INTEGRATED MANAGEMENT OF FOLIAR FUNGAL DISEASE OF CULINARY MELON (*Cucumis melo* L. var. *acidulus* Naudin)

by

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DECLARATION

I, hereby declare that this thesis entitled **"INTEGRATED MANAGEMENT OF FOLIAR FUNGAL DISEASE OF CULINARY MELON (*Cucumis melo* L. var. *acidulus* Naudin)"** 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 university or society.

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CERTIFICATE

Certified that this thesis entitled **"INTEGRATED MANAGEMENT OF FOLIAR FUNGAL DISEASE OF CULINARY MELON (*Cucumis melo* L. var. *acidulus* Naudin)"** is a record of research work done independently by **Ms. Narmadhavathy S** under my guidance and supervision that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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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
Nm	Nano meter
DAS	Days after sowing
et al.	And other co workers
Fig.	Figure
g	Gram
ha	Hectares
h.	Hours
g^{-1}	Per gram
i.e.	that is
kg.	Kilogram
t/ha.	Tonnes per hectare
l.	Litre
m	Meter
mm	Milli meter
mg	Milli gram
Ml	Milli litre
rpm	Rotations per minute
sec	Seconds

PDI	Percentage of disease index
sp. or spp	Species (Singular and plural)
viz.	Namely
dia.	Diameter
MT	Metric tonnes
AGR	Abortive Grain Rate
cv.	Cultivar
Kb	Kilobases
Mb	Megabases
RAPD	Random Amplified Polymorphic DNA
AFLP	Amplified Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
Max.	Maximum
Min.	Minimum
N	Normality
ITS	Internal Transcribed Spacer
DI	Disease Incidence
PDA	Potato Dextrose Agar
RH	Relative Humidity
min	Minutes
N	Nitrogen
P	Phosphorus
K	Potassium
PFP	Partial factor productivity

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Introduction

1. INTRODUCTION

Cucumis melo L. is an important vegetable crop cultivated in vast areas across the world. Africa is generally regarded as the centre of origin of *C. melo*, while India has been considered as an important centre of diversification. Melons of India have large variability for fruit shape, size, skin character, flesh colour, keeping quality and reaction towards insect pests and diseases.

Culinary melon (*Cucumis melo* L. var. *acidulus*; 2n = 2x = 24) commonly called "vellari" is being cultivated in India in the states of Kerala, Andhra Pradesh, Tamil Nadu and Karnataka. This is a popular vegetable crop in humid tropic regions of south India, and has a variety of common names *viz.,* vellari, melon, pickling melon, preserving melon, culinary melon etc. This vegetable is rich in vitamin C (Wehner and Maynard, 2003). A tremendous variability exists among landraces of culinary melon in southern India (Rakhi and Rajamony, 2003). The global production of melon has doubled within the last two decades to 26 million tons in 2007 (FAOSTAT, 2007). The countries with the highest levels of production worldwide are: Brazil (41%), Costa Rica (22%), Israel (13.5%) and Morocco (11.1%), (FAO, 2008).

Among several diseases affecting culinary melon plants, powdery mildew, downy mildew, anthracnose leaf spot and cucumber mosaic virus cause serious losses. Anthracnose leaf spot is a serious foliar disease, infecting the crop in India as well as other countries. This disease mainly affects the foliage causing necrotic lesions on the leaves which dry up resulting in considerable reduction in photosynthetic area of the plants and yield losses up to 55 per cent have been reported from the crop (Thompson and Jenkins, 1985). In addition to cucumber, anthracnose leaf spot disease affects cantaloupe, chayote, citron, gherkin, gourd, honeydew melon, muskmelon, watermelon, and many other species (Wasilwa *et al.,* 1993).

Culinary melon is often considered as a minor crop cultivated in the mixed cropping system of Kerala and therefore the incidence and severity of diseases are rarely documented. But it has often been observed that diseases cause much reduction in yield of the crop. Besides, the latent infections often to become focus of inoculum build up for subsequent seasons.

