botanical in disease suppression and yield improvement.

Not much research had been undertaken to study the effect of extracts of different weeds on *in vivo* yield improvement and disease management in economically important crops. Karthika (2017) revealed that fermented extract of *S. barbata* resulted in the improvement of both biometric and yield parameters of rice var. Uma.

5.4.3. Effect of arbuscular mycorrhizal fungus (AMF) on yield improvement

The potential of AMF through root colonization in plant growth promotion and yield improvement thereby, functioning as a biofertilizer had been proved beyond doubt. AMF had bioprotectant action against pathogenic fungi and nematodes (Cordier *et al.*, 1998; Morin *et al.*, 1999; Odebode *et al.*, 2001; Killani, 2010).

AMF increased the absorption of plant nutrients and also helped to expand the availability of soil for plants (Cordier *et al.*, 1996; Kahiluoto and Vestberg, 1998; Olsen *et al.*, 1999).

AMF improved the root structure and the functioning of plant vascular system, thereby maximizing nutrient absorption and crop yield (Stanley *et al.*, 1993; Miller *et al.*, 1997; Osonubi *et al.*, 1991). Absorption and transfer of carbon between plants were aided through the fungal mycelia of AMF (Simard *et al.*, 1997) which also prevented the competition between plants and microbes for different essential nutrients.

Phosphate ions were efficiently transported from soil to the plant system through the fungal hyphae of AMF (Hayman and Mosse, 1971). AMF significantly improved the nitrogen fixation efficiency of the root nodulation bacteria *Rhizobium* spp. (Dodd *et al.* (1990). Thus, the ability of AMF in increasing the availability as well as the absorption of soil nutrients form the basis for growth and yield improvement in AMF inoculated crops. The utilization of AMF along with plant growth promoting rhizobacteria could result in minimizing the use of inorganic fertilizers and in improving the quality and quantity of crop produce (Bona *et al.*, 2016).

Recently, the role of AMF in the suppression of fungal foliar pathogens has been unveiled (Fritz *et al.*, 2006). AMF exhibited induced systemic resistance as one of the mechanisms whereby, the growth improvement gradually led to the activation of plant defense mechanism. The role of AMF in the suppression of soil borne fungal and bacterial pathogens as well as nematodes was already proved (Killani, 2010).

Thus, the present work was an attempt to identify and manage the important fungal foliar diseases of yard long bean cultivated in polyhouse conditions. The study revealed that *Diaporthe* leaf spot and powdery mildew diseases were the most important fungal foliar diseases of the crop in the polyhouses of Thiruvananthapuram district. The leaf spot fungus was identified as *D. tulliensis* and the phylogenetic tree

revealed its close relationship with the Taiwan isolate of *D. tulliensis* from lotus and the China isolate 2 from kiwi fruit. The study revealed the antifungal potential of fermented *S. barbata* extract (10 %), an organic preparation and sodium bicarbonate (0.5 %), a non hazardous compound for the *in vitro* inhibition of *D. tulliensis*. *In vivo* studies also revealed the possibility of use of fermented *S. barbata* extract (10 %) and sodium bicarbonate (0.5 %) as alternatives to fungicides as their effects were on par with that of tebuconazole 50% and trifloxystrobin 25% (fungicide check) both under *in vitro* and *in vivo* conditions. Soil application of AMF at planting (5 g plant^{-1}) and foliar spray of fermented *S. barbata* extract (10 %) or sodium bicarbonate (0.5 %) at 20, 40 and 60 days after planting resulted in the maximum reduction in the disease incidence and severity of *Diaporthe* leaf spot and powdery mildew diseases respectively in yard long bean.

Summary

6. SUMMARY

The thesis work entitled "Eco-friendly management of major fungal foliar diseases affecting yard long bean in polyhouse" was undertaken at College of Agriculture, Vellayani and Integrated Farming System Research Station (IFSRS), Karamana during 2017-2019 to determine the major fungal foliar diseases affecting yard long bean grown in polyhouse and their management using natural resources and eco-friendly methods.

A survey was conducted in 15 polyhouses located in the various parts of Thiruvananthapuram district including Thirupuram, Vellanadu, Kuttichal, Tholicode, Malayinkeezhu, Vilavoorkal, Vembayam, Nedumangad, Pothencode, Manickal, and Nanniyode panchayats as well as in Thiruvananthapuram Corporation where yard long bean was cultivated.

