Etiology and Management of Ginger Yellows Disease

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Etiology and Management of Ginger Yellows Disease

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KERALA, INDIA

2014

DECLARATION

I hereby declare that this thesis entitled "**ETIOLOGY AND MANAGEMENT OF GINGER YELLOWS DISEASE"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µm	Micro meter
µl	Micro litre
@	At the rate of
°C	Degree Celsius
CD	Critical difference
cm	Centimeter
Ca	Circa (approximately)
et al.	And other co workers
Fig.	Figure
g	Gram
ha	Hectares
h	Hours
i.e.	that is
Kg	Kilogram
l.	Litre
mm	Milli meter
mg	Milli gram
ml	Milli litre
sec	Seconds
SE	Standard error
sp. or spp.	Species (Singular and plural)
viz.	Namely
dia.	Diameter
DI	Disease Incidence
Ppm	Parts per million
min.	Minutes

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Introduction

1. INTRODUCTION

Ginger (*Zingiber officinale* Rosc.) is one of the most important spice crops in India. The underground rhizome is much valued and it is used as raw, dried, powdered, oil and oleoresins. Besides its use as a flavouring agent in confectionaries and beverages, it is an indispensable ingredient in many ayurvedic and local medicines. Ginger has been used in Asian, Arabic and Indian cultures as a herbal medicine since ancient times. It is commonly used as a carminative to treat various types of "stomach problems," including motion sikness, upset stomach, gas, diarrhea, nausea caused by cancer treatment, nausea and vomiting after surgery, as well as loss of appetite.

India is a leading producer of ginger in the world and during 2010-11 the country produced 9.13 lakh tonnes of the spice from an area of 1.67 lakh ha (Spices Board, 2014). Ginger is cultivated in most of the states in India. However, states namely Kerala, Meghalaya, Arunachal Pradesh, Mizoram, Sikkim, Nagaland and Orissa together contribute 70 per cent to the total production of the country (Sasikumar *et al*., 2008).

Kerala is the leading ginger producing state in the country. The warm and humid climatic condition in Kerala is the best to grow ginger. Moreover it grows well in plains as well as in hills up to an altitude of 1500 meters above mean sea level. Ginger can be grown both under rain fed and irrigated conditions. Several high yielding cultivars of ginger are available and grown in parts of Kerala like Maran, Kuruppampadi, Ernad, Wynad, Himachal and Nadia. Exotic cultivars such as Rio-de-Janeiro have also become very popular among farmers (Sasikumar *et al*., 2008).

Ginger cultivation in Kerala is in a declining trend for the past two decades due to factors such as problems with input (land, labour, mulching materials, manures and fertilizers), biotic and abiotic stress (erratic rainfall, pests and diseases) and fluctuating market prices. Even the export

market is showing a downward trend. The stringent microbiological and quality standards set by the consumers worldwide have adversely affected the export potential of raw and dried ginger. Unhygienic processing conditions, unfavourable climate, and absence of mechanical processing may lead to higher moisture content in the finished product and consequently higher incidence of pest and diseases (George, 1996).

The area under ginger cultivation has drastically reduced from 10706 ha during 2001-02 to 4505 ha during 2012-13. Correspondingly the production too has come down from 40181 to 22064 tonnes. Wayanad district alone contribute to major share of ginger production in the state. The 2012-13 data showed that the dry ginger production from Wayanad district alone was 11846 tonnes from an area of 1893 ha, which accounts for nearly half of the total ginger production from the state (Anonymous, 2014).

Ginger is affected by various pests and diseases during its different growth stages. Majority of the foliar and rhizome diseases are found during the periods of South-West monsoon. Rhizome rot is the most complex determinable disease for ginger cultivation. The disease occurs in varying severity and intensity and may change with geographical location. Pests like *Aspidiella hartii* (rhizome scale) and *Conogethes punctiferalis* (shoot borer) can cause severe damage to the crop. Root knot (*Meloidogyne* spp.), burrowing (*Radopholus similis*) and lesion (*Pratylenchus* spp.) nematodes produce aerial symptoms like stunting, chlorosis, poor tillering and necrosis in ginger. Characteristic root galls and lesions that lead to rotting are generally seen in roots. Nematode infestation produce brown, water soaked areas in the outer tissues of rhizomes and aggravates the rhizome rot disease complex (Sasikumar *et al*., 2008).

Ginger yellows is a serious disease of ginger that cause rhizome rot in field and storage. It was first described by Simmonds (1955) from Queensland. It has been reported from all major ginger growing regions in India; Madhya Pradesh (Haware and Joshi, 1973); Karnataka (Kumar, 1977), Shillai, Rajgarh, Ronhat and Sirmur areas of Himachal Pradesh (Dohroo *et al*., 1988), Shimoga, Kodagu, Uttara Kannada, Chickmagalur and Bidar districts of Karnataka (Sagar *et al.*, 2008).

Vijayaraghavan and Mathew (2011) reported yellows disease from Wayanad district of Kerala. The disease was recently recorded from most of the commercial plantings with a severity of 50-90 %. The disease has caused significant losses to the commercial ginger industry here.

Ginger yellows occurs sporadically in ginger cropped area and it is often found associated with other disease causing pathogens like *Pythium* sp. (Drojee, 1986) and nematodes like root knot nematode *Meloidogyne* spp. (*M. javanica* and *M. indica*) (Lodha *et al*., 1994). The disease spread through infected soil and rhizome which are the primary sources of inoculum and through water and mechanical means as the secondary source (Sharma and Jain, 1978).

Many fungicides and biocontrol agents have been tested against ginger yellows around the world. Though the yellows disease has been found to be widespread in the major ginger growing area of Kerala *viz.*, Wayanad, no systematic study on symptomatology, etiology and management of the disease has been conducted. Many new fungicides are available now, which are ecofriendly and not yet tested on ginger. Hence the present study was undertaken with the following objectives,

- To study the etiology of yellows disease affecting ginger
- To develop a management strategy to contain the disease

Review of Literature

2. REVIEW OF LITERATURE

Ginger (*Zingiber officinale*) is an important commercial spice crop cultivated throughout India for its rhizome. Ginger is used as a flavoring agent, preservative used in pickling and ginger oil in soft drinks. India contributes 33 per cent of the world production of dry ginger accounting for substantial foreign exchange earnings (FAO, 2005). It has high medicinal value and is used in many decoctions in ayurveda.

Ginger plant is affected by many foliar and rhizome pathogens among which, those causing rhizome rot are of great significance, as it directly affects the economic part. The leaf spot caused by *Phyllosticta zingiberi* Ramkr., is an important foliar disease of ginger which can cause substantial yield loss (Singh *et al*., 2000). It was for the first time reported from India by Ramakrishnan (1942). Since then it has been reported from Philippines (Chanliongco, 1966) and Mauritius (Iyer, 1987).

The rhizome of ginger is affected by several fungal and bacterial pathogens in field as well as in storage conditions (Dohroo, 1993). This results in heavy post harvest loss (34%), thus affecting the quality and quantity of seed ginger (Sharma and Dohroo, 1982). Rhizome rot of ginger (*Z. officinale*) caused by *Pythium* spp. was known to exist from 1918 in the Malabar and South Kanara districts of South India (Thomas, 1938).

Bacterial wilt of ginger or 'Mahali' caused due to the pathogen *Ralstonia solanacearum* (Smith) is a serious problem which can cause 100 per cent yield loss. It is endemic to majority of the ginger growing regions of India like Kerala, Sikkim and north eastern regions of the country (Mathew *et al*., 1979).

P. aphanidermatum was found to be the most predominant pathogen isolated from different locations like Bidar, Uttar Kannada, Mysore, Chickmagalur districts of Karnataka (Sagar *et al.*, 2008). Other pathogens reported to cause rhizome rot are *Fusarium oxysporum* Schlecht. f. sp. *zingiberi* (Trujillo) from Madhya Pradesh (Haware and Joshi*.,* 1973) and Himachal Pradesh (Rana and Kumar, 1990); *Fusarium solani* (Mart.) Sacc. from Karnataka (Kumar, 1977); and *Pseudomonas solanacearum* Smith from Kerala (Sharma and Jain, 1978; Mathew *et al.,* 1979).

Ginger yellows has been reported from all major ginger growing regions in India; Madhya Pradesh (Haware and Joshi 1973); Karnataka (Kumar, 1977), Shillai, Rajgarh, Ronhat and Sirmur areas of Himachal Pradesh (Dohroo *et al*., 1988), Shimoga, Kodagu, Uttara Kannada, Chickmagalur and Bidar districts of Karnataka (Sagar *et al.*, 2008). Calicut and Wayanad district of Kerala (Dake and Edison, 1989; Vijayaraghavan and Mathew, 2011).

2.1. GINGER YELLOWS

Ginger yellows is a serious disease of ginger that affects the rhizome in the field and storage. It was first described by Simmonds (1955) from Queensland. Later this disease was reported from Hawaii (Trujillo, 1963) and India (Haware and Joshi, 1973). The causal agent for yellows disease have been identified as *F. oxysporum* Schlecht f. sp. *zingiberi* Trujillo (Haware and Joshi, 1973) and *F. solani* (Mart.) Sacc. (Kumar, 1977).

The disease occurs in patches with plants exhibiting yellow symptoms surrounded by green plants. The disease incidence and rhizome rot varies from plant to plant. The infected plants appear stunted with root rot and rhizome formation is affected. It is common to find yellow, stunted above-ground shoots among apparently healthy green shoots. The disease

incidence depends upon the initial inoculum in soil and infection in seed materials. The disease may start in the seed piece originally planted; also, it has been observed that the fungus can readily invade wounded healthy seed planted in infested soil (Trujillo, 1963).

The ginger yellows disease is a dry rot characterized by collapse of the cortical tissues of infected rhizome, occasionally accompanied by a purpling of the infected areas, and a white cottony mycelial growth on the cut surfaces of ginger. As the rot advances into newer rhizome sections, the cortex shows sunken areas. This is especially noticeable on infected rhizomes in storage (Trujillo, 1963).

F. oxysporum f. sp. *zingiberi,* the causal agent of *Fusarium* yellows in ginger is a fungus that can be carried in infected rhizomes. The fungus also produces resting structures (chlamydospores) in the decomposing tissues of infected rhizomes. Therefore, tissues from infected crops remaining in the field serves as a reservoir of the fungus. Once a ginger field becomes infected, the fungus can remain in the soil for many years. Ginger harvested from fields contaminated with the *Fusarium* yellows fungus may be infected and the fungus will continue to destroy the rhizome tissues in storage (Trujillo, 1963).

The disease can spread through infected soil and rhizome which are the primary sources of inoculum and through water and mechanical means as the secondary source. Disease development was found favourable at a temperature range of 15-38^{o}C. The infection increased at optimum temperatures of 23-29^{o}C with a high relative humidity of 87-95 per cent and presence of a thin film of water (Sharma and Jain, 1978).

There is a steady decline in area and production of ginger per unit area in Himachal Pradesh for the past seven to eight years and the major

constraint was identified as the quick and devastating spread of ginger yellows disease caused by *F. oxysporum* f. sp. *zingiberi* (Rana, 1991).

The ginger industry has experienced declining yield and significant crop losses in south-eastern Queensland, Australia due to a number of soil borne pathogens including *Fusarium* yellows (*F. oxysporum* f. sp. *zingiberi*) (Smith and Stirling, 2011).

Ginger yellows disease occurs sporadically in ginger cropped area and it is often found associated with other disease causing pathogens like *Pythium* sp. (Drojee, 1986) and nematodes like root knot nematode, *Meloidogyne* spp. (*M. javanica* and *M. indica*) (Lodha *et al*., 1994).

The ginger yellows caused due to *F. oxysporum* produces superficial mycelial growth of the fungus. The disease is also called as 'red rot' due to red colouration of internal tissues of infected rhizomes (Dohroo, 2001).

The yellows disease has been reported by Vijayaraghavan and Mathew (2011), from most of the commercial plantings in Wayanad district of Kerala. The disease severity ranged from 50-90% at different localities in this region. In Kerala the disease is known as "bellakedu" or "sarkarakedu" as the discolouration of the affected rhizome resembles the colour of jaggery.

The plants affected by the fungus *F. oxysporum* f. sp. *zingiberi* do not wilt rapidly as in bacterial wilt. Instead the infected plants are stunted and yellowed. The lower leaves dry out over an extended period of time. It is common to find yellows, stunted above ground shoots among apparently healthy green shoots. The plant finally dries out as the fungus invades the entire vascular system of the underground rhizomes (Taj and Kumar, 2013).

2.2. ISOLATION OF PATHOGEN

The ginger yellows infected specimens *viz.*, pseudostems, roots and rhizomes were collected from different parts of Karnataka by Sagar (2006), for isolation of the pathogen. The specimens were surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec. Ten pieces each of different plant parts were placed on Potato dextrose agar medium (PDA).

Taj and Kumar (2013) used sodium hypochlorite (1:1000) for surface sterilization of rhizomes from yellows infected ginger. The pieces were then placed in PDA medium and incubated for eight days at 25 ± 2^{o}C. Rangaswami and Mahadevan (2001) used Czapek Dox Agar medium for isolation of *F. solani* from rotted rhizomes of ginger.

2.3. PATHOGENICITY

Dake and Edison (1989) evaluated pathogenicity of the isolates of *Fusarium* spp. were evaluated on hypocotyl tissue of 30 days old sprouts grown in 12 x 15 cm size polythene bags on a standard pot mixture. Four hours before inoculation, bags were watered to field capacity in order to distribute the inoculum uniformly throughout the soil. Seven days old cultures of *Fusarium* spp. were harvested by washing mycelial growth with conidia and then suspended in 100 ml of tap water and was dispersed evenly using a magnetic stirrer. The mycelial/spore suspension was added to the bags around the hypocotyl @ 50 ml per bag.

Spore suspension (10^3 or 10^6 spores per ml) of *F. oxysporum* f. sp. *zingiberi* (BRIP 39298) was used to inoculate the seed pieces of cultivars Canton and Queensland for pathogenicity tests (Stirling, 2004). The seed materials were dipped in the spore suspension and were planted in pots

containing steamed peat-sand mix. The pots were placed in a shade-house and watered normally. Seed pieces dipped in water were used as control.

Ram and Thakore (2010) carried out pathogenicity tests on different fungal pathogens responsible for storage rot in ginger. The rhizomes were surface sterilized by 0.1 % mercuric chloride solution for one min. followed by three washings with sterilized water and then inoculated with different isolated fungi. The inoculum was obtained from five days old culture of fungal pathogens with two mm disc cut out from periphery of the fungal growth and then inoculated. Three rhizomes were separately inoculated with isolated fungi and incubated at room temperature 28 ± 2ºC for disease development. A separate lot of healthy rhizomes were injured and the cut end was covered with a piece of sterile cotton dipped in sterile distilled water to serve as control.

The ginger plants inoculated with *F. oxysporum* in a pot culture experiment developed initial symptoms of yellowing in about 45 days after inoculation (Rajan *et al.,* 2002). The yellowing of leaf margins was observed initially in the lower leaves, which progressed towards the top during later stages. The plants wilted subsequently leading to death of plants.

The morphological and cultural characterization of *F. oxysporum* f. sp. *lycopersici* causing wilt in tomato was carried out by Amini and Sidovich (2010), based on morphological criteria given by Gerlach and Nirenberg (1982). The mycelium was white cottony to pink, often with purple tinge or reddish coloration of the medium. Microconidia were born on simple phialides arising laterally and were abundant, oval-ellipsoid, straight to curved, 4–12 × 2.1-3.5 µm. Macroconidia were sparse to abundant, borne on branched conidiophores, thin walled, three to five septate, fusoid-subulate and pointed at both ends with a pedicellate base.

Three septate spores were more common. Chlamydospores, both smooth and rough walled, were abundant, formed terminally or intercalary.

Vijayaraghavan and Mathew (2011) studied the morphological and cultural characters of *F. oxysporum* f. sp. *zingiberi* causing yellows disease in ginger. The colony produced a cottony white mycelium initially which later turned pinkish purple after 10 days of incubation. On microscopic examination, single celled, hyaline, fusiform to ovoid microconidia measuring 5-12 × 2.7-3.0 µm were observed. Macroconidia were hyaline, 29-48 × 3.8-5.1 µm, multiseptate, fusiform to sickle shaped.

The *F. solani* isolate causing ginger yellows studied by Parveen and Sharma (2013) produced woolly to cottony with cream to white aerial mycelium on PDA and red colouration on the reverse side. They observed that the macroconidia were wide, thick walled, slightly curved and mostly five septate, apical cell was tapered and basal cell was foot shaped. It had three to four septa and a slightly blunted apical end. Microconidia were abundant, oval to kidney shaped, and developed on false heads (phialids).

Internal Transcribed Spacer (ITS) regions located in the rDNA gene complex show extensive sequence diversity among different species of fungi. This is used as a signature region for molecular analysis, characterization, identification and phylogeny of fungi. The PCR-RFLP analysis of the amplified region of 5.8S rDNA intervening the ITS region help to distinguish between the different fungal species (Ribes *et al*., 2000).

Molecular characterization of 48 isolates of *Fusarium* spp. from a conifer nursery was carried out by Stewart *et al*. (2006), to study the different pathogenic and non pathogenic isolates associated with root rot and damping off in Douglas fir seedlings. The characterization was carried out using Amplified Fragment Length Polymorphism (AFLP) and

DNA sequencing of nuclear rDNA (ITS including 5.8S rDNA), mitochondrial rDNA (small subunit [mtSSU]), and nuclear translation elongation factor 1-alpha. The sequences from all three regions and the combined data set showed that all highly virulent isolates clearly separated into a common clade that contained *F. commune*, which was recently distinguished from its sister taxon, *F. oxysporum*. All but one of the non pathogenic isolate grouped into a common clade was genetically similar to *F. oxysporum*.

Zaccardelli *et al*., (2008) studied different *forma specialis* (f. sp.) of *F. solani* isolates causing diseases in important crops such as root and fruit rot of *Cucurbita* spp., root and stem rot of pea, sudden death syndrome of soybean, foot rot of bean and dry rot of potato tubers during storage. Thirty four isolates of *F. solani* obtained were characterized by cultural, morphological and molecular criteria. The DNA sequences of 28S rDNA, Internally Transcribed Spacers (ITS) rDNA and elongation factor (EF-1α) were used to identify and distinguished the '*F. solani* complex' in to 50 sub specific lineages.

2.4. CULTURAL STUDIES

2.4.1. Growth on Different Solid Media

Five solid media *viz.* Potato Dextrose Agar (PDA), Czapeks Dox Agar medium (CDA), Corn Meal Agar medium (CMA), Cooke's Rose Bengal Agar medium (CRBA) and Oat Meal Agar medium (OMA) were used by Gupta *et al*. (2010), to study the radial growth and sporulation of *Fusarium* spp. (five isolates each of *F. oxysporum* f. sp. *psidii* and *F. solani*. The isolates were inoculation in the growth media and were incubated at 28 ± 2°C. The diameter of the each isolate was recorded at 24 h interval till full expansion and expressed in millimeters. The

measurements were taken in two directions at right angles to each other, and then average colony diameter was calculated.

Farooq *et al*. (2005) used eight different nutrient medium to study the growth of *F. oxysporum* f. sp. *ciceri, viz.,* chickpea seed meal extract agar (CSMA), PDA, CMA, malt extract agar (MEA), CDA, Sabouraud's agar medium, Waksman's agar medium, Richard's agar medium. The results of the experiment revealed that the CDA and CSMA media were the best for the radial growth of the fungus, which gave maximum growth of 85 and 80 mm, respectively, after seven days of inoculation. This was followed by CMA and MEA showed a growth of 70 and 65 mm, respectively.

Studies on sporulation of *F. oxysporum* f. sp. *psidii* and *F. solani* on different nutrient media was also undertaken by Gupta *et al*. (2010) and the number of spores per ml was calculated, using the formula by Pathak (1984).

2.4.2. Effect of Different Temperatures on Growth of *Fusarium* spp.

The effect of different temperatures on growth of *Lasiodiplodia theobromare* and *F. solani* in *in vitro* were studied by Kausar *et al.* (2009). In that study five mm culture discs were cut with sterilized cork borer from advancing margin of colonies of these two pathogens and inoculated on PDA plates separately and incubated at 20, 25, 30, 35 and 40ºC. The temperature of 25 ± 2ºC was found to be more suitable for growth of the pathogens.

The growth of ten isolates of *Fusarium* sp. (*F. oxysporum* f. sp. *psidii* and *F. solani*) at temperatures of 10, 16, 22, 28, 34 and 40 ± 1ºC were studied by Gupta *et al*. (2010). They observed the dia. on the seventh day after inoculations and the results showed that the radial

mycelial growth was maximum at 28°C (72.50) mm for both *F. oxysporum* f. sp. *psidii* and *F. solani*.

2.4.3. Effect of Light and Darkness on Mycelial Growth of *Fusarium* spp.

Kausar *et al.* (2009) in a study on the effect of light and darkness on mycelial growth of *L. theobromae* and *F. solani,* used five mm culture discs carbon paper to wrap the Petri dishes for darkness and fluorescent lamp for light exposure. The Petri dishes were incubated at 25 $\pm$ 2°C in quadruplicates under continuous light (24 h), alternating light and darkness (12 h light + 12 h darkness) and complete darkness (24 h). They observed that the growth under continuous light was the best followed by continuous darkness. The growth under alternate light and darkness was the least.

Ramteke and Kamble (2011) used different light spectra to study the growth of *F. solani* causing rhizome rot in ginger under *in vitro* condition on solid media. In the study, Petri plates containing CDA were inoculated with isolates of *F. solani* and wrapped with gelatine sheets of different colours. Plates kept in dark served as control. The experimental results showed that light had little influence on mycelial growth. Among different light spectra, growth was more under green and blue light. The mycelial growth was found to be the best under dark conditions.

2.4.4. Effect of p^H on Mycelial Dry Weight of *F. oxysporum* f. sp. *lentis*

Jaruhar and Prasad (2011) studied the effect of ten different p^H levels on *F. oxysporum* f. sp. *lentis.* The mean dry weight of the mycelium from each p^H level was determined as described by Prasad and Chaudhary (1966). They observed that the mycelial mat production and sporulation

of fungus was the maximum at p^H 6.0, where as they were low at extreme p^H levels.

2.5. MANAGEMENT OF FUSARIUM WILT DISEASE

2.5.1. *In vitro* Assay

Philip (1994) screened four commercial fungicides against *F. solani* and *F. oxysporum* causing wilt disease on *Pterocarpus indicus,* a way side and shade tree in Malaysia. The fungicides, thiabendazole, benomyl (benlate), metalaxyl and mancozeb were tested at different concentrations recommended by the manufacturer. The results of the study showed that Thiabendazole (0.12, 0.28 and 0.4%) and Benlate (0.4, 0.5 and 0.56%) inhibited the growth of *F. solani* and *F. oxysporum* at all concentrations, whereas metalaxyl (0.8, 1.00 and 1.20%) and mancozeb (0.16, 0.24 and 0.28%) showed no inhibition to both the fungus at any concentration.

Benlate has been reported to be most effective for checking the mycelial growth of *F. solani,* causing root infection in cucurbits (Ahmad *et al*., 1996). Benlate completely inhibited the growth of *F. solani* causing grain infection in sweet corn at 50 ppm (Baird *et al*., 1994; Mathre *et al,*. 1995; Nawar, 2007).

Sagar (2006) tested the per cent inhibition of mycelial growth of *F. solani* by systemic and contact fungicides by using poisoned food technique. She reported that among the different contact fungicides used, emisan completely inhibited the mycelial growth of *F. solani* in ginger. Thiram @ 0.3% gave mycelial growth inhibition of 87.03%. The least inhibition was recorded in Copper oxy chloride (67.40%) at 0.2%.