Generally farmers spray fungicides like mancozeb to control the disease or else they may even leave the disease unmanaged which in turn may cause appreciable losses in yield. However, of late, due to the growing demand for vegetables, even culinary melon has attained economic importance in Kerala and vegetable growers are therefore concerned about managing pest and diseases of the crop. But in case of selection of fungicides, they are left with very few options such as mancozeb, because copper based fungicides are seldom applied due to sensitivity of the crop to this element.

Several newer fungicide molecules such as those belonging to the class strobilurins have been introduced for crop disease management. These fungicides have been reported to be milder than previously used fungicides on account of their non-persistent nature and thereby eliminates the problem of retaining chemical residues on the plants, as encountered in fungicides that were previously used. However, the use of these newer chemical molecules is still in its infancy and has not been evaluated on crops like culinary melon, in Kerala

In addition to the fungicides, among the chemical methods of control, the impact of foliar fertilizers on plant diseases especially those affecting the foliage have been studied earlier and the evaluation of the same is still continuing as reported from several countries (Kuepper, 2003). Alternate methods of control such as the use of bio-control agents have attained widespread recognition in the field of crop disease

management as substantiated by the numerous reports and reviews about these microbial candidates.

In the light of these situations the present investigation was undertaken to screen certain fungicides, foliar fertilizers and bio-control agents to be included as promising components in the management of anthracnose leaf spot of culinary melon.

The initial part of the study consisted of survey in culinary melon fields for examining the major foliar diseases prevalent in the crop and also for making a comparative assessment of the diseases that were observed. Following this the major study of the thesis programme was undertaken which comprised of evaluating certain nutrients like foliar fertilizer NPK19:19:19 (0.5 per cent) and calcium nitrate (0.5 per cent); fungicides *viz.,* azoxystrobin (strobilurin) (0.15 ml/l) and mancozeb (dithiocarbamate) (0.4 per cent). The bio-control agents, *Pseudomonas fluorescens* and *Trichoderma viride* were also included for the evaluations which were conducted by laboratory assay, greenhouse experiments and field trials at the College of Agriculture, Vellayani. Finally, the most effective treatments that were screened from the previous trials were tested for confirming their efficacies in trials that were laid out in farmers' fields at different locations of Thiruvananthapuram district. Further in these confirmation trials the impact of the various treatments tested, on the microbial flora in the phyllosphere and rhizosphere of the treated culinary melon plants and also their effects on physiological parameters of the host plant were investigated.

Review of Literature

2. REVIEW OF LITERATURE

Culinary melon (*Cucumis melo* L. var. *acidulus* Naudin) is cultivated mainly for their vegetable production in India. There are several production constraints among which losses due to pest and disease are of much concern. Among the fungal diseases, leaf spot disease caused by *Colletotrichum* spp. is a major fungal disease which causes considerable yield loss.

The present study was focused on the integrated management of *Colletotrichum* leaf spot of culinary melon which is assuming importance as a serious disease. The review of literature pertaining to different aspects of the study such as disease assessment, isolation and identification of the pathogen, management of the disease involving chemicals and bio-control agents, biochemical and physiological changes induced by effective treatments are presented below:

2.1. Survey on incidence and intensity of culinary melon disease

Jenkins *et al.* (1983) observed that diseases of cucumber were mostly caused by fungi some of which are anthracnose (*Colletotrichum lagenarium*), downy mildew (*Pseudoperonospora cubensis*), powdery mildew (*Erysiphe cichoracearum*), scab (*Cladosporium cucumerinum*), target spot (*Corynesporacassicola)* and Alternaria leaf spot (*Alternaria cucumerinum*). These fungal pathogens may cause injury to all above-ground parts including the leaves, stems, petioles, peduncles and fruits. Cucurbitaceae is one of the largest family of vegetables, comprises mainly of bitter gourd (*Momordica charantia*), bottle gourd (*Lagenaria siceraria*), cucumber (*Cucumis sativus*), musk melon (*Cucumis melo* var. *reticulata*), pumpkin (*Cucurbita moschata*), snap melon (*Cucumis melo* var. *acidulus*), sponge gourd (*Luffa acutangula*) and summer squash (*Cucurbita pepo*). These crops are susceptible to a number of diseases caused by fungi, bacteria, nematodes and viruses (Kang and Sandhu, 2007) which render their cultivation uneconomical and relatively insecure.