NS 621 was the most commonly cultivated variety of yard long bean in 10 out of the 15 poly houses surveyed. Powdery mildew, *Diaporthe* leaf spot, *Cercospora* leaf spot, anthracnose and rust were the major fungal foliar diseases observed in the polyhouses; and leaf miner, red spider mite, thrips, aphids, mealy bug and leaf eating caterpillar were the major pests.

Powdery mildew and *Diaporthe* leaf spot were the most important diseases of yard long bean var. NS 621 cultivated in the polyhouses located at Thirupuram and Manickal panchayats with the maximum incidence (100 and 80 % respectively) and severity (60.70 and 45.77 % respectively) at final harvest stage of the crop.

The varietal screening of the five yard long bean varieties *viz.,* Lola, Vellayani Jyothika, Gitika, VS 50 and NS 621 conducted at IFSRS, Karamana revealed that there was no significant difference between the varieties with regard to the number of leaves, internodal length and length of lateral branches. The maximum plant height was observed in NS 621 (508.47 cm) followed by Vellayani Jyothika (481.18 cm).

Gitika and VS 50 recorded the maximum number of lateral branches (5.75 and 5.63 respectively) which were on par with each other.

VS 50 and NS 621 had the maximum pod length (61.39 cm and 60.89 cm respectively) which were on par with each other. The maximum pod weight was observed in NS 621 (25.95 g) followed by Gitika (25.41 g) and VS 50 (24.94 g). NS 621 recorded the maximum number of seeds per pod (21.63) followed by VS 50 (20.75). NS 621 and Gitika had the maximum number of pods per plant (49.25 and 46.13 respectively). The highest pod yield per plant was recorded in NS 621 (1.30 kg) followed by Gitika (1.12 kg). NS 621 took the minimum days for first harvest (42.06), followed by VS 50 (43.19). The maximum number of harvests was recorded in NS 621 (16.13) and Gitika (16.06) which were on par with each other. The maximum yield per harvest per plant was observed in NS 621 (0.081 kg) followed by Gitika (0.070 kg). Thus, the varietal screening revealed that NS 621 was the superior variety of yard long bean in terms of plant height and all yield attributes.

Diaporthe leaf spot was the most important disease of yard long bean followed by powdery mildew during the varietal screening trial. The maximum incidence and severity of *Diaporthe* leaf spot at 30 DAP (50 % and 16.11 %), 60 DAP (75 % and 32.67 %) and 90 DAP (100 % and 58.66 % respectively were observed in NS 621. The minimum incidence and severity of the disease was observed in VS 50 at 90 DAP (75 % and 34.66 %). The maximum incidence and severity of powdery mildew disease was observed in NS 621 at 30 DAP (37.50 % and 23.44 %), 60 DAP (50 % and 29.11 %) and 90 DAP (62.50 % and 50.89 %). VS 50 recorded the minimum incidence and severity of powdery mildew at 90 DAP (37.50 % and 11.11 %).

The new fungal disease, *Diaporthe* leaf spot identified in yard long bean was manifested as small, circular to irregular, off white lesions with characteristic brown margins which later coalesced to result in leaf blighting. Characteristic drying of the leaves were observed starting from the leaf tip leading to defoliation. The

pathogenicity studies with the fungus in NS 621 revealed characteristic circular to irregular, off white lesions with brown margins on 15th DAI on the inoculated leaves. Blighting and drying of the leaves were observed which later resulted in defoliation.

The growth of the fungus in PDA medium appeared as whitish mycelia turning to greyish white in colour. The fungus produced aerial mycelia and concentric grey coloured zonations on top view. The rear view appeared as off white growth with characteristic grey zonations. The fungus completed its growth in petri plates (9 cm) in four days when grown in PDA medium.

The fungal mycelia appeared as septate with an average width of 3.55 µm under 1000X magnification. Alpha conidia (4.41 - 5.11 µm × 1.57 - 2.24 µm) were single celled, hyaline, ovoid in shape with a submedian constriction. Beta conidia were not observed. The asexual fruiting bodies of the fungus *viz*., the conidiomata (0.4 cm x 0.3 cm) appeared on the 7th DAG in PDA medium as initially white, flat mass of mycelia, later turning to white blisters with black centre and honey dew secretions on top.

The molecular identification based on Internal Transcribed Spacer (ITS) regions, its sequencing and BLAST analysis in NCBI database confirmed the fungus to be *Diaporthe tulliensis*, which is the first report in yard long bean worldwide.