Sagar (2006) studied the effect of different fungicides on rhizome rot pathogens in ginger using pot culture studies. She reported that among the different systemic fungicides tested as rhizome treatment against rhizome

rot of ginger, carbendazim @ 0.2% was found to be most effective as there was good per cent germination (91.33), decrease per cent disease incidence (10.66) which was on par with combination product SAAF (mancozeb + carbendazim @ 0.2%) and Quintal (iprodione + carbendazim @ 0.2%). Rhizome treatment with thiram @ 0.3% showed poor germin...ation of 63.33 and increased disease incidence (22.66%) but significantly superior over control. The rhizome yield of ginger was significantly superior in all the treatments compared to control. Rhizome treatment with carbendazim @ 0.2 per cent recorded maximum yield (4.40 kg/pot) and was significantly superior over other treatment.

Sultana and Ghaffar (2010) studied the effect of different fungicides on mycelial growth of *F. solani* causing root infection in cucurbits. They observed significant effect on colony growth in all fungicidal concentration compared to control. Complete inhibition of colony growth of *F. solani* was observed where fungicides *viz.*, aliette, benlate and carbendazim @ 0.01% were used whereas mancozeb, ridomil, topsin-M and vitavax completely inhibited the colony growth at 0.1%. In field trial experiments, carbendazim completely eradicated seed borne infection of *F. solani* in bitter gourd and gave maximum disease control in cucumber and bottle gourd. Root infection was completely checked by benlate and carbendazim in bitter gourd. The disease was best controlled by aliette, topsin-M and carbendazim in bottle gourd and cucumber.

Amini and Sidovich (2010) studied the effect of six systemic fungicides: benomyl (Fundazol WP, 500 g/l), carbendazim (Kolfugo super SC, 200 g/l), prochloraz (Sportac EC, 450 g/l), fludioxonil (Maxim SC, 25 g/ l), bromuconazole (Bectra SC, 100 g/l) and azoxystrobin (Quadris SC, 250 g/l) were tested at different concentration (0.0001, 0.001, 0.01, 0.1, 1, 10, 100 µg/ml) individually to assess their effect on growth inhibition of *F. oxysporum* f. sp. *lycopersici*. They concluded that all fungicides except fludioxonil and azoxystrobin at 10 µg/ml, significantly reduced the

mycelial growth of pathogen *in vitro*. The fungicides prochloraz and bromuconazol proved to be the most effective in inhibiting mycelial radial growth of the pathogen, followed by benomyl and carbendazim.

Ramteke and Kamble (2011) studied the sensitivity of fungicide benomyl on *F. solani* causing rhizome rot disease in ginger. In the study, healthy ginger rhizomes were surface sterilized with 70% alcohol and eight mm well (15 mm deep) was prepared on it. Different concentrations of benomyl were poured into the well. The same rhizomes were inoculated next day with mycelial suspension of *F. solani*. The wells were closed with cylindrical rhizome tissues. These rhizomes were covered with moist paper towels and incubated at 26 ± 3^{o}C in dark. Rhizomes treated with sterile distilled water served as control. Diameter of infected portion was measured after eight days of inoculation. Each isolate showed varying levels of sensitivity to benomyl. The minimum inhibitory concentration of benomyl varied from 4-200 µg/ml, between different isolates causing rhizome rot in ginger.

2.5.2. Spore Germination Experiment

Begum *et al*. (2010) studied the spore germination of three fruit rot pathogens *F. oxysporum* f. sp. *capsici, Rhizopus artocurpi* and *Alternaria tenuis* using different fungicides. Spore suspension (10^3 conidia per ml) was made from seven day old cultures on PDA. Fungicides copper oxychloride (Cupravit), metalaxyl-M (Ridomil), iprodione (Rovral), wettable sulphur (Thiovit), mancozeb (Diathane M-45) and carbendazim (Bavistin) at five different concentrations (500, 1000, 1500, 2000 and 2500 ppm) were tested. The spore suspension was put in triplicate in fungicidal solution or suspensions in sterile water for 30 min. Five ml suspensions of each were taken in small sterilized Petri dishes (65 mm) and kept at 28^0C for 30 min. A drop of lacto phenol cotton blue was added to conidial suspension on the slides. The slides were examined

under microscope 400 X for recording the percentage of conidial germination. Among the fungicides tested, all the concentrations of Ridomil showed 100% inhibition of spore germination and the least inhibition was recorded in case of treatment with Bavistin for *F. oxysporium* f. sp. *capsici.* In case of *R. artocarpi* and *A. tenuis*, mancozeb showed good inhibitory effects but Thiovit had no inhibitory effect against *A. tenuis*.

2.5.3. Pot Culture Studies

Two month old ginger plants raised in plastic buckets containing sterile soil, were inoculated with pathogens *Pythium* spp. and *F. oxysporum*. Prior to inoculation, the top layer of soil was removed and the mycelial suspension was applied at the collar region @ 75 ml/pot and it was covered with soil (Rajan *et al*., 2002).

Stirling (2004) in a pot culture study using rhizome rot pathogens of ginger, used cornmeal-sand mix as an effective method for mass culturing of the pathogens. Five isolates of *Pythium* sp. were mass cultured in sterile cornmeal-sand mix (3 g cornmeal, 100 g washed river sand mixed with 15 ml water in glass jars and autoclaved for 20 min. at 121^0C on two consecutive days) for three weeks. The cornmeal–sand inoculum was then incorporated in steamed peat-sand mix at one or 10 g/l into which ginger seed-pieces were planted. Peat-sand mix amended with sterile cornmeal sand alone served as controls. The inoculated plants produced brown discoloration and rotting of seed-pieces. The newly emerged shoots turned yellow, wilted and eventually died. The seed pieces failed to germin...ate under severe infection. The disease assessments of rhizomes were made after growing the plants were raised in constant environment under glass house conditions.

2.5.4. Fungicide Trials

Ramachandran *et al*. (1989) studied the effect of ten different fungicide treatments on soft rot of ginger caused by *Pythium* spp. by using pot culture studies. Fungicides were applied as seed treatment and soil drench. Pretreated rhizomes of the ginger variety 'Maran' were sown in plastic containers and the first soil drench with fungicide solution (500 ml) was given soon after sowing. The second drench with fungicides was given at 28 days after the first application. The untreated control plants produced disease after 16 days of inoculation. The infection was delayed (28-38 days after planting) and reduced in case where Apron 35 W.S was used for seed treatment.

Rana (1991) steeped seed rhizomes in fungicide solution for 30 min. and dried them in shade before planting. Fungicides like mancozeb @ 0.25%, carbendazim + mancozeb @ 0.3% and metalaxyl @ 0.2% were found to reduce ginger yellows disease incidence. The treatment with mancozeb @ 0.25% yielded higher than the uninoculated check plant.

Chowdhury *et al.* (2009) studied the effect of different fungicides on ginger rhizome rot diseases under field conditions. The ginger rhizomes were treated in different fungicidal solutions for 30 min. before planting. Among the different treatments, 0.1% thiophanate-methlyl ethyl (Topsin M), hot water treatment at 51^{o}C for 20 min. and a combination of the hot water treatment followed by the fungicidal treatment were found to be effective in controlling rhizome rot and resulted in highest yield.

The inhibitory effect of triazole fungicide on radial mycelial growth of *F. oxysporum* f. sp. *zingiberi* was studied by Taj and Kumar (2013), using poisoned food technique. The fungicide tebuconazole (50% WG) completely inhibited the radial mycelial growth of *F. oxysporum* f. sp. *zingiberi* at 100 ppm. A combination of trifloxystrobin (25% WG) +

tebuconazole (25% WG) completely inhibited the radial growth of *F. oxysporum* f. sp. *zingiberi* at all concentrations (10, 25, 50 and 100 ppm). Carbendazim was found to be effective at all concentrations (10, 25, 50 and 100 ppm) where as captan was found to be least effective at 10 and 25 ppm. The inhibition of radial growth increases with increase in concentration in general.

2.5.5. Biocontrol Agents

Fungal antagonists such as *Trichoderma harzianum* and *Gliocladium virens* inhibited the mycelial growth of *F. oxysporum* f. sp. *zingiberi,* the incitant of ginger yellows (Dohroo, 1994). Sharma and Dohroo (1997) observed that seed treatment of ginger rhizomes with *T. harzianum* in combination with addition of pine needle as organic soil amendment could manage ginger yellows and increase the yield.

Mishra *et al*. (2004) tested the antagonistic potential of *T. virens* on *F. oxysporum* f. sp. *gladioli*, *in vitro* by using dual culture technique. A six mm disc of *T. virens* and *F. oxysporum* f. sp. *gladioli* were placed on a PDA Petri dish and incubated at 25 ± 1^{o}C in a BOD incubator. The growth of the advancing colonies of both fungus were observed and it was found that *T. virens* inhibited the mycelial growth of *F. oxysporum* f. sp. *gladioli*. The effect of culture filtrate and volatile compounds of *T. virens* on *F. oxysporum* f. sp. *gladioli* were also observed using standard procedures. It was observed that both the culture filtrate and volatile compounds released from young cultures of *T. virens* significantly inhibited the colony growth of *F. oxysporum* f. sp. *gladioli.*

In a field experiment, Chandel and Tomar (2008) used biocontrol agents such as *G. virens, T. harzianum* and *T. viride* to manage *Fusarium* wilt of carnation (*Dianthus caryophyllus* L.) caused by *F. oxysporum* f. sp. *dianthi* (Prill and Del.). The biocontrol agents were mass multiplied in

wheat bran-saw dust medium prepared at a ratio of 2:1. The fungus *F. oxysporum* f. sp. *dianthi* was mass cultured in sand-corn meal medium for inoculating the field beds of 1×1 m size @ of 100 g/ bed, 20 days prior to sowing of plants.

According to Taj and Kumar (2013), biocontrol agents such as *T. viride, T. harzianum, T. virens, T. koningii* and bacterial antagonist *Pseudomonas fluorescens* and *Bacillus subtilis* isolates could restrict growth of *F. oxysporum* f. sp. *zingiberi* on PDA in dual culture. Maximum inhibition of growth was observed in *T. viride* (87.77%) followed by *T. koningii* (86.67%) and *T. virens* (86.66%). Whereas the bacterial antagonist *P. fluorescens* (82.96%) inhibited the pathogen better than *B. subtilis* (60.74%).

2.5.6. Disease Scoring

Ramachandran *et al*. (1989) studied the disease incidence of rhizome rot on ginger in pot culture experiments. The observations were recorded as percentage of pseudostems or sprouts infected in each container at 10 days intervals and finally the mean of all observations for each container were worked out. Sufficient care was taken to avoid repeated counting of infected pseudostems on subsequent dates of observation. The yield per container was also recorded after harvest.

In an experiment in ginger, Smith and Stirling (2011) integrated various management practices to increase yield and control losses due to different diseases. Disease severity indices were developed to assess the percentage yield loss due to rhizome rot (*Pythium* sp.) and yellows (*F. oxysporum* f. sp. *zingiberi*). Ten percentage area of the entire trial plot was affected by ginger yellows. Observations of dead and severely yellowing shoots were taken and converted to a disease rating of 1 - 6, where a rating of 1 indicated that < 5% of shoots showed symptoms and a

rating of 6 indicated 45-55% of shoots were affected. Prior to harvest of the crop, the percentage of yellowing and necrotic shoots in the entire length of the bed was estimated, and plots were rated for disease severity (0 = all shoots green and healthy to 5 = total collapse and death of shoots). In the same experiment, they developed a separate scale was developed for assessing the suppression of infection by *Pythium* sp. Plants were assessed for disease severity after three months for each assay, on a 0–3 scale where 0 = no disease; 1 = some leaf yellowing; 2 = most shoots yellow or dead; 3 = rhizomes rotted and plants dead.

3. MATERIALS AND METHODS

The present investigation entitled "Etiology and Management of Ginger Yellows" was carried out in the Department of Plant Pathology, College of Agriculture, Vellayani during 2012-2014. The materials and methods followed in this study are described below.

3.1. SYMPTOMATOLOGY OF GINGER YELLOWS

The natural symptoms of ginger yellows occurring on aerial shoots and rhizomes of ginger plants were observed.

3.2. COLLECTION OF SAMPLES AND ISOLATION OF THE PATHOGEN

Samples of diseased ginger rhizomes were collected from different locations of Kerala and Karnataka for isolation of the pathogen. The technique followed by Taj and Kumar (2013), was used to isolate the pathogen from infected rhizome tissues of ginger. The specimens were washed and rinsed thoroughly in tap water. The diseased areas of plants were then cut into small pieces with a sterile blade and surface sterilized with sodium hypochlorite (1:1000) for one min. and washed in a series of sterile distilled water. The cut pieces were then placed on blotting paper to remove excess water. Such small pieces were transferred aseptically on Petri plate containing solidified PDA medium and were incubated for eight days at 25 ± 2^{o}C. The isolates thus obtained and purified by single spore isolation technique were identified with the help of relevant mycological literatures (Booth 1971, 1977). The cultures were maintained in culture tubes at 4^{o}C and used for further study whenever necessary.

3.2.1. Single Spore Isolation

The single spore isolation technique mentioned by Booth (1977) was followed here. A drop of sterile water was placed on a sterile slide under

dissecting microscope. An accumulation of spores obtained on the wet tip of a needle from the aerial mycelium was introduced into the drop of water on the slide and spores were observed to flow from the tip of the needle into the drop of water. The spore suspension on the slide was then picked up by a sterile loop and streaked across a clear agar plate, the position of the streaks were marked by a glass marker at the bottom of the Petri plate. The plate was then incubated for 12-16 h at 25 ± 2°C in a BOD incubator. By following the lines under 100X objective of a compound microscope, germin.ation of the conidia was observed and those which were positioned were removed by using a one mm cork borer on to a Petri dish containing PSA medium.

3.3. MAINTENANCE OF THE CULTURE

The isolates obtained were sub cultured on slants of potato sucrose agar (PSA) and were incubated at room temperature for five days. The slants were then preserved in refrigerators at 5°C. Sub culturing was done once in a month and such cultures were used for further studies. Virulence of the fungi was maintained by inoculating the isolates onto the host and reisolating them every three months.

3.4. IDENTIFICATION OF PATHOGEN

The identification of *Fusarium* sp. was done based on the spore morphology and colony characters of the fungus by referring to the book "The Genus Fusarium" by Booth (1971). The pure culture of the fungus was obtained by hyphal tip culture followed by single spore isolation under aseptic conditions. Such culture tubes were preserved in a refrigerator at 5°C and used for further studies.

3.5. CHARACTERIZATION OF THE PATHOGEN

3.5.1. Morphological Characterization

The morphological characters of the three isolates of the pathogen were studied by observing its growth in suitable culture medium, spore production and spore morphology. The cultures were sent to National Fungal Culture Collection Center of India, Agharkar Research Institute, Pune for morphological identification.

3.5.1.1. Colony characters

Five mm disc of each isolate was inoculated in the center of nine cm Petri dishes with PSA and were incubated at room temperature. Colony characters were studied seven days after incubation.

3.5.1.2. Conidial characters

The isolates were slide cultured and semi-permanent mounts were prepared as mentioned by Riddel (1974), for studying the conidial characters.

3.5.2. Molecular Characterization

In the present study, an attempt was made to sequence the Internal Transcribed Spacer (ITS) region of the three isolates of pathogens obtained, and study the identity and phylogeny of the three isolates by considering their sequence similarity using multiple alignment and phylogenetic tree. The amplicon of three isolates of the pathogen was sequenced in RGCB, Thiruvananthapuram (Rajiv Gandhi Center for Biotechnology). The protocol they followed was as given below.

3.5.2.1. DNA Isolation Using NucleoSpin® Plant II (Macherey-Nagel)

About 100 mg of the tissue was homogenized using liquid nitrogen and the powdered tissue was transferred to a microcentrifuge tube. Four hundred microlitres of buffer PL1 was added and vortexed for one min. Ten microlitres of RNase A solution was added and inverted to mix. The homogenate was incubated at 65ºC for 10 min. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for two min. The flow through liquid was collected and the filter was discarded. Four hundred and fifty µl of buffer PC was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for one min. and the flow through liquid was discarded. Four hundred µl buffer PW1 was added to the column, centrifuged at 11000 x g for one min. and flow though liquid was discarded. Then 700 µl PW2 was added centrifuged at 11000 x g and flow through liquid was discarded. Finally 200 µl of PW2 was added and centrifuged at 11000 x g for two min. to dry the silica membrane. The column was transferred to a new 1.7 ml tube and 50 µl of buffer PE was added and incubated at 65ºC for five min. The column was then centrifuged at 11000 x g for one min. to elute the DNA. The eluted DNA was stored at 4ºC.

3.5.2.2. Agarose Gel Electrophoresis for DNA Quality Check

The quality of the DNA isolated was checked using agarose gel electrophoresis. One µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5 µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.5.2.3. PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (contains 1.5 mM $MgCL_2$), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 10ng DNA, 0.4 µl of Phire Hot Start II DNA polymerase enzyme (Thermo scientific), 0.1 mg/ml BSA, 5 pM of forward and reverse primers.

3.5.2.4. Primers Used

Target	**Primer Name**	**Direction**	**Sequence (5' → 3')**	**Reference/ Remarks**
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTTGCGG	White *et al.*, 1990
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC	

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

3.5.2.5. PCR Amplification Profile

ITS

98 ºC - 30 sec

98 ºC - 5 sec
60 ºC - 10 sec } 4ºCycles
72 ºC - 15 sec

72 ºC - 60 sec

4ºC - ∞

3.5.2.6. Agarose Gel electrophoresis of PCR products

The PCR products were checked by following the procedure explained in 'Materials and Methods 3.5.2.2'.

3.5.2.7. ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five micro litres of PCR product is mixed with two µl of ExoSAP-IT and incubated at 37^{o}C for 15 min. followed by enzyme inactivation at 80^{o}C for 15 min.

3.5.2.8. Sequencing Using Big Dye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM
		(Forward or reverse)
Sequencing Mix	-	0.28 µl
5x Reaction buffer	-	1.86 µl
Sterile distilled water	-	make up to 10µl

The sequencing PCR temperature profile consisted of a first cycle at 96ºC for two min. followed by 3oCycles at 96ºC for 30 sec, 50ºC for 40 sec and 60ºC for four min. for all the primers.

3.5.2.9. Post Sequencing PCR Clean up

1. Make master mix I of 10 µl milli Q and 2 µl 125mM EDTA per reaction

2. Add 12 µl of master mix I to each reaction containing 10 µl of reaction contents and were properly mixed.

3. Make master mix II of 2 µl of 3 M sodium acetate pH 4.6 and 50 µl of ethanol per reaction.

4. Add 52 µl of master mix II to each reaction.

5. Contents are mixed by inverting.

6. Incubate at room temperature for 30 min.

7. Spin at 14,000 rpm for 30 min.

8. Decant the supernatant and add 100 µl of 70% ethanol

9. Spin at 14,000 rpm for 20 min.

10. Decant the supernatant and repeat 70% ethanol wash.

11. Decant the supernatant and air dry the pellet.

The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.5.2.10. Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the

obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al*., 2010).

3.5.2.11. Bioinformatics

The ITS sequence of the pathogens thus obtained were searched in NCBI (National Center for Biotechnological Information) database using BLAST- N (Basic Local Alignment Search Tool- Nucleotide) and the genetic similarity of the organisms was identified. The sequences were multiple aligned by using the software www.phylogeny.fr and a phylogenetic tree was constructed using Neighbour joining (NJ) method.

3.6. PATHOGENICITY

The technique followed by Ram and Thakore (2010), with slight modifications was followed here in proving the pathogenicity of the different isolates of the pathogen on ginger. Thirty days old healthy rhizome sprouts were surface sterilized using 0.1% Sodium hypochlorite followed by three washing with sterilized water. The inoculum was obtained from five days old culture of fungal pathogens. Two mm discs were cut out from periphery of the fungal growth and then inoculated on to the rhizomes by causing an injury. Three isolates of the pathogen were separately inoculated and incubated at room temperature 28 ± 2^{o}C for 48 h. A separate lot of healthy rhizomes were injured and the cut end was covered with a piece of sterile cotton dipped in sterile distilled water to serve as control.

Inoculated rhizomes were then planted in polythene bags of size 40 x 24 x 24 cm in a standard pot mixture. The plants were heavily mulched with leaves of *Pongamia pinnata* and were maintained under green house conditions. Control treatments were maintained separately. Pathogens were re-isolated when the inoculated plants started showing disease

symptoms. The identity of the pathogen was confirmed by using Booth's key (Booth, 1971, 1977).

3.6. CULTURAL CHARACTERS OF *F. SOLANI*

3.6.1. Growth and Sporulation on Solid Medium

Eight different nutrient media *viz.*, Potato Sucrose Agar (PSA), Potato Dextrose Agar (PDA), Corn Meal Agar (CMA), Malt Extract Agar (MEA), Host Extract Agar (HEA), Oat Meal Agar (OMA), Czapeks Dox Agar (CDA) and Carrot Agar (CA) were used to study the growth of *F. solani.* Twenty ml sterilized medium was poured into each Petri dish and inoculated with five mm dia. discs of each test isolate at the center. The Petri dishes were maintained in triplicates. After inoculation the Petri dishes were incubated at 28 ± 2°C. The dia. of the each of the test isolates was recorded in cm in two directions at right angles to each other, and then average colony dia. in cm was calculated and recorded. Measurements of growth were made at the interval of 24 h, till the full expansion of growth.

Sporulation of *F. solani* on different media was also carried out. A five mm disc of the fully grown culture was cut from the near center portion of the plate and put in sterilized water (10 ml) and shaken well, so that the spores were dislodged. One drop of this spore suspension was placed on a haemocytometer and the number of spores in each of the five squares was counted at random. The number of spores per ml was calculated with a haemocytometer, using the formula given by Aneja (2007).

$$\text{No. of spores per ml} = \text{Avg. no. of spores in one large square} \times 10^4 \text{ cm}^3/\text{ml}$$

3.6.2. Effect of Temperature on Growth of *F. solani*

The growth of *F. solani* at different temperatures of 20, 25, 30, 35 and 40 ±1°C were taken. Twenty ml of PSA were poured in the plates and used for different temperature studies. Mycelial discs of five mm in dia. were transferred from the margins of the seven day old growing colony of *F. solani* to the centre of each PSA plate. Each treatment was replicated five times. Observations on colony growth in dia. were recorded on the seventh day after inoculation.

3.6.3. Effect of Light and Darkness on Mycelial Growth of *F. solani*

Effect of light and darkness on radial mycelial growth of *F. solani* was carried out using the technique suggested by Kausar *et al.* 2009. The treatments used were continuous fluorescent light 500 lux (24 h), alternate light and darkness cycle (12 h light + 12 h darkness) and complete darkness (24 h). Carbon paper was used to wrap the Petri dishes for darkness. Fluorescent lamp was used for light exposure. Five mm culture discs were cut with the sterilized cork borer from the margins of *F. solani* and inoculated on Petri plates containing 20 ml PSA medium. Five replications were maintained for all treatments and the plates were incubated at a temperature of 25 $\pm$ 2°C.

3.7.4. Effect of p^H on Mycelial Dry Weight of *F. solani*

Different pH level ranging from 4.0 to 9.0 with a difference of 1.0 were used to study the effect on mycelial dry weight of *F. solani*. p^H was adjusted by using either N/10 HCl or NaOH before autoclaving. 100 mL of PS broth was taken in 250 mL conical flask and four replicate sets were used in each case. The solution was autoclaved at 15 psi for 15 min. The inoculation was done with five mm discs of the fungus culture cut with a sterilized cork borer from the margin of a seven days old colony PSA medium. Flasks were then incubated at 26ºC ± 2ºC for ten days. The

mean dry weight of the mycelium was determined by harvesting the mycelia mat and filtering the culture filtrate through Whatman No. 42 filter paper disc of 12.15 cm dia. The filter paper discs were dried to a constant weight at 60^{o}C in an electrical oven, prior to filtration. The filter papers along with the harvested mycelial mat were dried to a constant weight in an electrical oven at 60^{o}C and weighed immediately in an analytical electric balance. The mycelial dry weight was calculated by subtracting the weight of filter paper discs.