Cucumber production is seriously affected by several diseases which result in poor growth and yield (Agrios, 2005). Powdery mildew, downy mildew, anthracnose, alternaria blight and fusarium wilt are serious fungal diseases of cucumber (Sen *et al,* 2014). Kehinde (2011) reported that as with most minor crops in the mixed cropping system of farmers' in Nigeria, the incidence and severity of diseases in the vegetable crop melon, was rarely documented.

In a survey of farmers' fields across four states of southwestern Nigeria, major diseases of egusi melon were evaluated, their symptoms described and causal pathogens identified (Kehinde, 2011). Powdery mildew (*Erysiphe cucurbitarum*) appeared as round whitish spots on the lower surface which increased in size and later appeared as whitish talcum on the upper surface. Downy mildew (*Peronospora cucurbitarum*) appeared on the upper surface as small and pale green to yellow angular spot which became chlorotic and shriveled. Alternaria leaf spot (*Alternaria cucumerina*) appeared as small circular water soaked areas which turned dark brown to black and Cercospora leaf spot (*Cercospora citrullina*) appeared as circular spot with white to tan centers having dark margins. Foliar spots which began as small yellowish water-soaked areas on the veins that later turned circular, dried up, broke and became shattered were symptoms observed for the anthracnose disease (*Colletotrichum lagenarium*). Leaf blight, stem blight and fruit rot (*Didymella bryoaniae*) disease symptoms were observed on leaves, stems and fruits respectively. Leaf symptoms began as light brown, irregular spots surrounded by yellow borders that developed from the tip of the leaves and gradually progressed backwards. Brown lesions were observed on the stem which split open and turned dark. Lesions observed on fruits appeared as small, almost circular water-soaked areas which enlarged, resulting in dark, firm and leathery depressed areas. Wet rot of flower and fruit (*Choanephora cucurbitarum*) appeared as characteristic fungus growth, resembling numerous pins stuck in a pin-cushion on the infected surface. Anthracnose is a major and most common fungal disease of different angiospermic

plants throughout the world. Averre (1991) observed that the symptoms of anthracnose vary somewhat on different hosts. On cucumber leaves the spots started as water soaked area and expanded into brown spots which were roughly circular, reaching about 1/4 to 1/2 inch in diameter. Small, growing leaves may be distorted and severe spotting may cause entire leaves to blight. Symptoms included sunken spots or lesions (blight) of various colours in leaves, stems, fruits, or flowers, and some infections form cankers on twigs and branches. Anthracnose causes the wilting, withering, and dying of tissues, though the severity of the infection depends on both the causative agent and the infected species and can range from mere unsightliness to death (Thurston 1998). The cucumber plants both in greenhouse and field produced roughly circular to brown lesions that were usually large and more than 10 mm diameter on all the above –ground tissues including leaves, stems, petioles and fruits after infection by *Colletotrichum orbiculare* (Akem and Jovicich, 2011). Goldberg *et al.,* (2004) stated that anthracnose, caused by the fungus *Colletotrichum orbiculare*, was a relatively common fungal disease in humid areas and appeared only sporadically in New Mexico's dry environment; but when conditions were favorable the disease caused significant losses in New Mexico-grown cucurbits, especially in watermelons. Ferrin (2008) reported that lesions of the disease were brown to black with irregular margins often restricted by leaf veins. This disease first appeared as small, variously colored, circular spots on the older leaves, though it eventually spread to younger leaves, stems, pods and fruits. According to Shamsi and Naher (2015) the spots enlarged and merged, getting darker until the leaves dropped off and the plant was defoliated and died off. In some cases symptoms appeared as off-white, transparent lesions on leaves and other infected parts. Rampersad (2010) reported for the first time *C. gloeosporioides* as causing widespread anthracnose infection in pumpkin in Trinidad. He observed that although anthracnose was a serious threat to cucurbit production, infection was not common in pumpkin and squash. Foliar chlorosis and necrosis symptoms were observed in 15 commercial