In vitro inhibition studies of the fungus revealed that fermented *S. barbata* extract (10 %), sodium bicarbonate (0.5 %), combination of fermented egg lemon juice extract (10 %) and fermented *S. barbata* extract (10 %) and the treated control *viz*., tebuconazole (50 %) + trifloxystrobin (25 %) WG (0.04 %) resulted in 100 per cent mycelial inhibition of the fungus.

Soil application of AMF (5g plant^{-1}) at planting and foliar spray (20, 40 and 60 DAP) of fermented *S. barbata* extract (10%) resulted in the maximum plant height (561.98 cm), number of leaves (65.07) and number of lateral branches (8.07). The foliar spray of fermented *S. barbata* extract (10%) was on par with the above

treatment in terms of plant height (553.12 cm) and number of lateral branches (7.60). Soil application of AMF at planting was also on par with the above treatments in terms of number of lateral branches (7.53).

The maximum number of pods per plant (56.60), pod yield per plant (1.49 kg) and number of harvests (17.93) were recorded in the plants applied with AMF (5g $plant^{-1}$) in soil at the time of planting and the foliar spray of sodium bicarbonate (0.5 %). Soil application of AMF along with the foliar spray of fermented *S. barbata* extract (10 %) was on par with the above treatment in terms of yield per plant (1.47 kg) and was the next best treatment in terms of pods per plant (55.60).

The plants sprayed with tebuconazole (50%) + trifloxystrobin (25%) WG (0.04%) at 20, 40 and 60 DAP resulted in the maximum control of *Diaporthe* leaf spot (PDI - 4.89) and powdery mildew (PDI - 1.22) compared to untreated control (PDI - 60.40 and 50.17 respectively).

Among the treatments, soil application of AMF at planting and foliar spray of fermented *S. barbata* extract (10 %) resulted in the maximum control of *Diaporthe* leaf spot disease (PDI - 11.35) whereas, the soil application of AMF at planting and the foliar spray of sodium bicarbonate (0.5 %) resulted in the maximum control of powdery mildew disease (PDI – 11.55). Thus, the study revealed the possibility of an integrated disease management strategy in yard long bean, where the use of pesticides could be reduced to the minimum to produce safe-to-eat yard long bean and at the same time, ensuring the safety of the environment and its life forms.

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Appendices

Appendix- I

COMPOSITION OF MEDIA USED

1. PDA (POTATO DEXTROSE AGAR)

Potato	- 200 g
Dextrose ($C_6H_{12}O_6$)	- 20 g
Agar-agar	- 20 g
Distilled water	- 1000 ml

Potato extract was filtered and collected after boiling in 500 ml of distilled water. Twenty grams of agar-agar was separately dissolved in 500 ml of distilled water. The potato extract was mixed in molten agar and 20 g dextrose was dissolved into the mixture. Final volume was made upto 1000 ml with distilled water and the media was sterilized at 15 psi and 121^0C for 15-20 minutes.

2. Double strength PDA

Potato	- 400 g
Dextrose ($C_6H_{12}O_6$)	- 40 g
Agar-agar	- 40 g
Distilled water	- 1000 ml

Appendix- II

COMPOSITION OF LACTOPHENOL COTTON BLUE STAIN

Composition

Anhydrous lactophenol	- 67.0 mL
Distilled water	- 20.0 mL
Cotton blue	- 0.1 g

Anhydrous lactophenol prepared by dissolving 20 g phenol in 6 mL lactic acid and in 3 mL glycerol.

Appendix- III

SEQUENCE OF *DIAPORTHE TULLIENSIS*

Accession number MN267826

ACGGCAGGGCACCGCCAGGGCCTTCCAGAACGAGATATAACTACTACGCTCGGGGTCCTGGCGAGCTCGCCACTA
GATTTCAGGGCCCGCCCTTTTCCAAAGGCGGTGCCCCAACACCAAGCCAGGCTTGAGGGTTGAAATGACGCTCGA
ACAGGCATGCCCTCCGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTC
ACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTGATTCATT
ATGTTTTTTACTCAGAGATTCACTAAGAAACAAGAGTTTGTTTGGCCGCCGGCGGGCTGCTCCCTGTCTCCAGGGG
GCCTCGGTGAGGAGGCCGGCCAGCGCCGAGGCAACAGTATAGGTATAAGTTCACA