3.7. MANAGEMENT

3.7.1. *In vitro* Evaluation of Fungicides

Commercial fungicides were selected for *in vitro* evaluation against *F. solani* using poisoned food technique and spore germination inhibition studies.

3.7.1.1. In vitro Evaluation of Fungicides Using Poisoned Food Technique.

The technique used by Amini and Sidovich (2010) to study the effect of different fungicides on *F. oxysporum*, was followed here with slight modifications. Four concentrations of each seven commercial fungicides were evaluated *in vitro* against *F. solani*. The different concentrations of fungicides were prepared from commercial formulation and suspended in distilled water. Molten double strength PSA was used for the study. The fungicidal concentration was prepared in 50 ml of sterile distilled water and it was amended into the nutrient medium. Twenty ml of the poisoned medium was poured into sterile Petri dishes (9.0 cm) under aseptic conditions, and it was allowed to solidify (Table 1).

Each plate was inoculated with a four mm dia. disc was cut out form the periphery of actively growing five day old culture of *F. solani* and incubated in a BOD incubator at 28 ± 2ºC.

A control treatment amended with plain sterile distilled water was maintained separately to record the radial growth of the pathogen. Per cent inhibition of growth over control was calculated using the formula given by Vincent (1927).

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

Where,

I = per cent inhibition

C = dia. of *F. solani* in control

T = dia. of *F. solani* in treatment

3.7.1.1. In vitro Spore Germination Inhibition Study

The spore germination study was conducted by following the technique suggested by Peterson (1941). The spore suspension of *F. solani* was prepared from a seven day old culture by scraping off the mycelial growth with sterile distilled water and collecting the suspension in a sterile Petri dish. Ten ml solution of three different concentrations from each of seven fungicides solutions was prepared using sterile distilled water in test tubes. The fungicidal solution (0.1 ml was placed at the center of a clean sterilized glass slide and it was dried at room temperature (30-35^{0}C). The spore suspension (0.1 ml) was placed on the same spot where the fungicidal solution was placed. The entire experimental procedure was followed under aseptic conditions in a laminar air flow chamber (Table 2).

The slides were labeled and placed in moist chamber prepared using wet blotting papers placed in Petri dishes. The moist chambers were incubated at 28 ± 2°C in a BOD incubator. Observations of the number of spores germinated were recorded after 12 h of incubation under 400X objective microscope.

3.7.2. *In vitro* Evaluation of Biocontrol Agents Against *F. solani*

Two identified and standardized biocontrol agents, *T. viride* (MTCC 5694) and *P. fluorescens* (MTCC 5693) available in the Dept. of Microbiology was utilized for evaluating antagonistic action against *F. solani.* A four mm disc from an actively growing culture of *T. viride* was placed two cm away from the periphery of the Petri plate and at the opposite side, a similar disc of *F. solani* was placed two cm away from the periphery.

In the case of the bacterial biocontrol agent *P. fluorescens*, the antagonistic effect on *F. solani* was tested by the dual culture technique mentioned by Morton and Stroube (1955). The bacterial isolate was streaked two cm away from the periphery of the Petri plate and to the opposite side, a four mm disc of *F. solani* was placed two cm away from the periphery of the Petri plate. The Petri plates were sealed and incubated at 28 ± 2°C in a BOD incubator. Three replications were maintained for each assay. The cultures were observed daily for antagonistic action. Per cent inhibition of growth over control was calculated using the formula given by Vincent (1927). The bio control agent that showed better antagonistic action was selected for compatibility and pot culture studies.

3.7.3. Compatibility of Biocontrol Agent with Fungicides

The compatibility of bio control agents with fungicides were tested *in vitro* following the Poisoned food technique (Shravelle, 1961). All the fungicides were tested at their recommended dosages. Molten double

strength PSA was used for the study. The fungicidal concentration was prepared in 50 ml of sterile distilled water and it was amended into the nutrient medium. Twenty ml of the poisoned medium was poured into sterile Petri dishes (9.0 cm) under aseptic conditions, and it was allowed to solidify.

Each plate was inoculated with the biocontrol agent at the center. The treatments and replications were incubated in a BOD incubator at 28 ± 2^{o}C. A control treatment amended with plain sterile distilled water was maintained separately to record the growth of the pathogen. Per cent inhibition of growth over control was calculated using the formula given by Vincent (1927). The compatible combination between bio control agent and chemical fungicide was selected for pot culture studies.

3.7.4. Pot Culture Experiments

The pot culture experiment was laid out in CRD. Standard potting mixture was used for planting. Ginger cultivar 'Maran', a susceptible variety to ginger yellows was used. Healthy planting materials were procured from farmer, collected from field with no history of yellows disease incidence. Seeds were stored in shady area prior to planting. The seed rhizomes with two or three sprouts (weighing 70-100 g) were selected for planting. The pots were heavily mulched with leaves of *Pongamia pinnata* soon after planting. Second mulching was given at two months after first mulch. Plants were maintained under green house conditions with watering at regular interval (once in three days).

The technique used by Stirling (2004) was followed in this experiment with slight modifications. The soil was inoculated with the pathogen *F. solani*, 20 days prior to planting the seed rhizomes. Mass culturing of *F. solani* was done using sand-corn meal medium (9:1 ratio). The medium (400 g) was filled in Erlnmeyer flasks of one liter capacity and autoclaved at 1.05 kg/cm^2 pressure for fifteen min. for two

consecutive days. Two to three five mm discs from actively growing cultures of the fungus were added to the flasks and incubated for 14 days in a BOD incubator at 25 ± 2°C.

Healthy ginger seeds were treated in fungicide solution for 30 min. and shade dried for two hrs to remove the excess amount of fungicide. The seeds were then planted in pot mixture pre-inoculated with *F. solani* mass produced in sand-corn medium (100 g per pot).

The fungicidal treatments T1 (hexaconazole @0.2%), T2 (flusilazole @ 0.1%), T3 (tebuconazole @ 0.15%) and T4 (carbendazim + mancozeb @ 0.2%) were given as seed treatment followed by foliar spray + soil drench at two, three and four months after planting. Treatment T5 (talc based formulation of *T. viride*) was given as seed treatment, soil amendment of talc based formulation mass multiplied in cow dung + neem cake mixture (9:1) at the time of planting (100 g/ pot), followed by soil drench at two, three and four months after planting. In treatment T6 (talc based formulation of *T. viride* + mancozeb), mancozeb was applied as seed treatment and soil amendment of talc based formulation of *T. viride* mass multiplied in cow dung + neem cake mixture (9:1), this was followed by soil drench of talc based formulation (two per cent) at two, three and four months after planting (Table 3).

Observations regarding the effect of seed treatment with fungicides and biocontrol agents on the disease were taken at two months after planting soon after the first appearance of symptoms on plants. The first foliar spray + soil drench was applied soon after taking the first observations (two month after planting). Second and third observations were taken at three and four months after planting to find the effect of fungicides and biocontrol agents on disease by foliar spray + soil drench application. A score chart was developed with a 0 - 5 disease grade for

assessing the DI based on the prominent aerial symptoms like yellowing and stunting (Table 4 and Plate 1).

The rhizomes were harvested seven months after planting and each treatment and replication was labeled and fresh was weight taken. The per cent rhizome rot was also assessed with a 0 - 4 grade score chart to calculate DI (Table 5 and Plate 2).

3.8. STATISTICAL ANALYSIS

The data obtained from the studies conducted in laboratory and pot culture were subjected to analysis of variance (ANOVA) after appropriate transformations wherever needed.

Table 1.*In vitro* evaluation of fungicides using Poisoned food technique

Treatment	Fungicides	Concentrations (%)
T1	Carbendazim	0.2
		0.1
		0.05
		0.025
T2	Hexaconazole	0.3
		0.2
		0.1
		0.05
T3	Tebuconazole	0.3
		0.15
		0.075
		0.0375
T4	Flusilazole	0.2
		0.1
		0.05
		0.025
T5	Mancozeb	0.35
		0.3
		0.2
		0.1
T6	Captan + Hexaconazole	0.3
		0.2
		0.1
		0.05
T7	Carbendazim + Mancozeb	0.3
		0.2
		0.1
		0.05
T8	Control	

Table 2.*In vitro* spore germination inhibition study

Treatments	Fungicides	Concentrations (%)
T1	Carbendazim	0.1
		0.05
		0.025
T2	Hexaconazole	0.2
		0.1
		0.05
T3	Tebuconazole	0.15
		0.075
		0.0375
T4	Flusilazole	0.1
		0.05
		0.025
T5	Mancozeb	0.3
		0.2
		0.1
T6	Captan + Hexaconazole	0.2
		0.1
		0.05
T7	Carbendazim + Mancozeb	0.2
		0.1
		0.05
T8	Control	

Table 3.Schedule of application of treatments for pot culture experiments on ginger yellows

Treatments	**Rate of application (%)**	**Schedule of treatment application**		
		Seed treatment	**Soil amendment**	**Foliar spray & soil drench**
T1- Hexaconazole	0.2	30 min	-	2, 3, 4 month after planting
T2- Flusilazole	0.1	30 min	-	2, 3, 4 month after planting
T3- Tebuconazole	0.15	30 min	-	2, 3, 4 month after planting
T4- Carbendazim + Mancozeb	0.2	30 min	-	2, 3, 4 month after planting
T5- Talc based formulation of *T. viride*	100g/pot basal, 2 % foliar & soil drench	30 min	100 g enriched in FYM /pot at the time of planting	2, 3, 4 month after planting
T6- Talc based formulation of *T. viride* + mancozeb(seed treatment)	100g/pot basal, 2 % soil drench	30 min	100 g enriched in FYM /pot at the time of planting	2, 3, 4 month after planting
T7- Untreated control	-	-	-	-

Table 4. Score chart for yellows disease

Score	Symptoms
0	Green and healthy tillers
1	Green leaves with lower 1-2 leaves showing slight yellowing and slight stunting
2	Pale and stunted tillers with lower 2-3 leaves yellowed
3	Pale tillers with leaves highly reduced in size, general yellowing and lower leaves starting to wither
4	Severely stunted plants, completely yellowed and tillers beginning to wither, leaves highly reduced in size
5	Severe stunting and yellowing, whole plant withers

Table 5.Score chart for rhizome infection due to ginger yellows

Score	Symptoms
0	Healthy rhizomes
1	Creamy discolouration in the vascular tissue
2	Creamy discolouration in the vascular and cortical region
3	Creamy discolourationmummified rhizomeand rhizome beginning to rot
4	Completely mummified and rotten rhizome

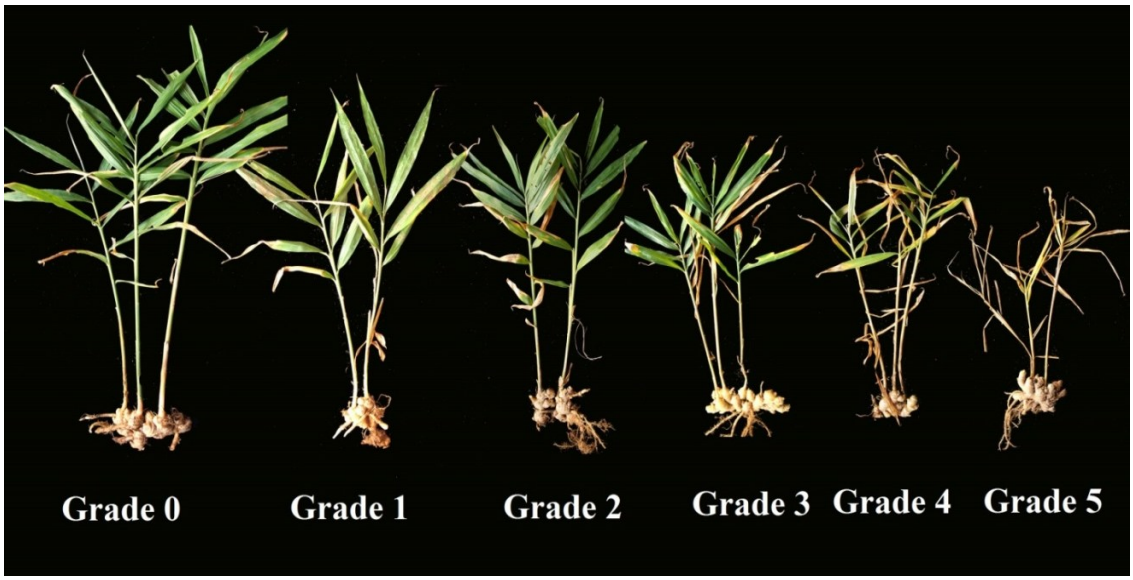

Plate 1. Score chart for ginger yellows disease

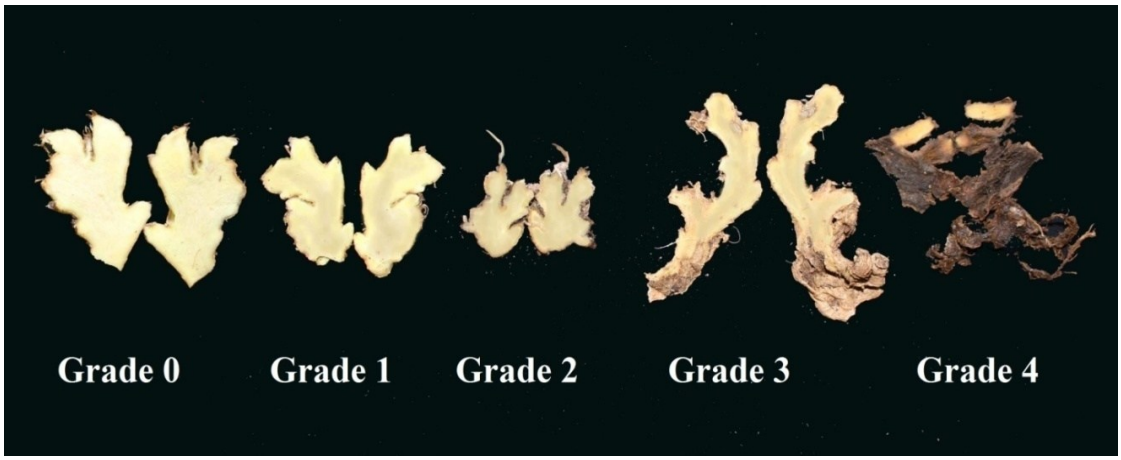

Plate 2. Score chart for rhizome infection due to ginger yellows

4. RESULTS

The present investigation entitled "Etiology and Management of Ginger Yellows" was carried out in the Department of Plant Pathology, College of Agriculture, Vellayani during 2012-2014. The results obtained from the laboratory and pot culture experiments are presented below.

4.1. SYMPTOMATOLOGY OF GINGER YELLOWS

The aerial symptoms of ginger yellows disease were yellowing followed by wilting of plants, stunting and reduced tillering. The under ground symptoms include creamy discolouration of vascular region of rhizome, rotting of vascular region which during later stages spread to the entire rhizome, leaving the shriveled outer rind intact, reduced root number and root length, reduced rhizome production.

4.1.1. Symptoms on Aerial Parts

The first visible symptoms of the disease, which appeared as yellowing of leaf margins occurred on lower leaves of the plants which gradually proceeded inwards. Yellowing is followed by gradual withering of leaf margins and finally the entire leaf withered (Plate 3). The lower leaf sheath showed yellowing and gradual withering and the whole plant appeared weak and yellowed which was the main characteristic of the disease. Unlike in bacterial wilt (*R. solanacearum*), the plants affected by yellows disease did not show marginal inward rolling and downward drooping of leaves. The yellows infected shoots neither fell on ground nor came out easily when pulled by hand unlike as in rhizome rot (*Pythium* spp.) or bacterial wilt.

The diseased plants also appeared stunted, when compared with healthy plants (Plate 4). The leaves that appeared on stunted plants were smaller in size and lesser in number. When the plant acquired infection

during the later stages, the primary tillers may remain healthy but secondary tillers may appear stunted.

Yellowing and stunting appeared distinctly or as together during the different stages of the crop period (Plates 5 and 6). In field condition, the infected plants took about three to four months to express initial symptoms of yellows disease. The disease symptoms were most severe when the plant neared its harvest period.

4.1.2. Symptoms on Rhizomes

The rhizome formation of infected plant was highly affected. The primary and secondary fingers developed from infected seed rhizomes were smaller in size and lesser in number. The intensity of disease varied highly under the field conditions. The infected plants showed varying degree of decay. During initial stages of infection, a creamy discolouration was observed in the vascular region. The cut ends of infected rhizomes resembled the colour of jaggery, which gives its name in regional languages as “Vellakkedu”, where “Vellam” means jaggery and “kedu” means decayed or rotten. The discolouration was more prominent in the vascular region between fingers (Plate 7).

During the later stages of infection, the rhizome gradually decayed and was invaded by pests and soil saprophytes. The rhizomes do not rot as in the case of soft rot, instead they appeared mummified (Plate 8). By the end of the cropping season the infected rhizomes were completely decayed leaving the outer rind intact (Plate 9).

The disease was carried over during storage in infected rhizomes. In stored ginger rhizomes, white cottony mycelial growth, which appeared as raised cushiony growth (sporodochia) could be seen developing on the surface and cut ends (Plate 10). During the later stages, the rhizomes

Plate 3.Yellows infected plant showing yellowing and withering of leaves

Plate 4. Comparison between yellows infected and a healthy plant

Plate 5. Infected plant showing severe yellowing

Plate 6. Infected plant showing severe stunting

Plate 7. Infected rhizomes showing discolouration of vascular region

Plate 8. Comparison between mummifiedrhizome and a healthy one

Plate 9. Severely infected rhizomes with intact outer rind

became dark and mummified. Such rhizomes neither fetch any market price nor could be used for planting.

4.2. ISOLATION OF PATHOGEN

Samples of infected ginger were collected from parts of Wayanad district of Kerala and Chamarajanagar district in Karnataka. Isolation of the pathogen was carried out following the procedures mentioned in the chapter 'Materials and Methods'. Three different isolates were obtained and they were designated as F1 (Mananthavady, Wayanad district.), F2 (Meenangadi, Wayanad district.) and F3 (Bheemanabeedu, Gundlupet, Chamarajanagar district.) (Plate 11).

4.3. CHARACTERIZATION

4.3.1. Morphological Characterization

Potato Sucrose Agar (PSA) was used to study the cultural and morphological characters of the different isolates. The spore shape and size were observed under Motic BA 210 Compound microscope under 400X objective magnification. The photographs of spores, phialides and chlamydospores were taken using Motic Image Plus (Version 2.0 ML) and the spore sizes were measured in µm.

4.3.1.1. Isolate F1

Colony covered nine cm in seven days. The aerial mycelium was floccose, white, became slightly yellowish creamy as it aged. Conidiophores were long, single, lateral monophialides in aerial mycelium, length (89- 110 µm), septate (3 septa) (Plate 12). Microconidia were found abundantly, singly borne (not in chains), non-septate/1 septum, (5-12 µm) × (2.5-3.5 µm) (Plate 13). Macroconidia were fusiform in shape, 3-4 septate, basal cell foot like, (12- 25µm) × (3-4 µm) (Plate 14).

Chlamydospores were 5-5.9 µm, intercalary and terminal, solitary and in chains (1-3 in number), smooth walled, hyaline (Plate 15).

4.3.1.2. Isolate F2

Colony covered nine cm in seven days, aerial mycelium was floccose, white, became slightly yellowish creamy as it aged. Conidiophores were long, single, lateral monophialides in aerial mycelium, length (71- 110 µm), septate (3 septa) (Plate 16). Microconidia were produced abundantly on long phialides, singly borne (not in chains), non-septate/ one septum, (12-27 µm) × (3.5 µm) (Plate 17). Macroconidia were fusiform in shape, 3-4 septate, basal cell foot like, (12-30.8 µm) × (2.5-3.5 µm) (Plate 18). Chlamydospores were found intercalary and terminal, 5-5.8 µm, 1-3 in number, smooth walled, hyaline (Plate 19).

4.3.1.3. Isolate F3

Colony covered nine cm in 12 days, aerial mycelium was floccose, white, became purple as it aged. Conidiophores were short, single lateral monophialides in aerial mycelium, length less than 12 µm (6.5- 8.2 µm), non-septate (Plate 20). Microconidia produced abundantly, singly borne (not in chains), non-septate/1 septum, cylindrical, straight to slightly curved, variable, oval-ellipsoid (5-12 µm) × (2.5-3.5 µm) (Plate 21). Macroconidia were fusiform, curved towards the tip, tip slightly pointed, 3-4 septate, basal cell pedicellate (12-15 µm) × (3-4 µm) (Plate 21). Chlamydospores were found as intercalary and terminal (5.5-5.9 µm) diameter, solitary and in chains (upto 3), smooth walled, hyaline (Plate 22).

The three isolates F1, F2 and F3 were identified by morphological characterization at National Fungal Collection Center of India (NFCCI), Agarkar Research Institute, Pune as *F. solani* (F1), *F. solani* (F2) and *F. oxysporum* (F3) (Table 6).