pumpkin fields and the severely infected plants were unable to support fruit maturation, which resulted in yield loss. The pathogen *C. gloeosporioides* isolated from surface-sterilized tissues of symptomatic plants produced on potato dextrose agar (PDA) white to cream colonies with gray spore masses in the center. Conidia were hyaline, cylindrical with rounded ends and aseptate.

In a study conducted in Southwestern Nigeria, Kehinde (2011) observed that anthracnose was a predominant foliar disease of melon with maximum disease incidence of 82%-100% among the different melon cultivars tested. He also implied that as with most minor crops in a mixed cropping system of farmers in Nigeria, the incidence and severity of diseases in the vegetable crop melon was also rarely documented.

Epidemics of anthracnose reduced yield when they were severe and occurred early in the season. Temperatures less than 90°F (32°C) and rain favored disease epidemics. Environmental conditions had a significant influence on the disease progression of anthracnose on cucumber which is less likely to infect cucumber when temperatures get above 86°F (30°C), even if rainfall occurs (Thompson and Jenkins, 1985). Shamsi and Naher (2015) observed that the disease can spread very quickly in warm (80F), wet weather, especially if air circulation is poor. Kehinde (2011) reported that the crop was more susceptible to this disease during rainy season which might have been due to the high relative humidity and also because the spores of the pathogen required periods of rainfall for dispersal, infection and disease development. Sherf and Macnab (1999) had also observed that the disease thrived well in places where wet growing seasons prevailed. Topit and Sovali (2010) also indicated that diversity, incidence and spread of fungal diseases increased during more humid growth periods.

Park *et al.* (1996) observed that anthracnose disease occurred severely in old fields of melon with successive cropping when compared with newly planted fields. The fungal pathogen was reported to be able to survive in soils for long periods, up to two years, in absence of substrate host (Averre, 1991 and Ferrin, 2008). Kehinde (2011) reported that the pathogen *Colletotrichum* had the ability to survive in fields on plant debris from previously infected plants or on contaminated seeds which may be the reason for severity of the disease.

Cylindrical and hyaline, conidia typical of those produced by *Colletotrichum gloeosporioides*, were observed on microscopic examination of the leaf samples that exhibited symptoms of anthracnose leaf spot. The fungus *Colletotrichum lagenarium* (syn. *C. orbiculare*) was reported as the causal agent of anthracnose disease on infected leaves of melon (Prakash *et al.*, 1974), Timchenko (1977); Peregrine *et al.* (1984); Wei *et al.* (1991) and Kehinde, (2011). The conidia were oval, or pill-shaped, clear, and had no cross-walls (Zitter *et al.*, 1998). *Colletotrichum orbiculare*, belonging to ascomycete, induces fatal anthracnose disease on cucumber (Pain *et al.* 1992), and has become a limiting factor in commercial production (Bi *et al.* 2007). The cucumber plants, both in the greenhouse and field, caused roughly circular, brown to reddish lesions on all above-ground tissues including leaves, stems, petioles, and fruits after attack by *C. orbiculare* (Lanston *et al.* 1999). *Colletotrichum* spp. is the causal organism of anthracnose disease (Douglas 2011). Akem and Jovicich (2011) reported that the foliar disease is caused by the plant pathogenic fungus *Colletotrichum orbiculare*. The fungus can infect cucumber, squash, pumpkin, melon and watermelon. Symptoms of anthracnose in cucumber may start with small round (2mm diam.) pale yellow leaf spots. The round lesions will increase in diameter (up to10 mm), as they turn from tan to dark brown. Anthracnose disease caused by *Colletotrichum orbiculare* has recently been considered to be particularly important wherever cucurbits are cultivated under highly controlled conditions. Severe