Abstract

ECOFRIENDLY MANAGEMENT OF MAJOR FUNGAL FOLIAR DISEASES AFFECTING YARD LONG BEAN IN POLYHOUSE

by

DEEPTHI S. NAIR

(2017-11-053)

ABSTRACT

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DEPARTMENT OF POST HARVEST TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM - 695 522

KERALA, INDIA

2019

ABSTRACT

The study entitled 'Ecofriendly management of major fungal foliar diseases affecting yard long bean in polyhouse' was conducted at College of Agriculture, Vellayani and Integrated Farming System Research Station (IFSRS), Karamana during 2017- 2019 with the objectives to determine the major fungal foliar diseases affecting yard long bean grown in polyhouse; and their management using natural resources and ecofriendly methods.

A survey was conducted in 15 polyhouses located at different parts of Thiruvananthapuram district, where yard long bean was cultivated. NS 621 was the most common variety of yard long bean cultivated in polyhouses. Powdery mildew (Disease Incidence (DI) - 100% and Per Cent Disease Index (PDI) - 60.70) and *Diaporthe* leaf spot (DI - 80% and PDI - 45.77) were the major fungal foliar diseases of the crop observed in these polyhouses.

Five varieties of yard long bean *viz*. Lola, Vellayani Jyothika, Gitika (KAU Varieties), VS 50 (KAU pre release culture) and NS 621 (Namdhari Seeds Private Limited) were screened in the polyhouse at IFSRS, Karamana in completely randomized design (CRD) with four replications, to assess the natural incidence and severity of different fungal foliar diseases. The most susceptible variety to natural incidence of powdery mildew (PDI - 50.89) and *Diaporthe* leaf spot (PDI - 58.66) diseases was NS 621, whereas VS 50 was tolerant to powdery mildew disease (PDI - 11.11).

In vitro evaluation of organic preparations *viz*, egg-lemon juice extract (10%) and sunflower oil (25 ml) + baking soda (10 g) + bar soap (10 g) + water (5 l), botanicals *viz*. fermented weed (*Setaria* barbata) extract (10%) and nimbecidine (0.5%), and non-hazardous compounds *viz*. potassium silicate (1%) and sodium bicarbonate (0.5%) against *Diaporthe tulliensis* by poisoned food technique in potato dextrose agar (PDA) medium revealed that fermented extract of *S. barbata* (10%),

sodium bicarbonate (0.5%) and a combination of egg-lemon juice extract (10%) and fermented extract of *S. barbata* (10%) resulted in cent per cent inhibition of the mycelial growth of the fungus, which were further used for *in vivo* evaluation. Fermented egg-lemon juice extract (10%), potassium silicate (1%) as well as *Psuedomonas fluorescens* (KAU isolate) had no inhibitory effect on the mycelial growth of the fungus.

A trial was conducted to assess the efficacy of selected treatments for the management of powdery mildew and *Diaporthe* leaf spot diseases in NS 621 in the polyhouse of IFSRS, Karamana in CRD with eight treatments replicated thrice. The plants sprayed with tebuconazole 50% + trifloxystrobin 25% (WG) (0.04%) (positive control) at 20, 40 and 60 days after planting (DAP) resulted in maximum control of *Diaporthe* leaf spot (PDI - 4.89) and powdery mildew (PDI - 1.22) diseases. Among the treatments, the combined application of arbuscular mycorrhizal fungi (AMF) (KAU isolate) @ 5g plant^{-1} at the time of planting along with the foliar application of fermented extract of *S. barbata* (10%) at 20, 40 and 60 DAP resulted in significant control of *Diaporthe* leaf spot (PDI - 11.35), whereas the combined application of AMF @ 5g plant^{-1} at the time of planting along with the foliar application of sodium bicarbonate (0.5%) at 20, 40 and 60 DAP resulted in significant suppression of powdery mildew disease (PDI -11.59). Soil application of AMF alone and foliar application of *P. fluorescens* (2%) had least effect in reducing the severity of both the diseases.

Thus, the present study revealed that powdery mildew and *Diaporthe* leaf spot were the most important diseases of yard long bean in polyhouses of Thiruvananthapuram district. Soil application of AMF @ 5g plant^{-1} at the time of planting and foliar spray of fermented extract of *S. barbata* (10%) or sodium bicarbonate (0.5%) at 20, 40 and 60 DAP were the most effective treatments against *Diaporthe* leaf spot and powdery mildew diseases respectively, which could be used as a green technology to produce safe-to-eat yard long bean.