Table 6. Cultural characters and pathogenicity of three isolates of ginger yellows

Isolates	Location	Characters	Time taken for symptom expression on aerial parts (Days)	Time taken for symptom expression on rhizomes (Days)	Time taken for full growth on PSA (Days)
F 1	Mananthavady, Wayanad district	White cottony growth, turns pale yellow when old	70	30	7
F 2	Meenangadi, Wayanad district	White cottony growth, turns pale yellow when old	45	22	7
F 3	Bheemanabeedu, Gundlupet, Chamarajanagar district	Pale purple, cottony mycelium, turns purplish when old	45	22	12

Table 7. Identification details of the three isolates of ginger yellows by NFCCI, Pune

Isolate	Cultural character	Identified organism	NFCCI deposition accession code
F 1	White cottony mycelium, turns slight pinkish when old	*F. solani*	**NFCCI-3427**
F 2	White cottony mycelium, turn slightly creamy when old	*F. solani*	**NFCCI-3428**
F 3	White – purple cottony aerial mycelium, turns dark purple when old	*F. oxysporum*	**NFCCI-3426**

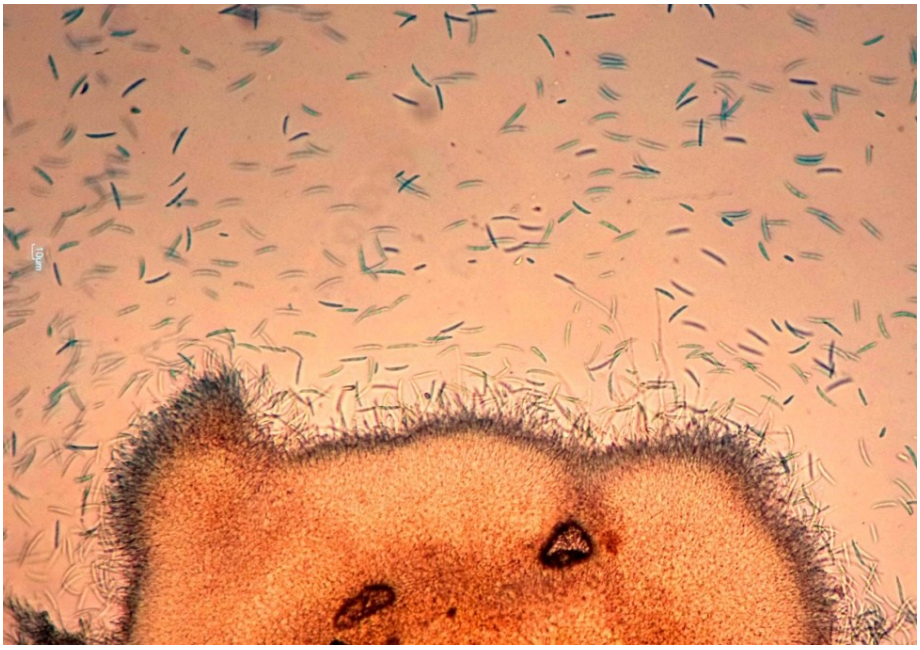

Plate 10. Sporodochia bearing macroconidia

Plate 11. Three isolates of *Fusarium* sp. F1, F2 and F3

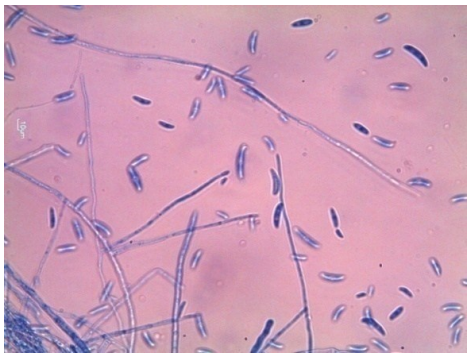

Plate 12. Phialids (isolate F1) (100X)

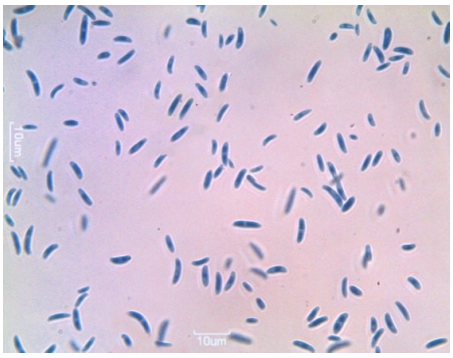

Plate 13. Microconidia (isolate F1) (400X)

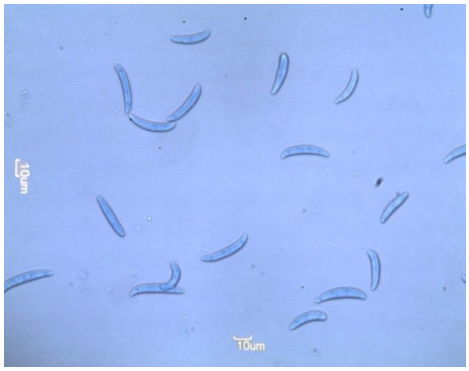

Plate 14. Macroconidia (isolate F1) (100X)

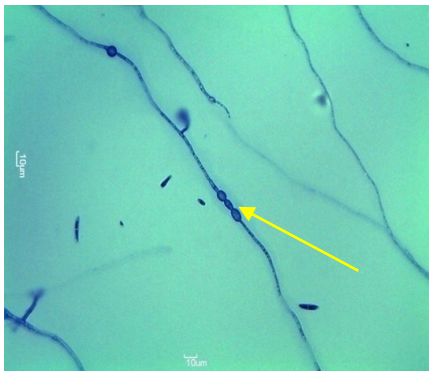

Plate 15. Chlamydospores (isolate F1) (100X)

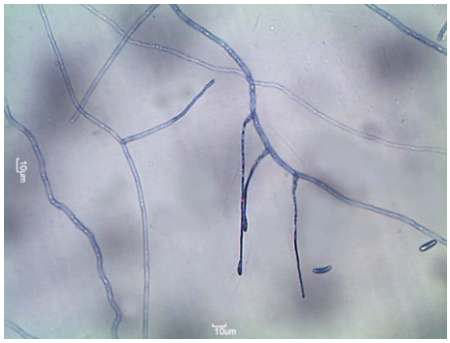

Plate 16. Phialids (isolate F2) (100X)

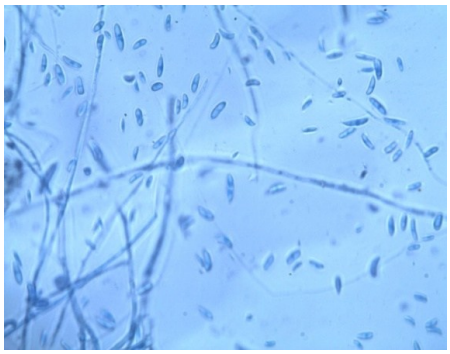

Plate 17. Microconidia (isolate F2) (400X)

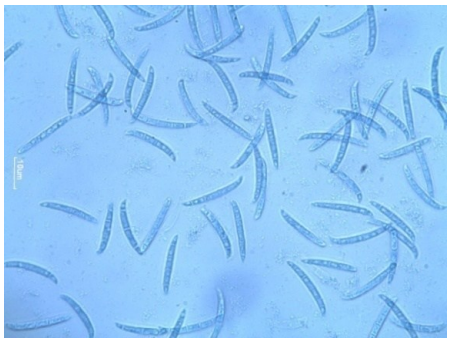

Plate 18. Macroconidia (isolate F2) (100X)

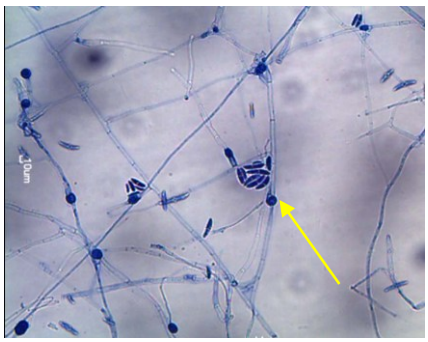

Plate 19. Chlamydospores (isolate F2) (100X)

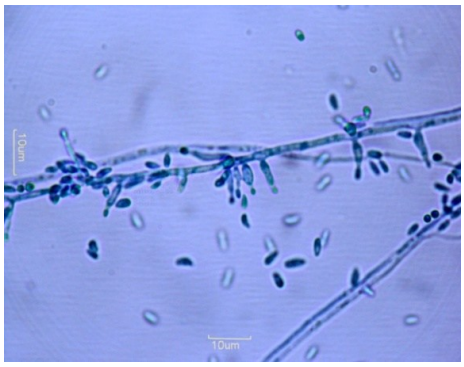

Plate 20. Phialids (isolate F3) (400X)

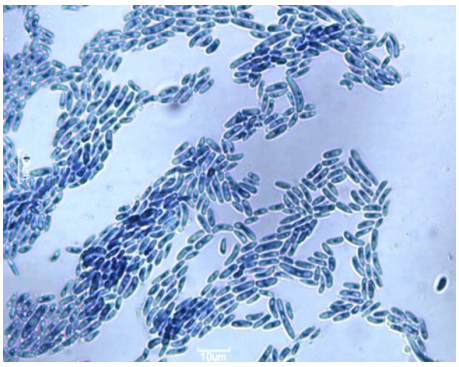

Plate 21. Micro & Macro conidia (isolate F3) (400X)

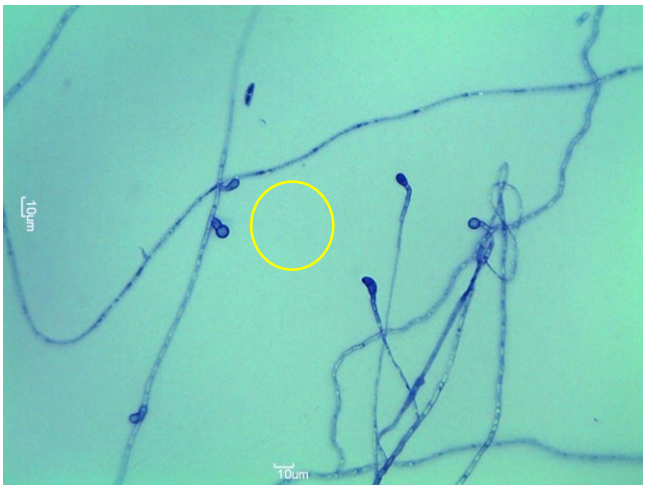

Plate 22. Chlamydospores (isolate F3) (100X)

4.3.2. Molecular Characterization

The internal transcribed spacer (ITS) region of 16S rDNA of the three isolates of *Fusarium* sp. were amplified using universal primers of ITS (ITS-1F and ITS-4R) in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) Sequencing was done using the Big Dye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1.

The sequences obtained by amplification of ITS region are given below.

Isolates of *Fusarium* sp.	**Sequences of ITS region**
F1	AGGGATCATTACCGAGTTATACAACTCATCAACC CTGTGAACATACCTATAACGTTGCCTCGGCGGGAACA GACGGCCCCGTAACACGGGCCGCCCCCGCCAGAGGA CCCCCTAACTCTGTTTCTATAATGTTTCTTCTGAGTAA ACAAGCAAATAAATTAAAACTTTCAACAACGGATCTC TTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCG ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG AATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGG CGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAGG CCCCCGGGCCTGGCGTTGGGGATCGGCGGAAGCCCCC TGCGGGCACAACGCCGTCCCCCAAATACAGTGGCGGT CCCGCCGCAGCTTCCATTGCGTAGTAGCTAACACCTC GCAACTGGAGAGCGGCGCGGCCACGCCGTAAAACAC CCAACTTCTGAATGTTGACCTCGAATCAGGTAGGAAT ACCCGCTGAACTTAA

F2	AGGGATCATTACCGAGTTATACAACTCATCAACC CTGTGAACATACCTATAACGTTGCCTCGGCGGGAACA GACGGCCCCGTAACACGGGCCGCCCCCGCCAGAGGA CCCCCTAACTCTGTTTCTATAATGTTTCTTCTGAGTAA ACAAGCAAATAAATTAAAACTTTCAACAACGGATCTC TTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCG ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG AATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGG CGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAGG CCCCCGGGCCTGGCGTTGGGGATCGGCGGAAGCCCCC TGCGGGCACAACGCCGTCCCCCAAATACAGTGGCGGT CCCGCCGCAGCTTCCATTGCGTAGTAGCTAACACCTC GCAACTGGAGAGCGGCGCGGCCACGCCGTAAAACAC CCAACTTCTGAATGTTGACCTCGAATCAGGTAGGAAT ACCCGCTGAACTTAA
F3	AGGGATCATTACCGAGTTTACAACTCCCAAACC CCTGTGAACATACCACTTGTTGCCTCGGCGGATCAGC CCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGAC CCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAA CCATAAATAAATCAAAACTTTCAACAACGGATCTCTT GGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGA TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA ATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGC GGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGC ACAGCTTGGTGTTGGGACTCGCGTTAATTCGCGTTCCT CAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGT AGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCC ACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGG ATCAGGTAGGAATACCCGCTGAACTTAA

The sequences obtained were BLAST-N searched to retrieve similar sequences available in the NCBI database. The identical sequences thus obtained were multipled aligned using the software www. Phylogeny.fr. The PCR profile of the amplified region of the three sequences shows identical sequence and length of ca. 600 bp (Plate 23). Multiple alignment of ITS region of the three *Fusarium* sp. showed sequence similarity between F1 (*F. solani*) and F2 (*F. solani*). Both these isolates were grouped under same clade and hence closely related. The isolate F3 (*F. oxysporum*) showed lesser similarity with the two previous isolates (Plate 24).

The molecular characterization studies also confirmed the identitiy of isolates F1, F2 and F3 as *F. solani* (F1), *F. solani* (F2) and *F. oxysporum* (F3). The sequences were deposited in NCBI Gen Bank and the accessions obtained are given in the Table 7.

4.4. PATHOGENICITY

F. solani isolate F2 produced symptoms of yellowing 45 days after inoculation, which was much faster compared to the isolates F3 and F1 (60 and 75 days after inoculation) (Plate 25). The infection in rhizomes of inoculated plants, showing the characteristic creamy discolouration was observed 22 days after inoculation in F1 and F2, whereas 25 days in case of F3 (Table 8). The plants initially exhibited yellowing in the lower leaves which later progressed upwards. The plants were generally stunted and pale compared to the uninoculated control plants. The isolate F2 was found to be more pathogenic and virulent hence it was selected for further studies (Plate 26).

Table 8. Molecular identification of the *Fusarium* isolates of ginger yellows

Isolate	Organism identified	Molecular size (bp)	Similarity percentage (%)	Gen Bank Accession numbers
F 1	***F. solani***	**600**	**100**	**KJ643901**
F 2	***F. solani***	**600**	**100**	**KJ643902**
F 3	***F. oxysporum***	**600**	**100**	**KJ643903**

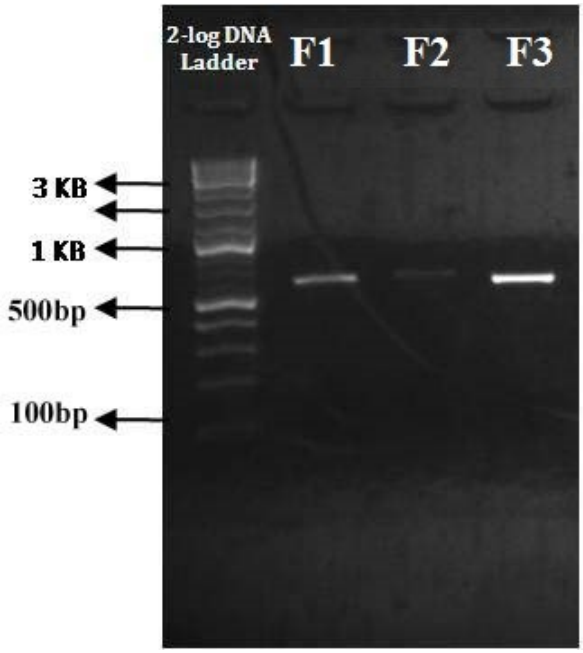

Plate 23. PCR profile of the amplified region

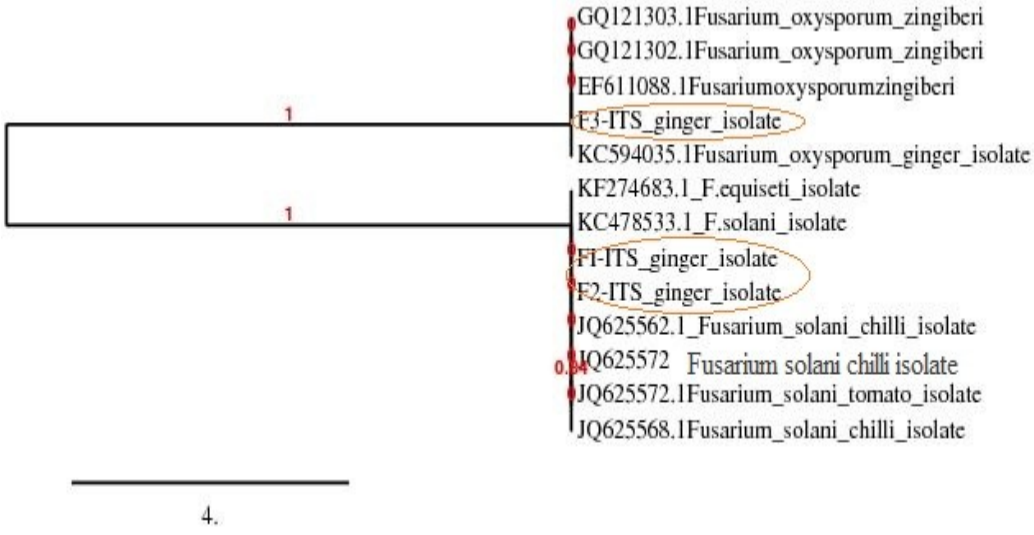

Plate 24. Cladogram showing phylogeny of different isolates

Plate 25. Pathogenicity test of three isolates (F3, F2 & F1) showing severely infected plants

Plate 26. Pathogenicity test (isolate F2)

4.5. CULTURAL CHARACTERS OF *F. SOLANI*

4.5.1. Growth and Sporulation on Different Solid Media

The radial mycelial growth of *F. solani* on eight different nutrient media was studied (Plate 27 and Table 9). The results showed that the radial mycelial growth was maximum (9.00 cm) in PSA and CMA after seven days of incubation. This was followed by HEA (8.60 cm), MEA (8.30 cm), OMA (8.00Cm) and CA (7.90 cm). The least growth were observed in PDA (7.30 cm) followed by CDA (6.60 cm) (Fig. 1).

Mycelial growth was cottony, fluffy and white with aerial mycelium in PSA, PDA, HA and CDA. The aerial mycelium was slight or absent in the case of MA, CMA, CA and OA (Table 9).

Sporulation was the best in PSA with good production of macro conidia followed by Host Extract Agar. The sporulation was average in PDA and CDA. Lesser sporulation was observed in MEA, CMA, CA and the least in OMA (Table 10).

4.5.2. Effect of Temperature on Growth of *F. solani*

The growth of *F. solani* at 20, 25, 30, 35 and 40 ± 2^{o}Cwas studied and there was significant difference in growth at these temperatures (Table 11). The radial mycelial growth was inhibited at extreme temperatures. The optimum temperature was found to be 30^{o}C, where the radial mycelial growth covered the nine cm plate in seven days of incubation. The mycelial growth at 20^{o}C was significantly lower (6.52 cm) when compared to the mycelial growth at 25^{o}C (8.64 cm). The temperature at 35^{o}C significantly lowered the radial mycelial growth (3.52 cm) and the growth was almost completely inhibited (1.0 cm) at 40^{o}C (Fig. 2).

The culture developed slight darker colour at the temperatures 25^{o}C and 20^{o}C (Plate 28). The cultures at the two above mentioned temperatures

produced chlamydospores in large number much earlier than at other temperatures.

4.5.3. Effect of Light and Darkness on Radial Mycelial Growth of *F. solani*

Effect of light and darkness on radial mycelial growth of *F. solani* on PSA was studied. The radial mycelial growth in complete darkness was 9.0 cm within seven days of incubation which was significantly different from the other two treatments (Table 12 and Plate 29). The treatment where alternate light (12 h) + dark cycle (12 h) gave a growth of 8.8 cm after seven days of incubation. The treatment with continuous fluorescent light (500 lux) gave the least radial growth of 8.0 cm (Fig. 3).

4.5.4. Effect of p^H on Mycelial Dry Weight of *F. solani*

The growth in p^H 7.0 was found to be highly significant with a mycelial dry weight of 0.547 g and it was found to be the best among the different p^H levels tested (Table 13 and Plate 30). Growth at p^H 6.0 (0.505 g) and p^H 8.0 (0.497 g) were found to be on par. This was followed by p^H 5.0 (0.450g) and p^H 9.0 (0.475 g) which were also found to be on par. The mycelial dry weight was found to be least in p^H 4.0 (0.417 g) (Fig. 4).

4.6. MANAGEMENT

4.6.1. *In vitro* Evaluation of Fungicides and Biocontrol Agents

4.6.1.1. Evaluation of Fungicides on F. solani by Poisoned Food Technique

Seven fungicides were evaluated at four concentrations each under *in vitro* conditions to study their effect on the growth of *F. solani* in PSA. The results are presented in (Table 14 and Plate 31). All fungicides inhibited the radial mycelial growth of *F. solani* at all tested Table 9. Mycelial characters of *F. solani* in different solid media

Sl. No.	Nutrient media	Colony characterstics	Radial growth (cm)*
1.	Potato Sucrose Agar (PSA)	Fluffy cottony mycelium slightly pinkish, aerial mycelium present, creamy colour on underside	9.00 (3.16) [a]
2.	Potato Dextrose Agar (PDA)	Fluffy white cottony mycelium, aerial mycelium present, creamy colour on underside	7.30 (2.88) [f]
3.	Corn Meal Agar (CMA)	White, lacks aerial mycelium	9.00 (3.16) [a]
4.	Malt Extract Agar (MEA)	White, slight aerial mycelium	8.30 (3.06) [c]
5.	Host Extract Agar (HEA)	White mycelium, slight aerial mycelium, white colour on underside	8.50 (3.09) [b]
6.	Oat Meal Agar (OMA)	White mycelium, slight aerial mycelium, white colour on underside	8.00 (3.00) [d]
7.	CzapeksDox Agar (CDA)	Fluffy white cottony mycelium, aerial mycelium present, creamy colour on underside	6.60 (2.77) [g]
8.	Carrot Agar (CA)	White, lacks aerial mycelium	7.90 (2.98) [e]
	C D (0.05)		0.04

*Observations taken after seven days of incubation

**Mean of three replications

Table 10. Sporulation of *F. solani* on different solid media

Nutrient media	Total spore count (spores/cm^3)*	Macro conidia (spores/cm^3)	Micro conidia (spores/cm^3)
PSA	38.8×10^4	7.6×10^4	31.2×10^4
PDA	29×10^4	3.8×10^4	25.2×10^4
CMA	3×10^4	0.8×10^4	2.2×10^4
MEA	10.2×10^4	1×10^4	9.2×10^4
HEA	37.8×10^4	8×10^4	29.8×10^4
OEA	4.8×10^4	0.2×10^4	5×10^4
CDA	18.6×10^4	3×10^4	15.6×10^4
CA	5×10^4	3×10^4	2×10^4

*Mean of three replications

Table 11.Effect of temperature on radial mycelial growth of *F. solani*

Sl. No.	Temperature (^{0}C)	Radial mycelial growth (cm)*
1.	40	1.00^e
2.	35	3.52^d
3.	30	9.00^a
4.	25	8.64^b
5.	20	6.52^c
	C. D. (0.05)	0.16

*Mean of four replications
**Observations taken after seven days of incubation
***Figures followed by the same letter do not differ significantly according to one way ANOVAat P=0.05

Table 12. Effect of light and darkness on radial mycelial growth of *F. solani*

Sl. No.	Treatments	Radial mycelial growth (cm)*
1.	Fluorescent light	8.00 ^{c}
2.	Light + Dark cycle (12 h ambient light + 12 h darkness)	8.80^b
3.	Complete darkness	9.00 ^{a}
	C.D (0.05)	0.0234

*Mean of five replications
**Observations taken after seven days of incubation
***Figures followed by the same letter do not differ significantly according to one way ANOVAat P=0.05

Table 13. Effect of pH on mycelial dry weight of *F. solani*

Sl. No.	pH levels	Mycelial dry weight (g)*
1.	4.0	0.417[abe]
2.	5.0	0.450[b]
3.	6.0	0.505[c]
4.	7.0	0.547 [d]
5.	8.0	0.497[e]
6.	9.0	0.475[f]
	C.D (0.05)	1.98

*Mean of four replications
**Observations taken after 10 days of incubation
***Figures followed by the same letter do not differ significantly according to one way ANOVAat P=0.05

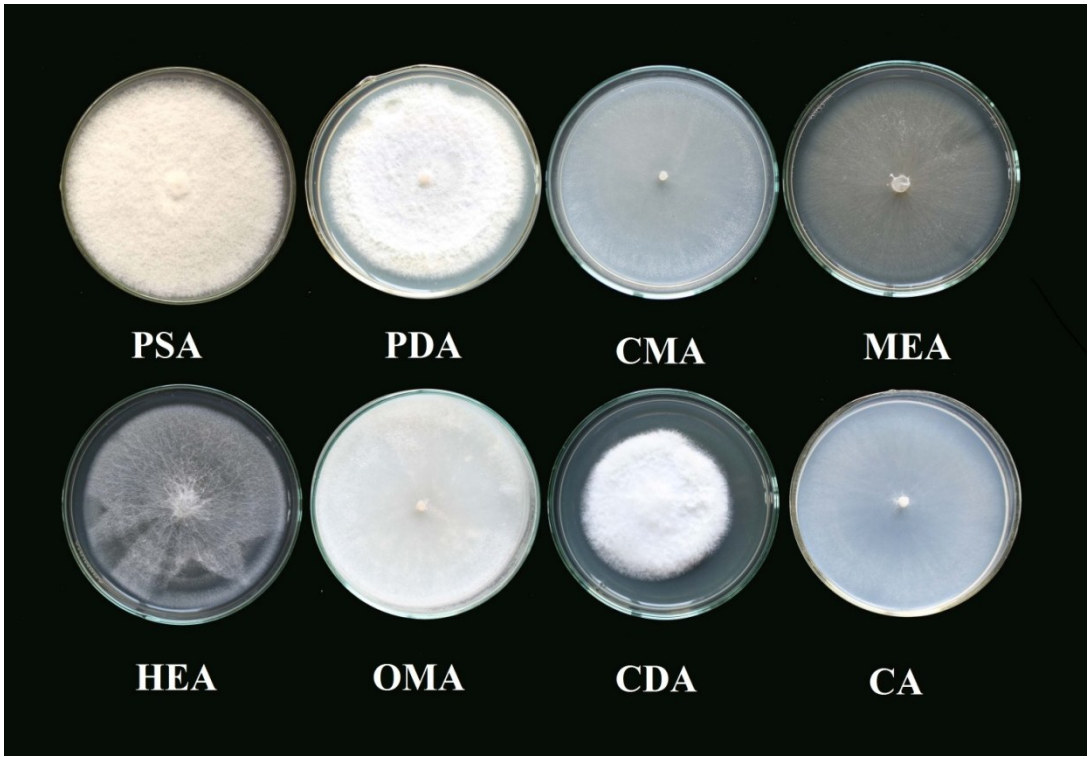

Plate 27. Growth of *F. solani* (F2) in different solid media

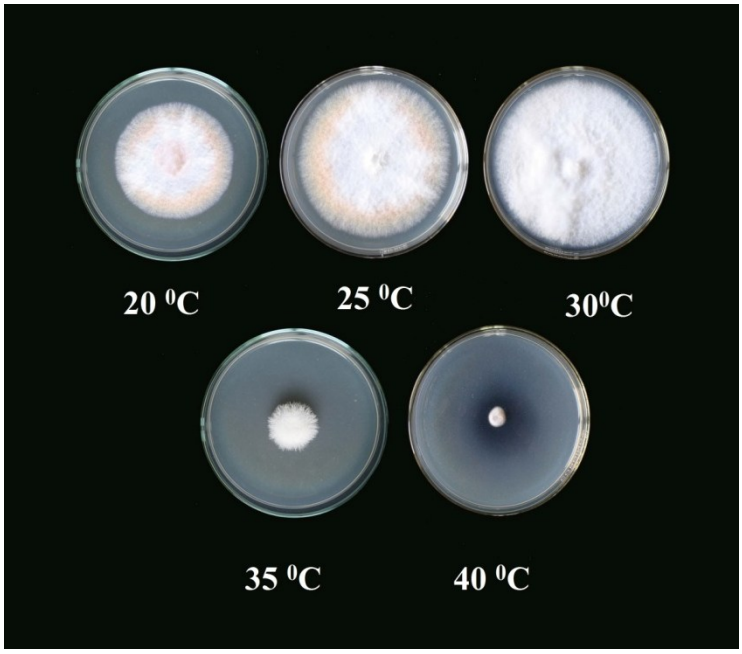

Plate 28. Effect of different temperatures on growth of *F. solani* (F2) on PSA

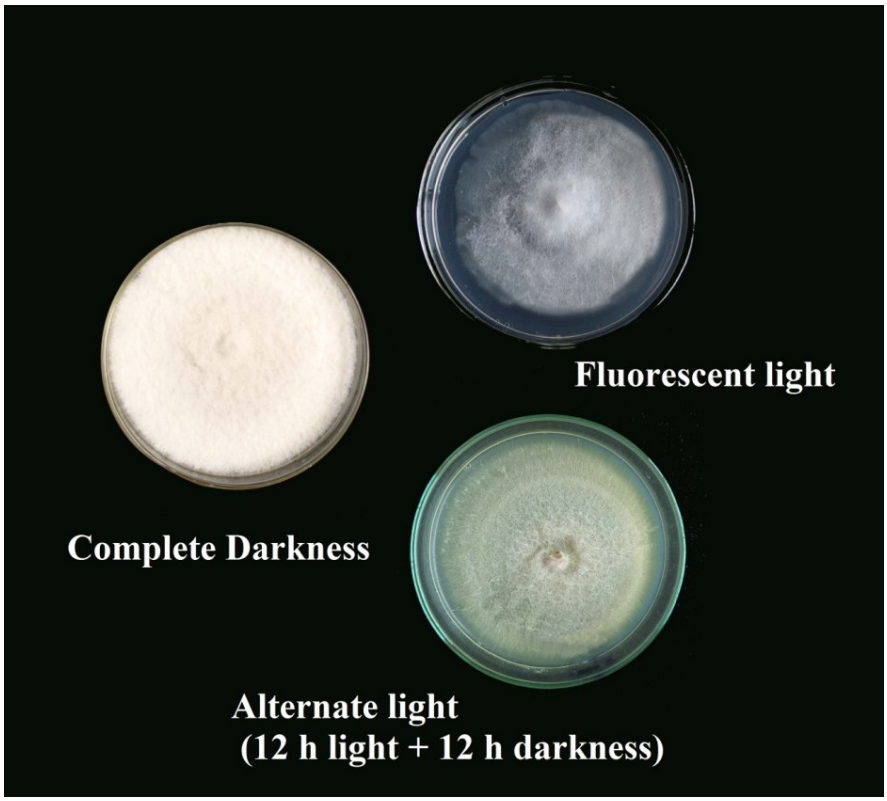

Plate 29. Effect of light and darkness on radial mycelial growth of *F. solani* (F2)

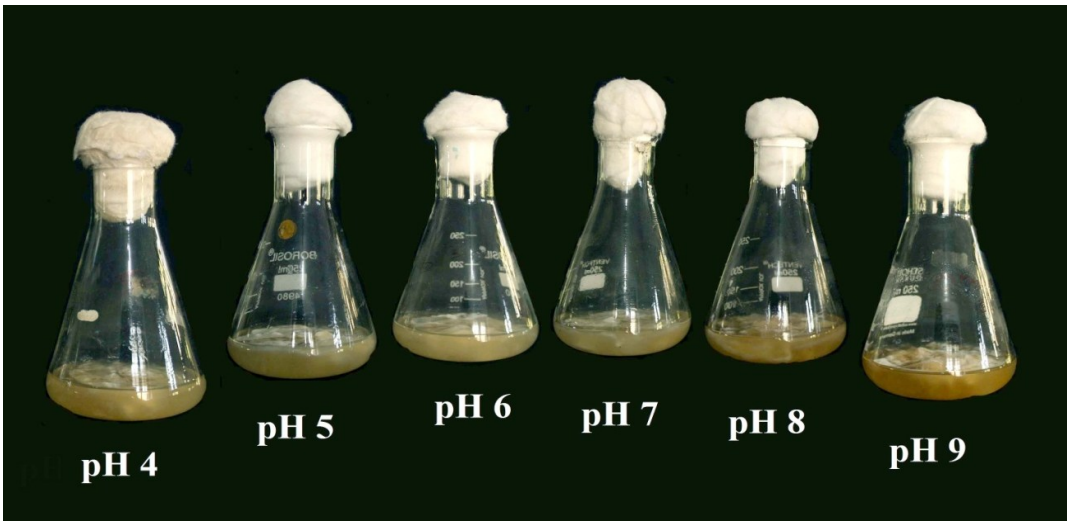

Plate 30. Effect of p^H on mycelial dry weight of *F. solani* (F2)

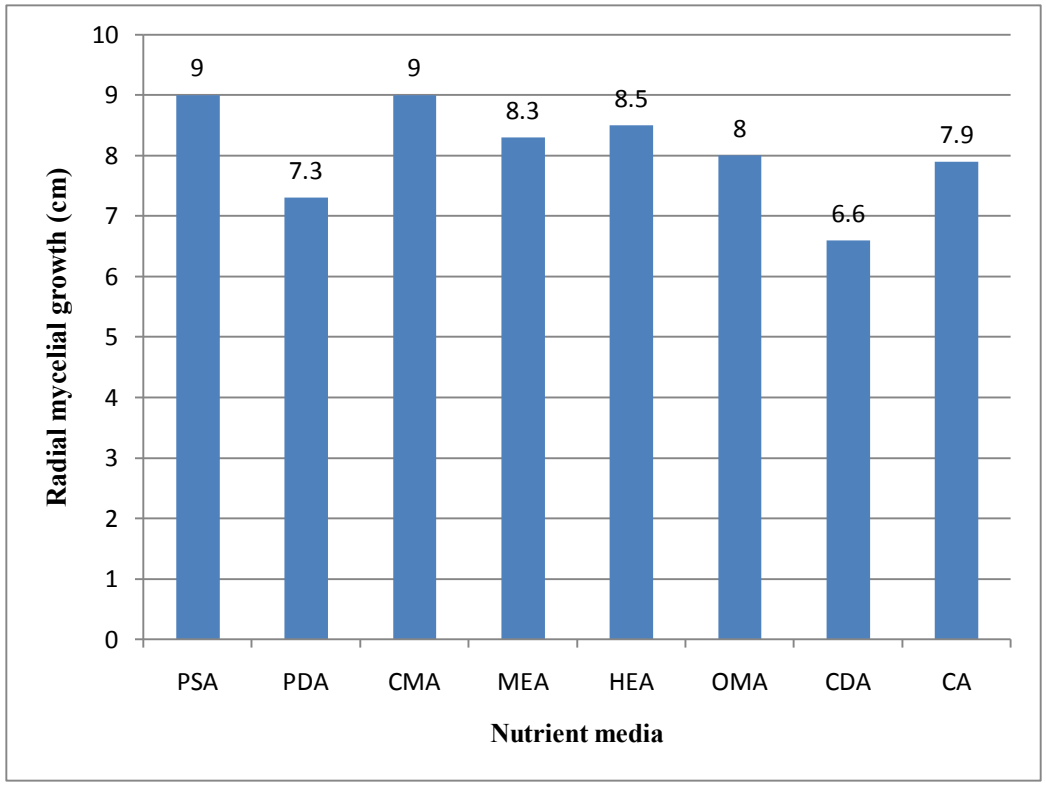

Fig. 1. Growth of *F. solani* (F2) in different nutrient media

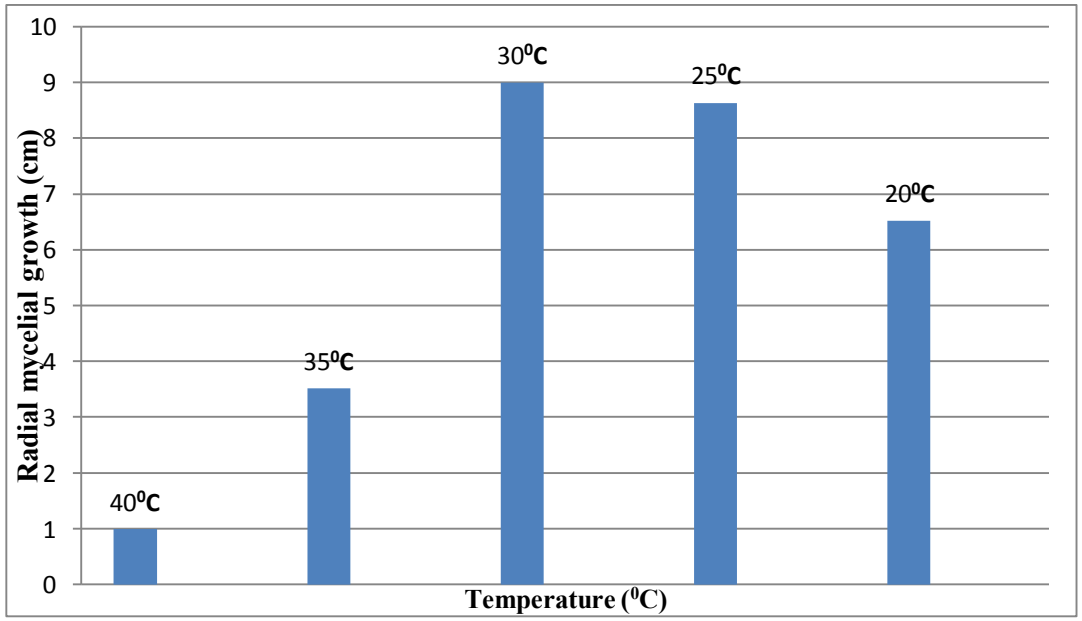

Fig. 2. Effect of temperature on radial mycelial growth of *F. solani* (F2)

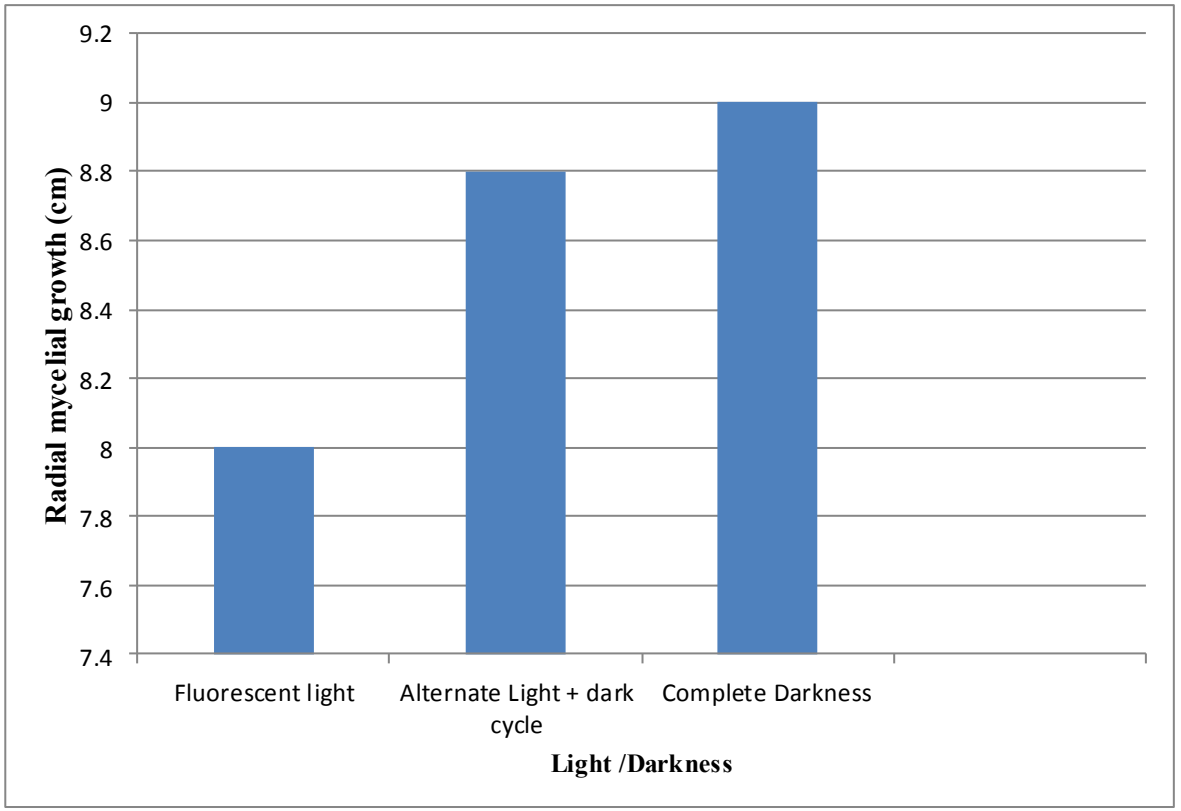

Fig. 3. Effect of light and darkness on radial mycelial growth of *F. solani* (F2)

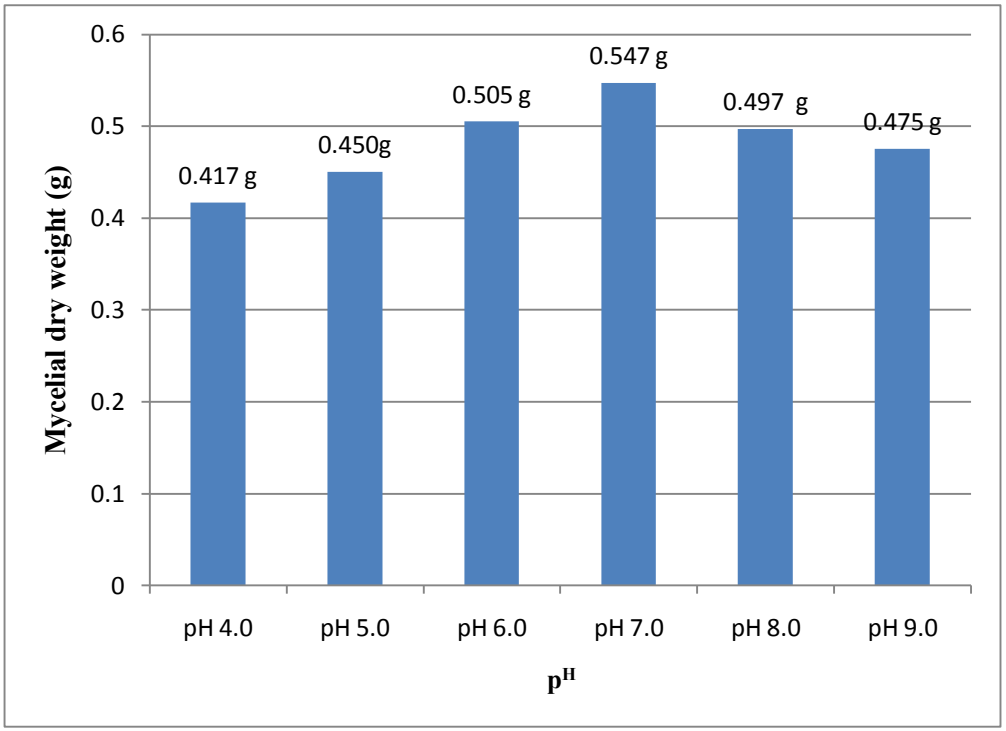

Fig. 4. Effect of p^H on mycelial dry weight of *F. solani* (F2)

concentrations. The inhibition generally increased with increasing concentration (Fig. 5).

The recommended dose, tebuconazole @ 0.15% completely inhibited the growth (100 %) of *F. solani* and was significantly better than all other fungicides. This was followed by flusilazole @ 0.1% where there was 86.60% inhibition to the growth. The fungicides hexaconazole @ 0.2% and carbendazim + mancozeb @ 0.2% recorded 83.30% inhibition and were on par. This was followed by carbendazim @ 0.1% and captan + hexaconazole @ 0.2% which recorded 80.00% and 77.70% inhibition respectively and were also on par. The least inhibition (64.40%) was recorded in mancozeb @ 0.3%.

A similar trend was observed in half the recommended dose also. Tebuconazole @ 0.075% completely inhibited the growth of *F.solani* and was significantly better than all other fungicides. This was followed by flusilazole @ 0.05% and carbendazim + mancozeb @ 0.1% which recorded 85.50% and 84.40% inhibition and were on par. The fungicides carbendazim @ 0.05 % and hexaconazole @ 0.1% recorded 83.30% and 81.10% inhibition and were also on par. This was followed by captan + hexaconazole @ 0.1% which recorded 77.70% inhibition and was significantly superior to mancozeb @ 0.2%, which recorded the lease inhibition of 44.40%.

At one fourth the recommended dose, tebuconazole @ 0.037% completely inhibited the growth of *F.solani* and was significantly superior to all other fungicides. This was followed by flusilazole @ 0.025% which recorded 85.50% inhibition to growth of the fungus. The fungicide carbendazim + mancozeb @ 0.05% recorded 76.60% inhibition and were significantly better than hexaconazole @ 0.05% which recorded an inhibition of 75.50%. This was followed by carbendazim @ 0.025% which recorded 74.40% inhibition and was significantly better than captan

+ hexaconazole @ 0.05% which recorded 72.20% inhibition and were significantly different. Mancozeb @ 0.1% recorded the least inhibition of 44.40%.

Among the seven fungicides tested, tebuconazole showed complete inhibition to radial mycelial growth at all concentrations tested (0.037, 0.075, 0.15 and 0.3%) and was found to be the best for suppressing the mycelial growth of *F. solani*. This was followed by flusilazole with per cent mycelial growth inhibition of 85.50, 86.60 and 94.40% at concentrations 0.05, 0.1 and 0.2% respectively. The next best fungicide which showed good inhibition to mycelial growth was carbendazim + mancozeb @ 0.05, 0.1, 0.2 and 0.3% which showed inhibition of 76.60, 84.40, 83.30 and 94.40% respectively. This was followed by hexaconazole @ 0.05, 0.1, 0.2 and 0.3% which recorded growth inhibition of 75.50, 81.10, 83.30 and 94.40% respectively. The least inhibition was showed by mancozeb at concentrations 0.1, 0.2 and 0.3% with growth inhibition of 44.40, 44.40 and 64.40% respectively. Whereas at higher dose (0.4%), mancozeb recorded complete inhibition (100%) to the growth of the fungus.

4.6.1.2. In vitro Spore Germination Inhibition Study

Seven fungicides at three levels of concentrations were evaluated to study their effect on inhibition of spore germination of *F. solani*. The inhibition to spore germination generally increased with higher concentration (Table 15 and Fig. 6).

At recommended dose, captan + hexaconazole @ 0.2% and tebuconazole @ 0.15% recorded 94 and 93% inhibition to spore germination respectively and were on par. This was followed by carbandazim + mancozeb @ 0.2% and mancozeb @ 0.3% which recorded 90 and 88% inhibition to spore germination. Both the fungicides were on par with tebuconazole @ 0.15%. Hexaconazole @ 0.2% and carbendazim

@ 0.1% recorded 65% inhibition to spore germination and were on par. The least inhibition (25%) to spore germination was observed with flusilazole @ 0.1%.

At double the recommended dose, tebuconazole @ 0.3% and carbandazim + mancozeb @ 0.3% completely inhibited (100%) the spore germination and were on par. This was followed by captan + hexaconazole @ 0.3% and mancozeb @ 0.4% which recorded 96 and 93% inhibition and were on par. Hexaconazole @ 0.3% recorded 88% inhibition to spore germination and was significantly superior to carbendazim @ 0.2% which recorded 80% inhibition to spore germination. The least inhibition (50%) to spore germination was observed in flusilazole @ 0.2%.

At half the recommended dose, tebuconazole @ 0.075% inhibited the spore germination (91%) and was significantly superior to all other fungicides. This was followed by carbandazim + mancozeb @ 0.1% and captan + hexaconazole @ 0.1%, both of which recorded 86 % inhibition each and was on par. This was followed by and mancozeb @ 0.2% and carbendazim @ 0.05% which recorded 71 and 69% inhibition to spore germination respectively and were on par. Hexaconazole @ 0.1% recorded 22% inhibition to spore germination and was significantly superior to flusilazole @ 0.05% which recorded the least inhibition (0%) to spore germination.

Among all the fungicides tested, tebuconazole was found to be the best in inhibiting the spore germination of *F. solani*, followed by carbendazim + mancozeb and captan + hexaconazole. Flusilazole showed the least inhibition to spore germination at all concentrations.

Table 14.*In vitro* evaluation of fungicides on growth inhibition of *F. solani*

Fungicides	Per cent growth inhibition *F. solani*			
	¼ recommended dose	**½ recommended dose**	**Recommended dose**	**Higher than recommended dose**
T1- Carbendazim	0.025%	0.05%	0.1%	0.2%
	74.40(8.60)^{e}	83.30 (9.00)^{c}	80.00 (8.87)^{d}	88.80(9.42)^{c}
T2- Hexaconazole	0.05%	0.1%	0.2%	0.3%
	75.50 (8.64)^{d}	81.10 (8.96)^{c}	83.30 (9.06)^{c}	94.40(9.71)^{b}
T3- Tebuconazole	0.037%	0.075%	0.15%	0.3%
	100(10.00)^{a}	**100(10.00)^{a}**	**100(10.00)^{a}**	**100(10.00)^{a}**
T4- Flusilazole	0.025%	0.05%	0.1%	0.2%
	85.50 (9.18)^{b}	85.50 (9.20)^{b}	86.60 (9.36)^{b}	94.40 (9.71)^{b}
T5- Mancozeb	0.1%	0.2%	0.3%	0.4%
	44.40(6.60)^{g}	44.40 (6.66)^{e}	64.40(8.11)^{e}	**100(10.00)^{a}**
T6- Captan + hexaconazole	0.05%	0.1%	0.2%	0.3%
	72.20 (8.47)^{f}	77.70 (8.83)^{d}	77.70 (8.85)^{d}	88.80(9.42)^{c}
T7-Carbendazim + mancozeb	0.05%	0.1%	0.2%	0.3%
	76.60 (8.77)^{c}	84.40 (9.14)^{b}	83.30 (9.06)^{c}	94.40 (9.71)^{b}
C.D (0.05)	0.12	0.12	0.12	0.12
SE (m) ±	0.04	0.04	0.04	0.04

*Mean of four replications
**Observations taken after seven days of incubation
***Figures followed by the same letter do not differ significantly according to one way ANOVAat P=0.05
Values in the parentheses are square root transformed.

Table 15.Effect of different fungicides on inhibition of spore germination of *F. solani*

Fungicides	Per cent spore inhibition *F. solani*		
	½ recommended dose	**Recommended dose**	**Double the recommended dose**
T1- Carbendazim	0.05%	0.1%	0.2%
	69(56.17)[c]	65(53.73)[c]	80(63.43)[d]
T2-Hexaconazole	0.1%	0.2%	0.3%
	22(27.97)[d]	65(53.73)[c]	88(69.73)[c]
T3- Tebuconazole	0.075%	0.15%	0.3%
	91(72.54)[a]	**93 (74.66)[ab]**	**100 (90.00)[a]**
T4- Flusilazole	0.05%	0.1%	0.2%
	0 (0.0)[e]	25(30.00)[d]	50(45.00)[e]
T5- Mancozeb	0.2%	0.3%	0.4%
	71 (57.42)[c]	88 (69.73)[b]	93 (74.66)[b]
T6- Captan + Hexaconazole	0.1%	0.2%	0.3%
	86(63.08)[b]	**94(75.82)[a]**	96(78.46)[b]
T7- Carbendazim + Mancozeb	0.1%	0.2%	0.3%
	86(63.08)[b]	90(71.57)[b]	**100 (90.00)[a]**
C.D (0.05)	3.53	3.28	4.57

*Mean of three replications
**Observations taken after 24 h of incubation
***Figures followed by the same letter do not differ significantly according to one way ANOVAat P=0.05
Values in the parentheses are square root transformed.

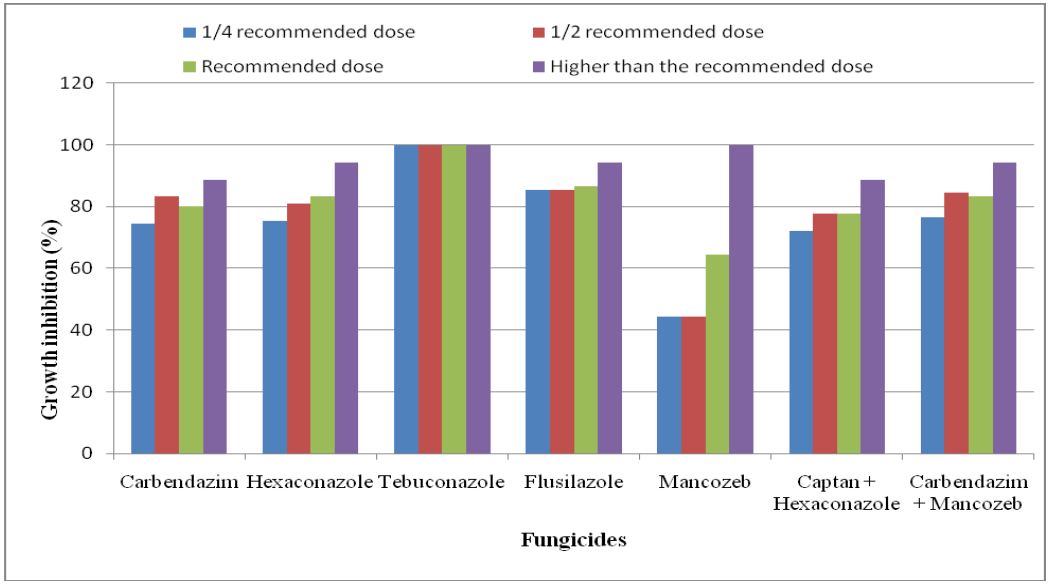

Fig. 5.*In vitro* evaluation of fungicides on growth inhibition of *F. solani* (F2)

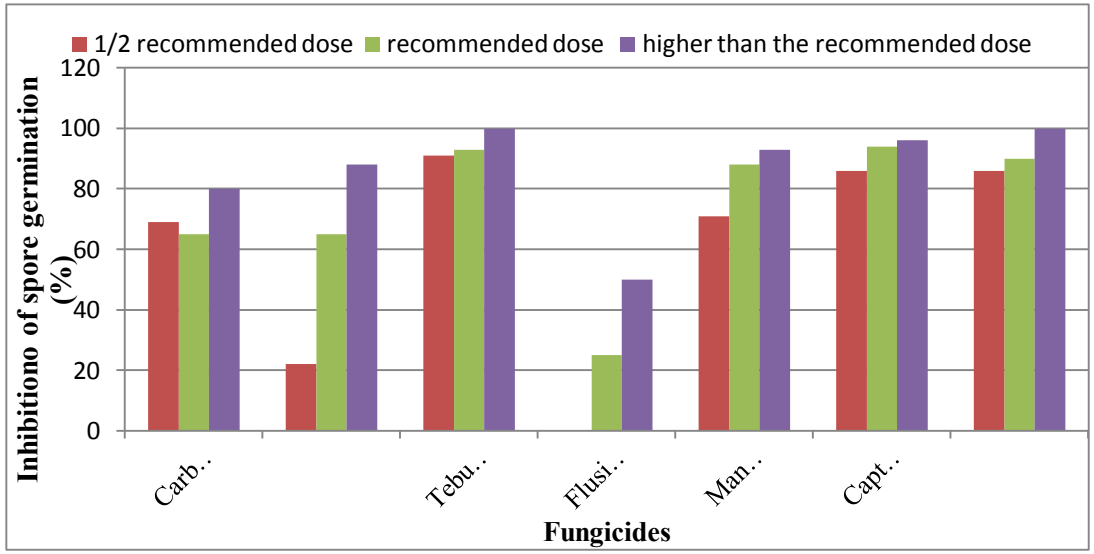

Fig. 6. Effect of different fungicides on inhibition of spore germination of *F. solani* (F2)

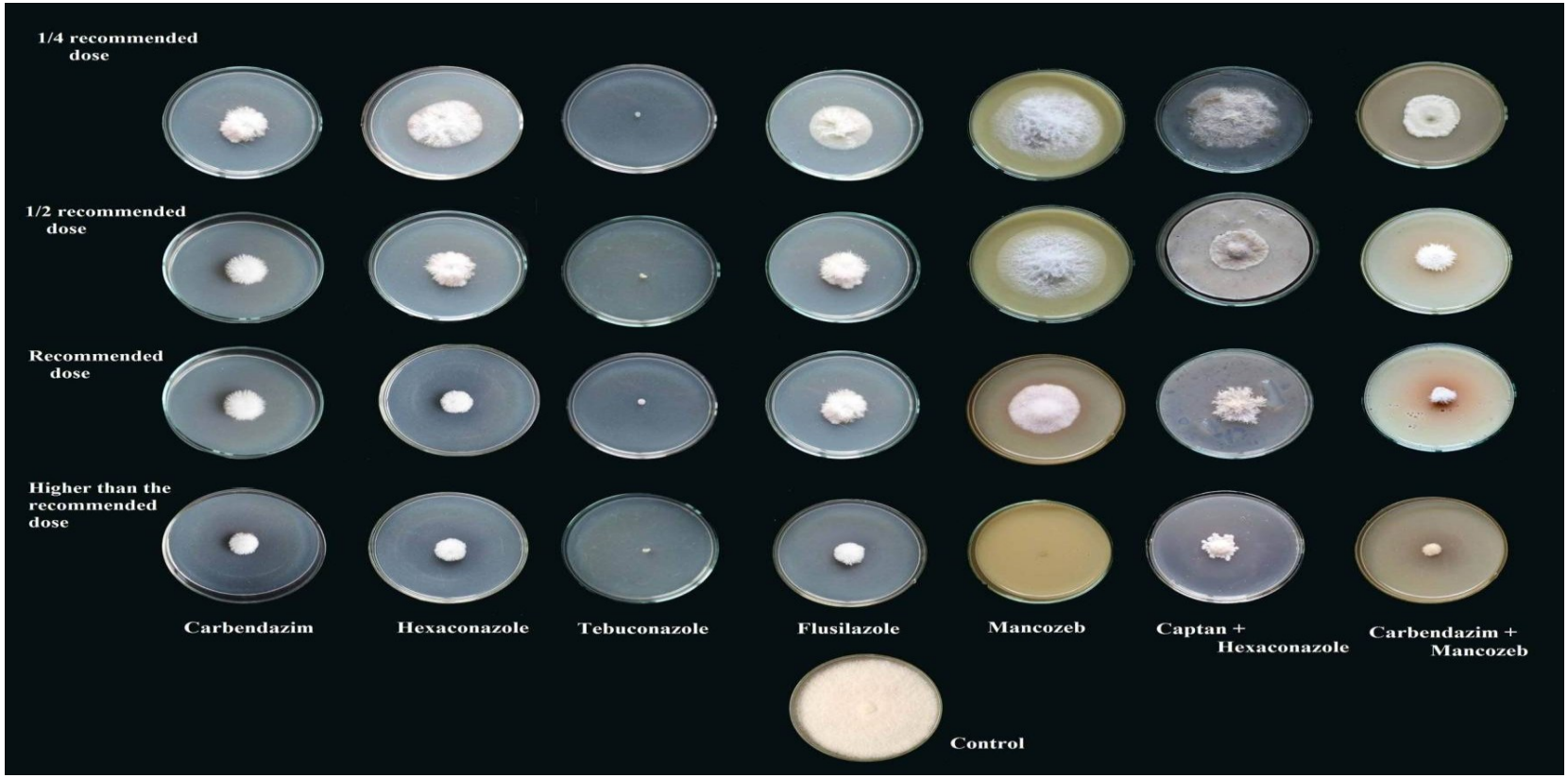

Plate 31.*In vitro* evaluation of fungicides on growth inhibition of *F. solani*

4.6.2. *In vitro* Screening of Biocontrol Agents Against *F. solani*

The fungal antagonist *T. viride* (KAU culture) completely suppressed the growth of *F. solani.* The growth of *T. viride* in the culture was faster and it overgrew *F. solani* in three days. (Plate 32 and 33). *P. fluorescens* (KAU culture) inhibited the growth of *F. solani* through anitbiosis. A clear zone was observed at the region of interaction and the inhibition was 30 per cent on the seventh day of incubation (Plate 34 and 35). *T. viride* was found to be a strong antagonist according to the scale developed by Bell *et al.* (1982) and hence it was found to be more effective in controlling the pathogen *in vitro*.

4.6.3. Compatibility of Biocontrol Agent with Fungicides

The compatibility between *T. viride* and different test fungicides were studied. The result showed that all fungicides completely inhibited the radial mycelial growth of *T. viride* and none were found to be compatible (Plate 36). Hence no treatment combinations were selected for pot culture experiments.

4.6.4. Pot Culture Experiments

4.6.4.1. Evaluation of Fungicides and Bio control Agent Against Ginger Yellows

Seven treatments containing three fungicides, one combination fungicide, one bio control agent and a combination of fungicide and bio control agent were tested against ginger yellows disease in pot culture experiment (Plate 37). Treatments and schedule of application of treatments for pot culture experiment were given as per Table 3 of 'Materials and Methods'.

The effect of seed treatment and soil amendment with fungicides and biocontrol agent on ginger yellows were recorded two month after

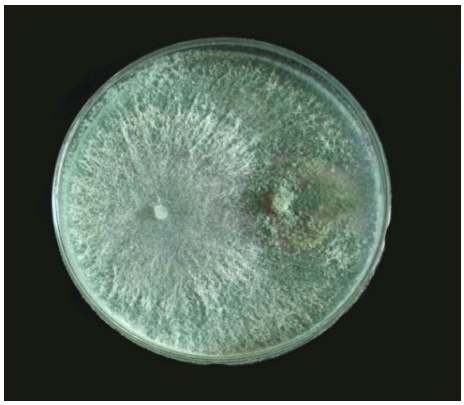

Plate 32. *T. viride* showing overgrowth on *F. solani* (F2)

Plate 33. Control plate of *F.solani* (F2)

Plate 34. *P. fluorescens* showing inhibition on *F. solani* (F2)

Plate 35. Control plate of *F. solani* (F2)

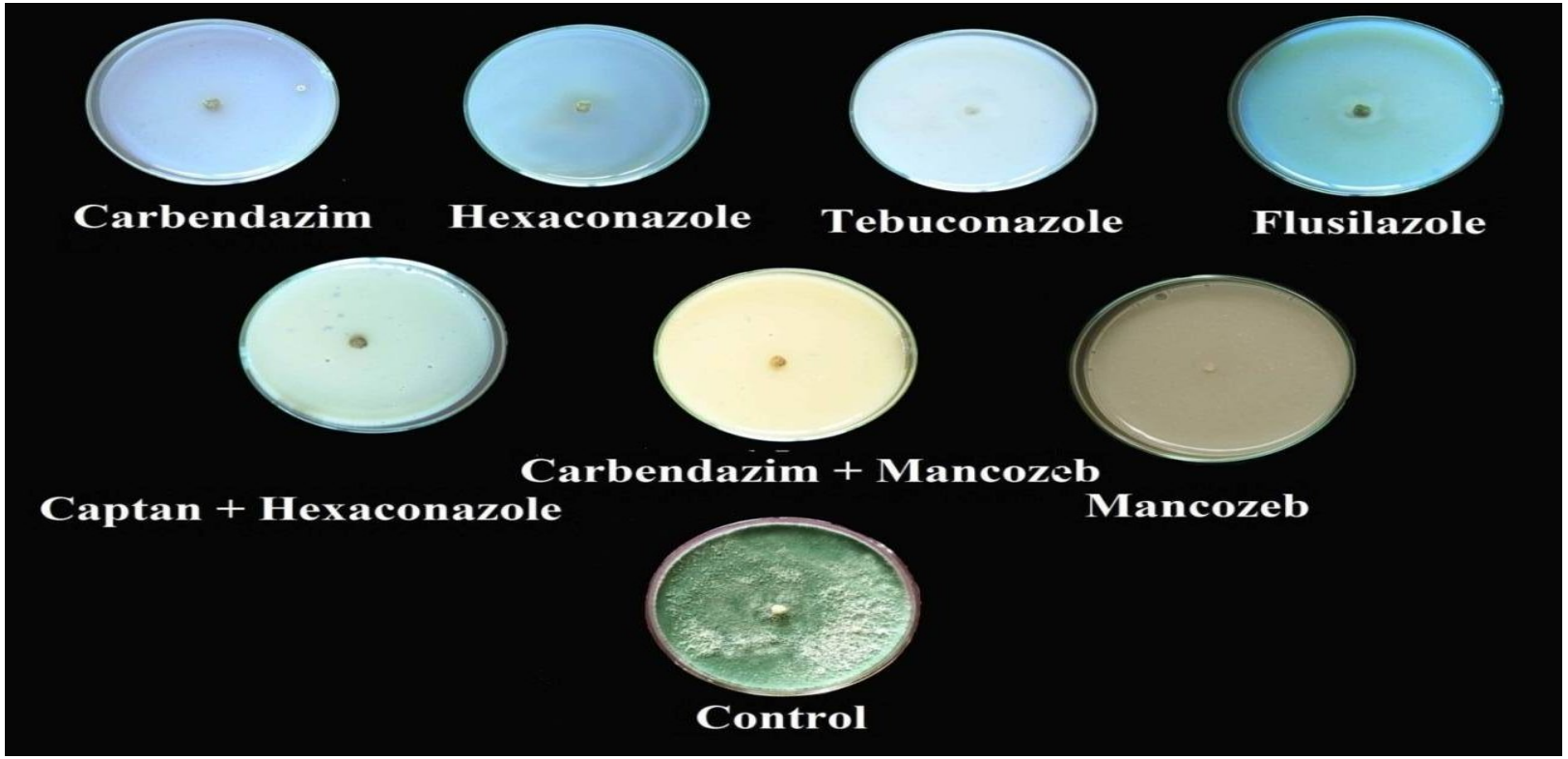

Plate 36. Compatibility of *T. viride* with fungicides

planting. The results are presented in Table 16 and Fig. 7. The treatments T3 and T6 where tebuconazole @ 0.15% applied as seed treatment and mancozeb @ 0.3% applied as seed treatment + soil amendment of talc based formulation of *T. viride* enriched in FYM (cow dung + neem cake mixture in the ratio 9:1) at the time of planting recorded the lowest disease index (0 and 0.29). The treatments were on par and recorded disease reduction of 100 and 98.72% respectively. The treatment T5 and T1 where seed treatment (slurry) with talc based formulation of *T. viride* + soil amendment (enriched in cow dung + neem cake mixture in the ratio 9:1) at the time of planting and seed treatment with hexaconazole @ 0.2% also showed lower disease index of 1.21 and 1.52 respectively. The treatments were on par and recorded disease reduction of 94.66 and 93.29% respectively. This was followed by the treatments T2 and T4 where flusilazole @ 0.1% and carbendazim + mancozeb @ 0.2% were given as seed treatment recorded disease index of 8.67 and 13.41 respectively. The treatments were significantly different with disease reduction of 61.75 and 40.85% respectively. The untreated control showed highest disease index of 22.67.

The effect of fungicide and biocontrol agents on ginger yellows after first foliar spray and soil drench were recorded at three month after planting. The results showed that treatment T6 where talc based formulation of *T. viride* was applied as soil drench @ 2% at two month after seed treatment (mancozeb @ 0.3%) recorded the lowest disease index of 1.79. The treatment recorded a disease reduction of 95.50 % and was significantly better than all other treatments. The treatment T5 also recorded lower disease index of 2.82, where in talc based formulation of *T. viride* was given as soil drench @ 2% at two months after seed treatment with *T. viride* slurry. The treatment was also significantly superior to all other treatments and recorded disease reduction of 92.92%. The treatment T3 and T4, where tebuconazole @ 0.15% and carbendazim + mancozeb @

0.2 %, applied as foliar spray + soil drench at two month after first treatment recorded disease index of 5.48 and 6.20 respectively and were on par and the treatments recorded disease reduction of 86.24 and 84.44% respectively. The treatments T2 and T1, where flusilazole @ 0.1% and hexaconazole @ 0.2% were applied as seed treatment + soil drench recorded higher disease index of 19.34 and 19.95 respectively. The treatments were on par and recorded lower disease reduction of 51.46 and 49.93% respectively. The highest disease index was recorded in the control treatment (39.85).

A second foliar spray and soil drench using fungicides and soil drench aone with biocontrol agents were given at three month after planting and its effect on ginger yellows was scored at fourth month after planting. The treatment T6 where mancozeb (0.3%) was applied as seed treatment, soil amendment of talc based formulation of *T. viride* enriched in FYM at the time of planting, followed by soil drench with 2% talc based formulation of *T. viride* at two and three months after planting showed lowest disease index (2.14) and maximum disease reduction (97.06%) (Plate 43). The treatment was found to be the best and was significantly better than all other treatments. This was followed by the treatment T5 where, talc based formulation of *T. viride* was given as seed treatment (slurry) + soil amendment at the time of planting, followed by soil drench with 2% at two and three months after planting, showed a low disease index of 5.16 and disease reduction of 92.91%. (Plate 42)

Among the treatments with fungicides alone, the treatment T3 where tebuconazole @ 0.15% was given as seed treatment followed by foliar spray + soil drench at two and three months after planting also lowered the disease index (9.72). The treatment T3 was significantly lower than T5 and it recorded a disease reduction of 86.65%. (Plate 40)

Fungicides like hexaconazole @ 0.2% applied as seed treatment, foliar spray + soil drench at two and three month after planting recorded a disease index of 20.54 and disease reduction of 71.79% (Plate 38). This was followed by carbendazim + mancozeb @ 0.2% applied as seed treatment, foliar spray + soil drench at two and three after planting which recorded a disease index of 26.31 and disease reduction of 63.86%. (Plate 42). The highest disease score and the least disease reduction among different fungicides were recorded in flusilazole @ 0.1% (32.27 and 55.68% respectively). (Plate 39) The treatment T7, which was the untreated control showed the highest disease index (72.82). (Plate 44)

A third foliar spray + soil drench with fungicides and biocontrol agent was given at fourth month after planting. The rhizomes were harvested at sixth month after planting and the effect of treatments on rhizome infection and yield were scored using the score chart developed for the purpose (Table 5 and Plate 2).

4.6.4.2. Effect of Fungicides and Biocontrol Agent on Fresh Weight of Ginger

The effect of fungicides and biocontrol agent on fresh weight of ginger was recorded by harvesting the rhizomes at six month after planting. The rhizome weight of three replications within each treatments were weighed and pooled to get the per pot rhizome weight of different treatments (Table 17 and Fig. 8). The treatment T6 where mancozeb @ 0.3% was applied as seed treatment, soil amendment of talc based formulation of *T. viride* enriched in FYM at the time of planting, followed by soil drench with 2% talc based formulation of *T. viride* at two, three and four months after planting showed the highest yield of ginger in terms of fresh weight (289.77 g). This treatment was on par with treatments T5 (283.07 g) where talc based formulation of *T. viride* was given as seed treatment (slurry), soil amendment with talc based formulation of *T. viride*

enriched in FYM, followed by soil drench with 2% at two, three and four months after planting and the treatment T3 (249.10 g) where tebuconazole @ 0.15% was given as seed treatment followed by foliar spray + soil drench at two, three and four months after planting.

The treatment T4 where carbendazim + mancozeb @ 0.2% was given as seed treatment followed by foliar spray + soil drench at two, three and four months after planting recorded 229.71 g fresh weight and was on par with treatment T1 where hexaconazole @ 0.2% was given as seed treatment followed by foliar spray + soil drench at two, three and four months after planting (192.00 g). The treatment T2 where flusilazole @ 0.1% was given as seed treatment followed by foliar spray + soil drench at two, three and four months after planting recorded the lowest fresh weight (183.23 g) among the fungicides. The lowest yield in terms of fresh weight was recorded in the untreated control plant (92.00 g).

4.6.4.3. Effect of fungicides and bio control agent on rhizome infection of ginger due to ginger yellows

The effect of fungicides and biocontrol agent on rhizome infection due to ginger yellows was recorded by longitudinally splitting the rhizomes and scoring the disease index by using the score chart developed for assessing the rhizome infection (Table 18 and Fig. 9). The treatment T6 was recorded as the best treatment for reducing rhizome infection of ginger. The treatment included application of mancozeb @ 0.3% as seed treatment, soil amendment with talc based formulation of *T. viride* enriched in FYM at the time of planting, followed by soil drench with 2% with talc based formulation of *T. viride* at two, three and four months after planting with the lowest index for rhizome infection (2.86). This treatment was on par with treatments T5 where talc based formulation of *T. viride* was given as seed treatment (slurry), soil amendment with talc based formulation of *T. viride* enriched in FYM, followed by soil drench

with 2% with talc based formulation of *T. viride* at two, three and four months after planting (4.47).

The treatment T3 where tebuconazole @ 0.15% was given as seed treatment followed by foliar spray + soil drench at two, three and four months after planting recorded a disease index of 16.59. This treatment was found to be on par with the treatments T2 (31.62), T4 (33.17) and T1 (33.41) where carbendazim + mancozeb @ 0.2%, flusilazole @ 0.1% and hexaconazole @ 0.2% were given as seed treatment followed by foliar spray + soil drench at two, three and four months after planting. The highest rhizome infection due to ginger yellows was recorded in the untreated control plant (46.22).

Table 16.Effect of fungicides and bio control agents on ginger yellows disease incidence in pot culture

Treatments	Per cent Disease Index					
	2 month after planting	**Disease reduction (%)**	**3 month after planting**	**Disease reduction (%)**	**4 month after planting ***	**Disease reduction (%)**
T1- Hexaconazole	1.52(1.58)[b]	93.29	19.95(4.57)[d]	49.93	20.54(4.64)[d]	71.79
T2- Flusilazole	8.67(3.11)[c]	61.75	19.34(4.51)[d]	51.46	32.27(5.76)[f]	55.68
T3- Tebuconazole	**0(1.00)[a]**	100	5.48(2.54)[c]	86.24	9.72(3.27)[c]	86.65
T4- Carbendazim + mancozeb	13.41(3.79)[d]	40.84	6.20(2.68)[c]	84.44	26.31(5.22)[e]	63.86
T5- Talc based formulation of *T. viride*	1.21(1.48)[b]	94.66	2.82(1.95)[b]	92.92	5.16(2.48)[b]	92.91
T6- Talc based formulation of *T. viride*+ mancozeb(seed treatment @ 0.3%)	**0.29(1.13)[a]**	98.72	**1.79(1.67)[a]**	95.50	**2.14(1.77)[a]**	97.06
T7- Control	22.67(4.86)[e]	-	39.85(6.39)[e]	-	72.82(8.59)[g]	-
C.D(0.05)	0.23		0.23		0.23	
SE (m) ±	0.08		0.08		0.08	

*Mean of three replications

**Figures followed by the same letter do not differ significantly according to one way ANOVA at P=0.05.

Values in the parentheses are square root transformed.

Table 17. Effect of fungicides and bio control agents on fresh weight of ginger

Treatments	Fresh weight/pot (g)*
T1- Hexaconazole (0.2%)	192.00 ^{cd}
T2- Flusilazole (0.1%)	183.23^{d}
T3- Tebuconazole (0.15%)	**249.10^{ab}**
T4- Carbendazim + mancozeb (0.2%)	229.71^{bc}
T5- Talc based formulation of *T. viride*	**283.07^{a}**
T6- Talc based formulation of *T. viride*+ mancozeb(seed treatment @ 0.3%)	**289.77^{a}**
T7- Control	92.00 ^{e}
C.D (0.05)	44.19
SE (m) ±	14.57

*Mean of three replications
**Observations taken at six months after planting
***Figures followed by the same letter do not differ significantly according to one way ANOVAat P=0.05

Table 18.Effect of fungicides and biocontrol agents on rhizome infection due to ginger yellows

Treatments	Rhizome infection index *
T1- Hexaconazole (0.2%)	33.41(5.86)[b]
T2- Flusilazole (0.1%)	31.62(5.71)[b]
T3- Tebuconazole (0.15%)	16.59(4.19)[b]
T4- Carbendazim + mancozeb (0.2%)	33.17(5.84)[b]
T5- Talc based formulation of *T. viride*	**4.47(2.54)[a]**
T6- Talc based formulation of *T. viride* + mancozeb (seed treatment @ 0.3%)	**2.86(1.96)[a]**
T7- Control	46.22(6.87)[c]
C.D (0.05)	1.92
SE (m) ±	0.63

*Mean of three replications
**Observations taken at six months after planting
***Figures followed by the same letter do not differ significantly according to one way ANOVAat P= 0.05
Values in the parentheses are square root transformed.

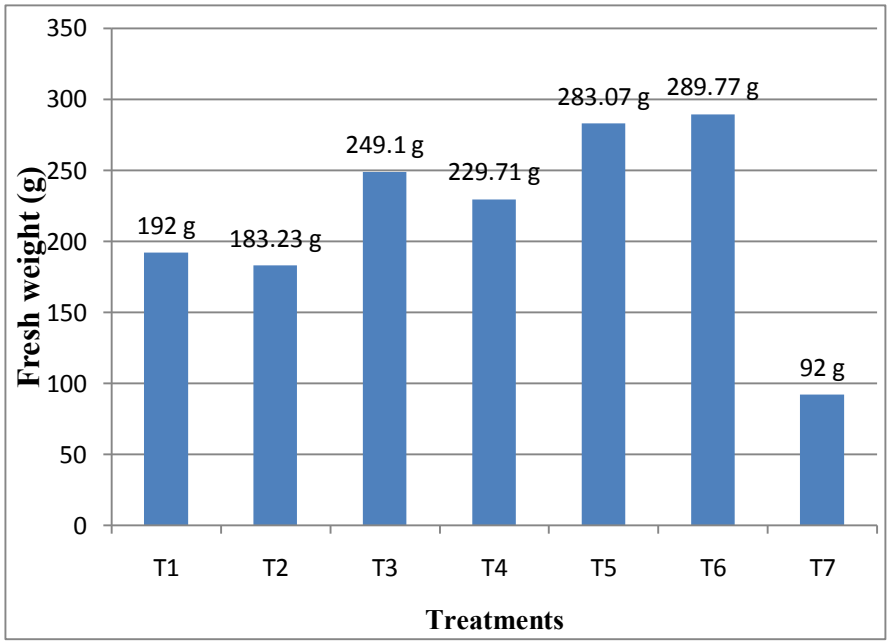

Fig. 8. Effect of fungicides and bio control agent on fresh weight of ginger

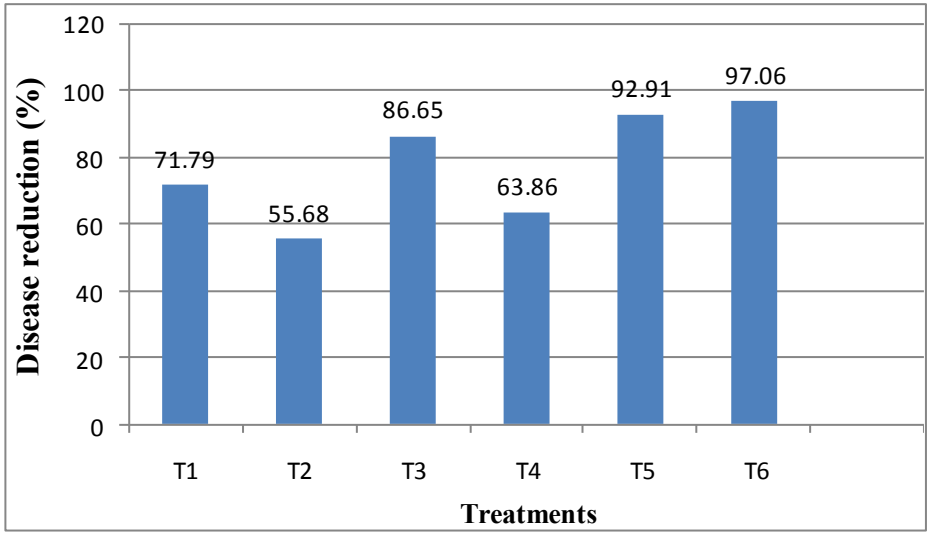

Fig. 7.Effect of fungicides and bio control agent on per cent disease reduction

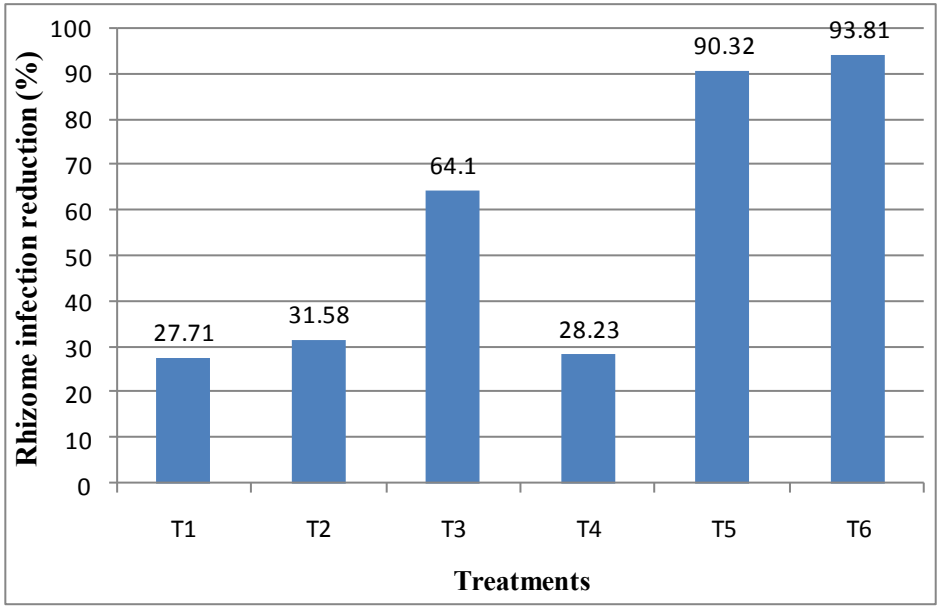

Fig. 9. Effect of fungicides and bio control agent on per cent reduction of rhizome infection

Plate 37. Overview of pot culture experiment

Plate 38.Uninoculated, treatment T1 & inoculated control

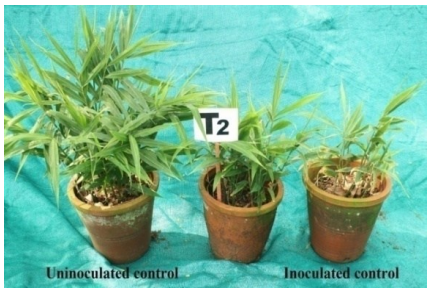

Plate 39.Uninoculated, treatment T2 & inoculated control

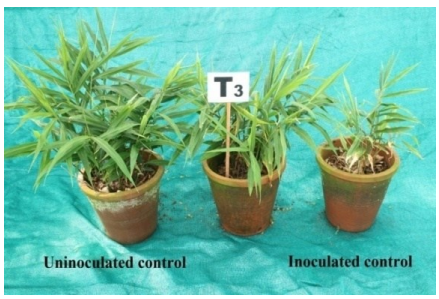

Plate 40.Uninoculated treatment T3 inoculated control

Plate 41. Uninoculated, treatment & T4 & inoculated control

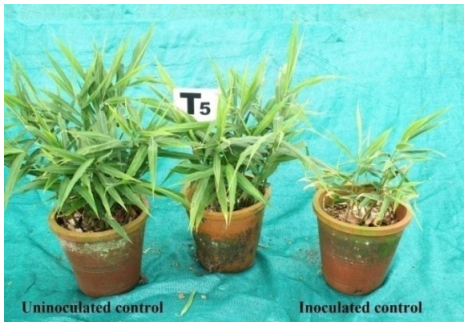

Plate 42.Uninoculated, treatment T5 & inoculated control

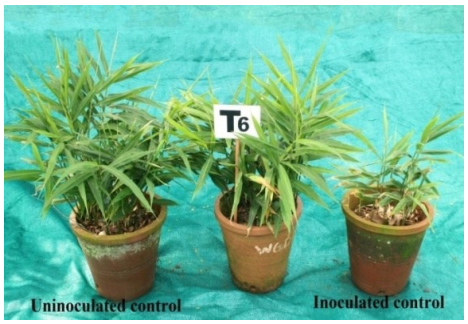

Plate 43.Uninoculated, treatment T6 & inoculated control

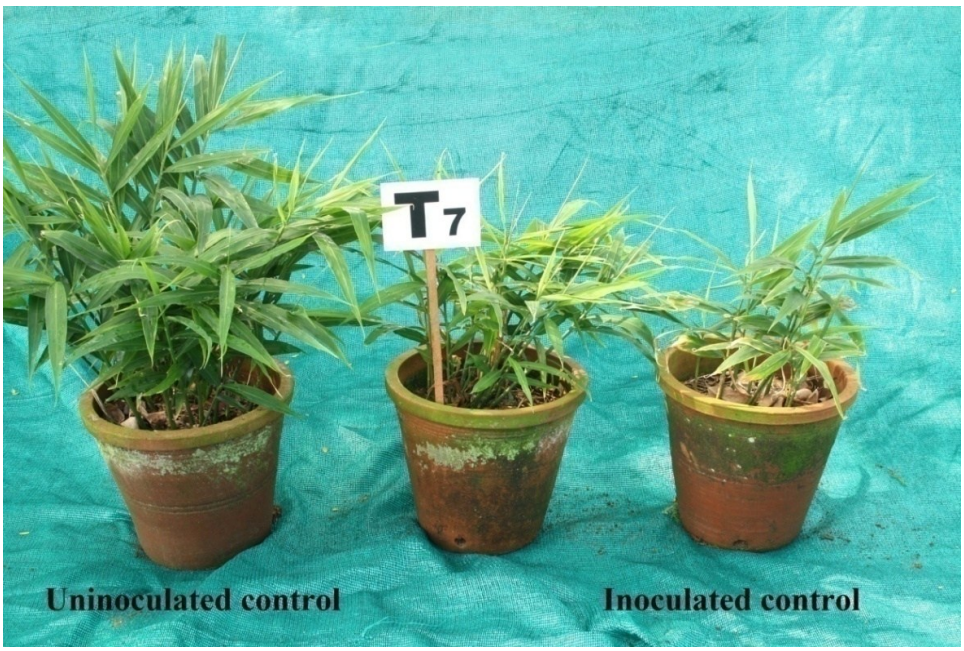

Plate 44.Uninoculated, treatment T7 & inoculated control

Discussion

5. DISCUSSION

Ginger is one of the most economic spice crop cultivated in India. There is a strong domestic as well as international market for raw and processed ginger. Rhizome rot is one of the major constraints for ginger cultivation. Many pathogens including bacteria, fungi, and nematodes are found to be associated with the rotting and it is often found as a complex disease.

Ginger yellows is a disease that can affect ginger rhizomes in the field and during storage. Ginger yellows disease occurring in ginger cultivated regions of Wayanad district showed symptoms on aerial parts as well as in rhizomes. The aerial symptoms include yellowing that initially appears on leaf margins later proceeds inwards. Yellowing is followed by gradual withering of leaf margins and finally the entire leaf becomes withered. The shoots wilt and wither gradually; the whole plant appears weak and yellowish which is the main character of the disease. Unlike in bacterial wilt (*R. solanacearum*), the plants affected by yellows disease do not show marginal downward drooping of leaves. The diseased shoots neither falls on ground nor comes out easily when pulled by hand unlike as in rhizome rot (*Pythium* spp.) or bacterial wilt.

The symptomatology of ginger yellows described by Trujllo (1963), is in accordance with the present study. The symptoms of ginger yellows are appearance of yellow, stunted above-ground shoots among apparently healthy green shoots. The disease incidence depends upon the initial inoculums in soil and infection in seed materials. The fungus can also readily invade wounded healthy seeds planted in infested soil. Taj and Kumar 2013, also reported that the plants affected by yellows disease do not wilt rapidly as in bacterial wilt or soft rot. Instead the infected plants are stunted and yellowed. The lower leaves dry out over an extended period of time. It is common to find yellows, stunted above

ground shoots among apparently healthy green shoots. The plant finally dries out as the fungus invades the entire vascular system of the underground rhizomes.

Stunting was observed in infected plants and it appeared during two distinct stages. In case of seed borne infection, the primary tillers itself appeared severely stunted and yellowed, whereas the plant acquired infection during the later stages, the primary tillers may remain healthy but secondary tillers may appear stunted and yellowed. Taj and Kumar (2013) explained that the diseased plants also appeared stunted, when compared with healthy plants and the leaves that appeared on stunted plants were smaller in size and lesser in number.

The rhizome formation of infected plant was highly affected. The primary and secondary fingers developed from infected seed rhizomes were smaller in size and lesser in number. The intensity of disease varied highly under the field conditions. The infected plants showed varying degree of decay. During initial stages of infection, a creamy discolouration was observed in the vascular region. The cut ends of infected rhizomes resembled the colour of jaggery. The discolouration was more prominent in the cortical region between fingers. Vijayaraghavan and Mathew (2011) reported ginger yellows from Kerala for the first time and the symptoms observed in the affected rhizomes were in accordance with the present study.

The rhizomes did not rot as in the case of soft rot, instead they appeared mummified. Similar symptoms were observed in yellows infected rhizomes by Trujillo, (1963). The yellows disease is a dry rot characterized by collapse of the cortical tissues of infected rhizome, occasionally accompanied by a purpling of the infected areas, and a white cottony mycelial growth on the cut surfaces of ginger. As the rot advances

into newer rhizome sections, the cortex shows sunken areas. This is especially noticeable on infected rhizomes in storage.

Seed rhizomes were the primary sources of pathogen in the field. During the later stages of infection, the rhizome gradually decayed and was invaded by soil saprophytes. By the end of the cropping season the infected rhizomes were completely decayed leaving the outer rind intact. The presence of large number of chlamydospores was observed in the completely rotten rhizomes as well as in the outer rind of the infected ginger. This may be one of the major survival mechanisms of the pathogen in the fields. The rotten rhizomes are often discarded on the field by farmers causing the inoculum to perpetuate in the field, season after season. Trujillo (1963) reported that the ginger harvested from fields contaminated with yellows will continue to destroy the rhizome tissues in storage. The findings of Sharma and Jain, (1978) are also in accordance with the present study. The disease was reported to spread through infected soil and rhizome which are the primary sources of inoculums and through water and mechanical means as the secondary source. The pathogen causing ginger yellows in ginger produces resting structures (chlamydospores) in the decomposing tissues of infected rhizomes. Therefore, tissues from infected crops remaining in the field serve as a reservoir of the fungus. Once a ginger field becomes infected, the fungus can remain in the soil for many years.

Three different isolates were obtained and they were designated as F1 (Mananthavady, Wayanad district), F2 (Meenangadi, Wayanad district) and F3 (Bheemanabeedu, Gundlupet, Chamarajanagar district). The causal agent has been identified as *F. oxysporum* f. sp. *zingiberi* (Haware and Joshi, 1973) and *F. solani* (Kumar, 1977).

In the present study, the inoculated plants produced symptom of yellowing on aerial parts at 45- 75 days after inoculation. The time taken

for production of symptom varied with different isolate. Similar results were observed by Rajan *et al.* (2002), where the ginger plants inoculated with *F. oxysporum* developed initial symptoms at about 45 days after inoculation. The infection resulted in yellowing of plants especially at leaf margins, which progressed from bottom to top. The plants wilted subsequently leading to the death of plants.

The pathogens causing ginger yellows were isolated and the pure culture of the isolates was obtained by hyphal tip culture followed by single spore isolation. The identification of *Fusarium* sp. was done based on the spore morphology and colony characters of the fungus by referring to "The Genus Fusarium" by Booth (1971).

The morphological characteristics of the three isolates such as conidia, chlamdospores, and phialids were observed under Motic BA 2.10 compound microscope under 400X objective magnification. The isolate F1 covered 9 cm Petri dish in seven days on PSA. The aerial mycelium was floccose, white, became slightly yellowish creamy as it aged. Conidiophores were long, single, lateral monophialides in aerial mycelium, length (89- 110 µm), septate (3 septa). Microconidia were found abundantly, singly borne (not in chains), non-septate/1 septum, (5-12 µm) × (2.5-3.5 µm). Macroconidia were fusiform in shape, 3-4 septate, basal cell foot like, (12- 25µm) × (3-4 µm). Chlamydospores were 5-5.9 µm in diameter intercalary and terminal, solitary and in chains (1-3 in number), smooth walled, hyaline.

The isolate F2 covered 9 cm Petri dish in seven days on PSA. It produced aerial floccose, white mycelium, became slightly yellowish as it aged. Conidiophores were long, single, lateral monophialides in aerial mycelium, length (71- 110 µm), septate (3 septa). Microconidia were produced abundantly on long phialides, singly borne (not in chains), non-septate/ one septum, (12-27 µm)×(3.5 µm). Macroconidia were fusiform in

shape, 3-4 septate, basal cell foot like, (12-30.8 µm) × (2.5-3.5 µm). Chlamydospores were found intercalary and terminal, (5-5.8 µm) in diameter, 1-3 in number, smooth walled, hyaline.

The isolate F3 covered 9 cm in 12 days on PSA. The aerial mycelium was floccose, white, becomes purple as it aged. Conidiophores were short, single lateral monophialides in aerial mycelium, length less than 12µm (6.5- 8.2 µm), non-septate. Microconidia produced abundantly, singly borne (not in chains), non-septate/1 septum, cylindrical, straight to slightly curved, variable, oval-ellipsoid (5-12 µm) ×(2.5-3.5 µm). Macroconidia were fusiform, curved towards the tip, tip slightly pointed, 3-4 septate, basal cell pedicellate (12-15 µm) × (3-4 µm). Chlamydospores were found as intercalary and terminal (5.5-5.9 µm) diameter, solitary and in chains (upto 3), smooth walled, hyaline.

The three isolates of *Fusarium* sp. were identified by NFCCI, Agarkar Research Institute, Pune as, *F. solani* (F1), *F. solani* (F1), *F. oxysporum* (F3). The three isolates were deposited at NFCCI with accession numbers NFCCI 3427 (F1), NFCCI 3428 (F2) and NFCCI 3426 (F3). The identities of the pathogens were confirmed by molecular characterization. The ITS region of the three isolates were sequenced and the sequences were deposited at NCBI gene bank, with accession numbers KJ643901 (F1), KJ643902 (F2) and KJ643903 (F3).

The study by Vijayaraghavan and Mathew (2011) showed that, the colonies of *F. oxysporum* f. sp. *zingiberi* produced a cottony white mycelium initially and later after 10 days of incubation, a pinkish purple pigmentation was noticed. On microscopic examination, single celled, hyaline, fusiform to ovoid microconidia measuring 5-12 × 2.7-3.0 µm were observed. Macroconidia were hyaline, 29-48 × 3.8-5.1 µm, multiseptate, fusiform to sickle shaped. Parveen and Sharma (2013) studied the morphological and cultural characters of *F. solani* causing

rhizome rot in ginger and the results are in accordance with the present study.

The radial mycelial growth of *F. solani* on eight different nutrient showed that the radial mycelial growth was maximum (9.00Cm) in PSA after seven days of incubation. Booth (1977) reported that PSA can be used as the general growth medium for *Fusarium* spp. The reason for PSA to be the most suitable medium as observed in the present study, for the growth of *Fusarium* spp. could be attributed to the factor that sucrose is one of the best carbon sources for *Fusarium* spp. (Farooq *et al*., 2005).

Mycelial growth of *F. solani* was cottony, fluffy and white with aerial mycelium in PSA, PDA, HEA and CDA. Good sporulation was observed in PSA followed by PDA and CDA. Kulkarni (2006) reported that *F. oxysporum* f. sp. *gladioli* produced abundant sporulation in PDA and OMA but moderate sporulation was observed in CDA.

The optimum temperature for growth of *F. solani* was found to be 30 ^{0}C, where the radial mycelial growth covered the nine cm plate in seven days of incubation. The mycelial growth at 20^{o}C was 6.25 cm which was significantly lower when compared to the mycelial growth at 25^{o}C (8.64 cm). The temperature at 35^{o}C significantly lowered the radial mycelial growth (3.52 cm) and the growth was almost completely inhibited (1.00 cm) at 40^{o}C. Kausar *et al.* (2009) reported that 25 ± 2^{o}C as more suitable for growth of *F. solani*. The present study showed that 30^{o}C as the best for radial mycelial growth of *F. solani*. Khilare and Ahamed (2012) reported that temperatures from 25 to 35^{o}C were most favorable for the growth of *F. oxysporum* f. sp. *ciceri*, whereas the 30^{o}C was the best for the growth of the pathogen.

Effect of light and darkness on radial mycelial growth of *F. solani* showed that the growth was maximum in complete darkness. Alternate light (12 h) + dark cycle (12 h) recorded a growth of 8.80 cm after seven

days of incubation. The treatment with continuous fluorescent light (500 lux) gave the least radial growth of 8.00 cm. The experimental results of Ramteke and Kamble (2011), on the effect of different light spectra on the growth of *F. solani* showed that the mycelial growth was maximum under dark conditions.

The growth in p^H 7.0 was found to be highly significant and the best among the different p^H with a mycelial dry weight of 0.547 g. Growth at p^H 6.0 (0.505 g) and p^H 8.0 (0.497 g) were found to be on par. This was followed by p^H 5.0 (0.450g) and p^H 9.0 (0.475 g) which were also found to be on par. The mycelial dry weight was found to be least in p^H 4.0 (0.417 g). Farooq *et al*. (2005) reported that the growth of *F. oxysporum* f. sp. *ciceri* was obtained at all p^H levels (5.0- 9.0) tested but it was maximum at p^H 7.0 where it was eight cm after seven days of inoculation. p^H 6.0 (7.4 cm) and p^H 8.0 (6.5 cm) were also favourable. Growth of the fungus was found to decrease at extreme p^H levels, which is in accordance with the results of the present study.

Seven fungicides were evaluated at four concentrations under *in vitro* conditions to study their effect on the growth of *F. solani* in PSA. All fungicides inhibited the radial mycelial growth of *F. solani* at all tested concentrations. The inhibition generally increased with increasing concentration.

The fungicide tebuconazole showed complete inhibition to radial mycelial growth at all concentrations tested (0.037, 0.075, 0.15 and 0.3 per cent) and was found to be the best. This was followed by flusilazole with per cent mycelial growth inhibition of 85.50, 86.60 and 94.40 % at concentrations 0.05, 0.1 and 0.2 % respectively. The results of the present study are in accordance with the findings of Taj and Kumar (2013), where tebuconazole (50% WG) at 100 ppm (0.1 %) completely inhibited the radial mycelial growth of *F. oxysporum* f. sp. *zingiberi*. A combination of

trifloxystrobin (25% WG) + tebuconazole (25% WG) completely inhibited the radial growth of *F. oxysporum* f. sp. *zingiberi* at all concentrations (25, 50 and 100 ppm). The inhibition of radial growth increases with increase in concentration.

The next best fungicide which showed good inhibition to mycelial growth was carbendazim + mancozeb @ 0.05, 0.1, 0.2 and 0.3 % which showed inhibition of 76.60, 84.40, 83.30 and 94.40 % respectively. Chavan *et al*. (2009) reported that carbendazim and carbendazim + mancozeb gave 100 per cent inhibition of mycelial growth of *F. solani* at 0.2 and 0.3% concentrations.

Hexaconazole @ 0.05, 0.1, 0.2 and 0.3 % against *F. solani* recorded growth inhibition of 75.50, 81.10, 83.30 and 94.40 % respectively. The study by Kulkarni (2006), on inhibition of mycelial growth of *F. oxysporum* f .sp. *gladioli* using hexaconazole recorded 81.40, 82.63, and 86.40 % inhibition @ 0.025, 0.05 and 0.1 % respectively.

Carbendazim @ 0.1% recorded 80.00 % inhibition to mycelial growth of *F. solani,* whereas the study by Sultana and Ghaffar (2010) revealed that, complete inhibition of colony growth of *F. solani* was observed in fungicides like aliette, benlate and carbendazim @ 0.1 %. Kulkarni (2006) recorded 93.62 % growth inhibition of *F. oxysporum* f .sp. *gladioli* with 0.1 % carbendazim using *in vitro* poisoned food technique.

Captan + hexaconazole @ 0.05, 0.1, 0.2 and 0.3 % recorded 72.20, 77.70, 77.70 and 88.80 % inhibition to *F. solani in vitro* in the present study. Kulkarni (2006) recorded 19.67, 39.56 and 47.55 % inhibition to the growth of *F. oxysporum* f .sp. *gladioli* with 0.1, 0.2 and 0.3 % captan. The use of combination of fungicide captan + hexaconazole in the present study was found to show better inhibition to *Fusarium* sp. rather than using captan alone.

Studies using biocontrol agents revealed that the fungal antagonist *T. viride* completely suppressed the growth of *F. solani.* The growth of *T. viride* in the culture was faster and overgrowth was observed in three days of inoculation. *P. fluorescens* inhibited the growth of *F. solani* through anitbiosis and 30% inhibition of F. solani was observed. Similar results were recorded by Kulkarni (2006), where the fungal antagonists *T. harzianum, T. koningii, T. virens* and *T. viride* significantly reduced the growth of *F. oxysporum* f. sp. *gladioli* either by over growing or by exhibiting inhibition zones. However, *P. fluorescens* was least effective in inhibiting mycelial growth and an inhibition of 38.54 % was observed against the pathogen.

Rini and Sulochana (2007) tested 56 isolates of *P. fluorescens* against *F. oxysporum* infecting tomato. *P. fluorescens* could not successfully inhibit *F. solani* when cultured *in vitro*. The antagonist could not successfully inhibit the test pathogen in PDA. The nutrient source or its concentration in the medium might have affected the production of antifungal compounds that in turn are responsible for the antagonistic activity of fluorescent pseudomonads in different media, as reported by Hebbar *et al.* (1992).

In pot culture experiments, the treatment where mancozeb (0.3%) was applied as seed treatment, soil amendment of talc based formulation of *T. viride* enriched in FYM (cow dung + neem cake mixture in the ratio 9:1) at the time of planting, followed by soil drench with 2% talc based formulation of *T. viride* at two and three months after planting showed lowest disease index (2.14) and maximum disease reduction (97.06%). This treatment was found to be the best and significantly higher from all other treatments. Rana (1991) reported that the spread of ginger yellows caused by *F. oxysporum* f. sp. *zingiberi,* can be checked through proper seed treatment. Mancozeb can reduce disease at harvest up to 11.33% when seed treatment was carried out prior to planting. Seed rhizomes

were steeped in mancozeb @ 0.25% for 30 min. yielded higher than the uninoculated check plant. Ram and Thakore (2009) reported that pretreatment of ginger rhizomes before storing with mancozeb, carbendazim, metalaxyl or thiophanate methyl @ 0.2% reduced the decay of rhizomes by *F. solani* and increased recovery of healthy rhizomes during storage.

The treatment where, talc based formulation of *T. viride* was given as seed treatment (slurry) + soil amendment at the time of planting (enriched in cow dung + neem cake mixture in the ratio 9:1), followed by soil drench with 2% at two and three months after planting, showed a low disease index of 5.16 and disease reduction of 92.91% which was the second best treatment. The results are in accordance with the results of the pot culture study conducted by Rajan *et al.* (2002). *T. harzianum* effectively controlled rhizome rot, root rot in ginger caused by *F. oxysporum* and increased the yield /pot. The treatment where *T. harzianum* was amended to soil gave a yield of 202.5 g/pot. Rana (1991) reported that proper selection of seed rhizomes can completely check ginger yellows. Sarma *et al.*, 1996 reported that seed dressing with *Trichoderma* and *Gliocladium* mixtures reduced storage rot of ginger seed rhizomes considerably. Application of biocontrol agents to soil after soil solarization resulted in reduced rhizome rot incidence and increased yield. Sharma and Dohroo (1997) reported that, treatment of ginger rhizomes with *T. harzianum* in combination with organic soil amendment with pine needles and soil application with *T. harzianum/ Gigaspora margarita* reduced the incidence of ginger yellows and increased the yield, which is in consonance with the results of the present study.

Summary

6. SUMMARY

An investigation of etiology and management of ginger yellows disease was carried out with reference to symptomatology, isolation, proving pathogenicity, morphological, molecular and cultural studies of the pathogen. The results are summarized below.

The aerial symptoms of ginger yellows disease were yellowing followed by wilting and withering of plants, stunting and reduced tillering. The under ground symptoms include creamy discolouration of vascular region of rhizome, rotting of vascular region which during later stages spread to the entire rhizome, leaving the shriveled outer rind intact, reduced root number and root length, reduced rhizome production.

Three isolates were obtained and they were designated as F1 (Mananthavady, Wayanad district), F2 (Meenangadi, Wayanad district) and F3 (Bheemanabeedu, Gundlupet, Chamarajanagar district). The isolate F2 (*F. solani*) covered nine cm in seven days of incubation and it was the most virulent isolate among the three. Produced floccose, white aerial mycelium which became slightly yellowish creamy as it aged. Conidiophores were long, single, lateral monophialides in aerial mycelium, length (71-110 µm), septate (3 septa). Microconidia were produced abundantly on long phialides, singly borne (not in chains), non-septate/ one septum, (12-27 µm) × (3.5 µm). Macroconidia were fusiform in shape, 3-4 septate, basal cell foot like, (12-30.8 µm) × (2.5-3.5 µm). Chlamydospores were found intercalary and terminal, 5-5.8 µm, 1-3 in number, smooth walled, hyaline. They were identified by cultural, morphological and molecular characterization as *F. solani* (F1), *F. solani* (F2) and *F. oxysporum* (F3).

The cultures were deposited at NFCCI, Pune and the accession codes are NFCCI-3427 (F1), NFCCI-3428 (F2) and NFCCI-3426 (F3). Amplification and sequencing of the ITS region of the isolates confirmed

the identity of the isolates. Phylogenic study revealed that the two isolates of *F. solani* belonged to one clade, and showed sequence similarity to that of *F. solani* causing wilt in chilli and tomato. The *F. oxysporum* isolate was grouped under a seperate clade and showed sequence similarity with that of *F. oxysporum* f. sp. *zingiberi*. The sequences of the ITS regions of three isolates were deposited at NCBI database and the Gen Bank accession numbers are KJ643901 (F1), KJ643902 (F2), and KJ643903 (F3).

F. solani recorded maximum radial mycelial growth on Potato Sucrose Agar and Corn Meal Agar. It produced white fluffy aerial mycelium and maximum sporulation on PSA, hence further studies were conducted in this medium. A temperature of 30^{o}C and complete darkness favoured maximum growth of *F. solani* and it covered the nine cm in seven days of incubation. The neutral p^{H} 7.0 produced maximum mycelial dry weight of 0.547g.

Among the seven fungicides tested under *in vitro* conditions, tebuconazole @ 0.15% completely inhibited the growth (100%) of *F. solani* and was the best among the fungicides. This was followed by flusilazole @ 0.1% where there was 86.60% inhibition to the growth. The fungicides hexaconazole @ 0.2% and carbendazim + mancozeb @ 0.2% recorded 83.30 % inhibition and were on par. This was followed by carbendazim @ 0.1% and captan + hexaconazole @ 0.2% which recorded 80.00% and 77.70% inhibition respectively and were also on par. The least inhibition (64.40 %) was recorded in mancozeb @ 0.3%.

Seven fungicides at three concentrations were evaluated to study their effect on inhibition of spore germination of *F. solani*. Captan + hexaconazole @ 0.2% and tebuconazole @ 0.15% recorded 94 and 93% inhibition to spore germination respectively and were on par. This was followed by carbandazim + mancozeb @ 0.2% and mancozeb @ 0.3%

which recorded 90 and 88% inhibition to spore germination. Both the fungicides were on par with tebuconazole @ 0.15%. Hexaconazole @ 0.2% and carbendazim @ 0.1% recorded 65% inhibition to spore germination and were on par. The least inhibition (25%) to spore germination was observed with flusilazole @ 0.1%.

T. viride suppressed *F. solani in vitro* by over growth. *P. fluorescens* inhibited the growth of *F. solani* (30%) through anitbiosis. Based on the study *T. viride* was found to be a better antagonist and hence it was selected for pot culture experiment. The compatibility between *T. viride* and different test fungicides were studied and none of them were found to be compatible.

The results of the pot culture experiment conducted showed that the treatment where mancozeb (0.3%) was applied as seed treatment, soil amendment of talc based formulation of *T. viride* enriched in FYM (cow dung + neem cake mixture in the ratio 9:1) at the time of planting, followed by soil drench with 2% talc based formulation of *T. viride* at two and three months after planting showed lowest disease index (2.14) and maximum disease reduction (97.06%). The treatment was found to be the best and was significantly superior to all other treatments.

The effect of fungicides and biocontrol agent on fresh weight of ginger was recorded by harvesting the rhizomes at six month after planting. The treatment where mancozeb @ 0.3% was applied as seed treatment, soil amendment of talc based formulation of *T. viride* enriched in FYM, followed by soil drench with 2% talc based formulation of *T. viride* at two, three and four months after planting showed the highest yield of ginger in terms of fresh weight (289.77 g). This was found to be on par with the treatment where talc based formulation of *T. viride* was used for seed treatment (slurry), soil amendment with talc based formulation of *T. viride* enriched in FYM, followed by soil drench with

2% at two, three and four months after planting and the treatment where tebuconazole @ 0.15% was given as seed treatment followed by foliar spray + soil drench at two, three and four months after planting.

The effect of fungicides and biocontrol agent on rhizome infection due to ginger yellows was recorded by longitudinally splitting the rhizomes and scoring the disease index by using the score chart developed for assessing the rhizome infection. The treatment where mancozeb @ 0.3% was applied as seed treatment, soil amendment of talc based formulation of *T. viride* enriched in FYM (cow dung + neem cake mixture in the ratio 9:1) at the time of planting, followed by soil drench with 2% with talc based formulation of *T. viride* at two, three and four months after planting recorded the lowest rhizome infection (2.86).

Future line of work

1. Survey and isolation of pathogens from farmers fields to find out the predominant species of *Fusarium* sp. causing ginger yellows in Kerala.
2. Study the host range of the pathogen and identify alternate hosts to find how the disease perpetuates in the field condition.
3. Conduct field experiment on management of ginger yellows to confirm the results of the present study.
4. Conduct trials incorporating more treatments and treatment combinations to develop an integrated management package for managing the rhizome rot disease, since the disease is a complex one and involves pathogens like *Fusarium* spp., *Pythium* spp., *Rhizoctonia* sp., *R. solanacearum* and nematodes.

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*Original not seen

Appendices

APPENDIX-I

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar (PDA)

Peeled and sliced potatoes	- 200 g
Dextrose ($C_6H_{12}O_6$)	- 20 g
Agar-agar	- 20 g
Distilled water	- 1000 ml

Potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was dissolved separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in to the mixture. The volume was made upto 1000 ml with distilled water and medium was sterilized at 15 psi and 121 °C for 15 min.

2. Potato Sucrose Agar (PSA)

Peeled and sliced potatoes	- 200 g
Sucrose	- 20 g
Agar-agar	- 20 g
Distilled water	- 1000 ml
Agar-agar	- 16 g
Distilled water	- 1000 ml

3. Czapek-Dox Agar (CDA)

$NaNO_3$	- 2 g
K_2HPO_4	- 1 g
$Mg(SO_4).7H_2O$	- 0.5 g
KCl	- 0.5 g

$FeSO_4$	- 0.1 g
Sucrose	- 30 g
Agar-agar	- 20 g
Distilled water	- 1000 ml

4. Oat Meal Agar (OMA)

Oats	- 30g
Agar-Agar	- 20g
Distilled water	- 1000ML

5. Carrot Agar (CA)

Carrot	- 20g
Agar-Agar	- 20g
Distilled Water	- 1000ml

6. Corn Meal Agar (CMA)

Corn flakes	- 60 g
Agar-Agar	- 20g
Distilled Water	-1000ml

7. Malt Extract Agar (MEA)

Malt extract	- 20g
Agar-agar	- 20g
Distilled water	- 1000ml

8. Host Extract Agar (HEA)

Healthy rhizomes	- 200g
Agar-agar	- 20g
Distilled water	- 1000ml

APPENDIX - II

Composition of stain used

1. Lactophenol –Cotton blue

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

Anhydrous lactophenol prepared by dissolving 20 g phenol in 16 ml lactic acid in 3ml glycerol.

APPENDIX - III

Details of fungicides tested against *F. solani*

Sl. No.	Common name	Trade name	Formulation
1.	Hexaconazole	Contaf	5% EC
2.	Flusilazole	Nustar	40% EC
3.	Folicur	Tebuconazole	25.9% EC
4.	Carbendazim	Bavistin	50% DF
5.	Mancozeb	Indofil M-45	75% WP
6.	Carbendazim + Mancozeb	SAAF	12% + 74% WP
7.	Captan + Hexaconazole	Taquat	70% + 5% WP

APPENDIX - III

Sequences of *Fusarium* sp. from GenBank used for comparison with the sequences generated in the study

>KC478533.1|:28-556 Fusarium solani isolate RSPG_231 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

AGGGATCATTACCGAGTTATACAACTCATCAACCCTGTGAACAT
ACCTATAACGTTGCCTCGGCGGGAACAGACGGCCCCGTAACAC
GGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTATAATG
TTTCTTCTGAGTAAACAAGCAAATAAATTAAAACTTTCAACAAC
GGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGC
GATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTT
TGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGT
TCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGCGTTGGG
GATCGGCGGAAGCCCCCTGCGGGCACAACGCCGTCCCCCAAAT
ACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAGCTAACA
CCTCGCAACTGGAGAGCGGCGCGGCCACGCCGTAAAACACCCA
ACTTCTGAATGTTGACCTCGAATCAGGTAGGAATACCCGCTGAA
CTTAA

>gi|239819651|gb|GQ121302.1| Fusarium oxysporum isolate GIFUDHAN5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

GGGGGCTTCCGGGGGTTTCACTCCAACCCCTGTGACATACCACT
TGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGG
CCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTG

AGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCT
TGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGT
CATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTAA
TTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATA
GCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACG
CCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGG
AATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAGGT

>gi|239819652|gb|GQ121303.1| Fusarium oxysporum isolate GIFUDHAN6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

GGGGGCTTCCGGGGGTTTCACTCCAACCCCTGTGACATACCACT
TGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGG
CCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTG
AGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCT
TGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGT
CATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTAA
TTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATA
GCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACG
CCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGG
AATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAGGT

>gi|421991549|gb|JQ625562.1| Fusarium solani isolate FWC27 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

TAAGAGGAAGTAAAAGTCGTAACAAGGTTTCGTTGGTGAACCA
GCGGAGGGATCATTACCGAGTTATACAACTCATCAACCCTGTG
AACATACCTATAACGTTGCCTCGGCGGGAACAGACGGCCCCGT
AACACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTA
TAATGTTTCTTCTGAGTAAACAAGCAAATAAATTAAAACTTTCA
ACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
AATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT
GCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGC
GTTGGGGATCGGCGGAAGCCCCCTGCGGGCACAACGCCGTCCC
CCAAATACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAG
CTAACACCTCGCAACTGGAGAGCGGCGCGGCCACGCCGTAAAA
CACCCAACTTCTGAATGTTGACCTCGAATCAGGTAGGAATACCC
GCTGAACTTATGCATATCAATAAGCGGAGGAAAAGAAACCAAC
AGGGATTGCCCCAGTA

>gi|421991555|gb|JQ625568.1| Fusarium solani isolate FWC124 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

TAAGAGGAAGTAAAAGTCGTAACAAGGTTTCGTTGGTGAACCA
GCGGAGGGATCATTACCGAGTTATACAACTCATCAACCCTGTG
AACATACCTAAAACGTTGCCTCGGCGGGAACAGACGGCCCCGT
AACACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTA
TAATGTTTCTTCTGAGTAAACAAGCAAATAAATTAAAACTTTCA

ACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
AATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT
GCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGC
GTTGGGGATCGGCGGAAGCCCCCTGCGGGCACAACGCCGTCCC
CCAAATACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAG
CTAACACCTCGCAACTGGAGAGCGGCGCGGCCACGCCGTAAAA
CACCCAACTTCTGAATGTTGACCTCGAATCAGGTAGGAATACCC
GCTGAACTTATGCATATCAATAAGCGGAGGAAAAGAAACCAAC
AGGGATTGCCCCAGTA

gi|421991559|gb|JQ625572.1| Fusarium solani isolate FWT6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

TAAGAGGAAGTAAAAGTCGTAACAAGGTTTCGTTGGTGAACCA
GCGGAGGGATCATTACCGAGTTATACAACTCATCAACCCTGTG
AACATACCTATAACGTTGCCTCGGCGGGAACAGACGGCCCCGT
AACACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTA
TAATGTTTCTTCTGAGTAAACAAGCAAATAAATTAAAACTTTCA
ACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCATCGA
AATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG
AATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT
GCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGC
GTTGGGGATCGGCGGAAGCCCCCTGCGGGCACAACGCCGTCCC
CCAAATACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAG
CTAACACCTCGCAACTGGAGAGCGGCGCGGCCACGCCGTAAAA
CACCCAACTTCTGAATGTTGACCTCGAATCAGGTAGGAATACCC
GCTGAACTTATGCATATCAATAAGCGGAGGAAAAGAAACCAAC
AGGGATTGCCCCAGTA

>gi|148733625|gb|EF611088.1| Fusarium oxysporum strain Z9 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GGGGGTTTCGAGTTAACTCCAACCCCTGTGACATACCACTTGTT
GCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCG
CCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTA
AAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTT
CTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTG
CGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTT
CAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTAATTCGC
GTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTA
GTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTT
AAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATA
CCCGCTGAACTTAAGCATATCAAAAGCCGGAGGAA

>gi|526843900|gb|KF274670.1| Fusarium solani isolate FsolaniKSA13-01 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

GTTGGTGAACCAGCGGAGGGATCATTACCGAGTTATACAACTC
ATCAACCCTGTGAACATACCTATAACGTTGCCTCGGCGGGAAC
AGACGGCCCCGTAACACGGGCCGCCCCCGCCAGAGGACCCCCT
AACTCTGTTTCTATAATGTTTCTTCTGAGTAAACAAGCAAATAA
ATTAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTAT
TCTGGCGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAGGCC
CCCGGGCCTGGCGTTGGGGATCGGCGGAAGCCCCCTGCGGGCA
CAACGCCGTCCCCCAAATACAGTGGCGGTCCCGCCGCAGCTTCC
ATTGCGTAGTAGCTAACACCTCGCAACTGGAGAGCGGCGCGGC

CACGCCGTAAAACACCCAACTTCTGAATGTTGACCTCGAATCAG
GTAGGAATACCCGCTGAACTTAAGCATATCAATAA

>gi|507000240|gb|KC594035.1| Fusarium oxysporum isolate 4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

TAACTCCAACCCCTGTGACATACCACTTGTTGCCTCGGCGGATC
AGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCT
AAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAA
ATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGA
AGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTAT
TCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCA
CAGCTTGGTGTTGGGACTCGCGTTAATTCGCGTTCCCCAAATTG
ATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCT
CGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTC
TGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTA
AGCATATCAAAAGGCCGGGAGGAA

Etiology and Management of Ginger Yellows Disease

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(2012-11-120)

Abstract of the thesis

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Kerala Agricultural University

Department of Plant Pathology

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM - 695 522

KERALA, INDIA

2014

ABSTRACT

The study entitled **"Etiology and management of ginger yellows disease"** was conducted during the period 2012-14 at College of Agriculture, Vellayani, Thiruvananthapuram. The objectives are to study the etiology of yellows disease affecting ginger and to develop a management strategy to contain the disease.

The causal agent of ginger yellows disease was isolated from diseased specimens collected from various locations in Kerala and Karnataka and three isolates were obtained, pure cultured and pathogenicity proved. Two isolates were identified as *F. solani* (F1 and F2) and one isolate as *F. oxysporum* (F3). *F. solani* was found to be causing ginger yellows in Kerala. Based on the growth characters and symptom expression, *F. solani* isolate F2 was selected for further studies.

Potato Sucrose Agar (PSA) and Corn Meal Agar (CMA) were found to be the most suitable medium. PSA was the best medium for growth and sporulation.

Seven chemical fungicides; carbendazim, hexaconazole, tebuconazole, flusilazole, mancozeb, captan 70%+ hexaconazole 5.0%, carbendazim 12% + mancozeb 74% and two bio control agents *Trichoderma viride* and *Pseudomonas fluorescens* obtained from Dept. of Microbiology were tested *in vitro* against the pathogen. All the chemical fungicides inhibited the growth of *F. solani* at different concentrations. Tebuconazole @ 0.037, 0.075, 0.15 and 0.3% completely inhibited the growth of the pathogen (100%).

Among bio control agents *T. viride* effectively inhibited the growth of the pathogen. A score chart was developed to assess the disease intensity on aerial parts and in rhizomes.

The results of pot culture trial indicated that seed treatment with mancozeb (0.3% for 30min...), soil amendment with *T. viride* enriched in

FYM followed by soil drench with 2% talc based formulation of *T. viride* at two, three and four months after planting recorded the maximum reduction of yellows disease and recorded highest yield of 289.77 g/pot.

The results of the investigation showed that *F. solani* is the causal agent of ginger yellows in Kerala. The study indicated the possibility of using the fungicide mancozeb @ 0.3% along with the biocontrol agent *T. viride* in order to manage the disease and obtain higher yields. Tebuconazole @ 0.15% can also be used for seed treatment and foliar spray + soil drench in case of severe infection.