infections may cause formation of numerous leaf lesions and vine defoliation resulting in poor quality fruit and yield loss (Egel, 2014). *C. gloeosporioides* infects about 470 different host genera some among which are economically important crops such as: avocado, mango, beans, cashews, cassava, citrus plant, cotton, cow-pea, cucumber, eggplant, green gram, mango, onion, pepper, pumpkin, papaya, sorghum, soybean, tomato, watermelon, wheat, yam, zucchini, cereals, legumes and spinach (Sharma *et al.,* 2015).

Downy mildew is a destructive disease of cucumber, muskmelon, and watermelon. Occasionally it causes damage to gourd, pumpkin, and squash and is favored by warm, moist weather. It is most prevalent in regions where rain falls during the growing season. The disease is incited by the fungus *Pseudoperonospora cubensis* (Middleton and Bohn, 1953). Downy mildew caused by *Pseudoperonospora cubensis* (Berk. et Curt.) Rost. is a major disease of cucurbits in temperate regions of the world that considerably reduces the production and greatly affects both yield quantity and quality (Ahmed *et al.,* 2000). Downy mildew is one of the most important melon diseases in Northeast Brazil. It causes up to 60 percent reduction in fruit production (Cardoso *et al.*, 2002a) and 49% in the content of soluble solids (Cardoso *et al.*, 2002b). Seebold, (2010) observed that symptoms of downy mildew first appeared as pale-to-bright yellow spots on the upper surface of leaves in the crown area of the plant. Leaf spots were irregular or "blocky" in appearance and were limited by leaf veins. As lesions expanded and the number of lesions increased, leaves became necrotic and plants appeared scorched. On the underside of leaves, lesions were water-soaked and slightly sunken and profuse sporulation light to dark gray or purple in color was evident as a fuzzy or "downy" growth on lower leaf surfaces when humidity was high. Downey mildew symptoms started showing up in older leaves, as angular light-yellow spots, limited by the veins, following which lesions coalesced and caused rotting of the tissue which assumed a bronze to brown hue. Severe infection resulted in early leaf dropping, producing malformed and

underdeveloped fruits (Michereff *et al.,* 2009). Kehinde (2011) reported that at the early stage of downy mildew infection, symptoms appeared on the upper surface of the oldest leaves near the crown, as small and pale-green to yellow angular spots. The underside of the leaves opposite the yellow spots became covered with layers of grey mycelial growth. The leaf veins confined the spots and at advanced stage, severely infected leaves became chlorotic, turned light brown and shrivelled.

Pseudoperonospora cubensis, the causal agent of cucurbit downy mildew, is responsible for devastating losses worldwide of cucumber, cantaloupe, pumpkin, watermelon and squash. The pathogen has a wide geographical distribution and has been reported in over 70 countries, including environments ranging from semi-arid to tropical. Other economically important hosts of *P. cubensis* are melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), and squash (*Cucurbita* spp.) (Whitaker and Davis, 1962). In addition, *P. cubensis* has a wide host range, infecting approximately 20 different genera of cucurbits (Palti and Cohen, 1980; Urban and Lebeda, 2007) including 19 species in the genus *Cucumis.* Cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.), water melon (*Citrullus lanatus*) and marrow or summer squash (*Cucurbita pepo*) are the major hosts that harbour the pathogen. The other cucurbit hosts include sponge gourd (*Luffa aegyptiaca*), ridge gourd (*Lagenaria acutangula*), bottle gourd (*L. siceria*), wax gourd (*Benincasa hispida*), bitter gourd (*Momordica charantia*), pumpkins (*Cucurbita moschata*), and round melon (*Citrullus vulgaris* var. *fistulosus*).

In India, cucumber, musk melon, sponge gourd and ridge gourd are severely affected than other cucurbits (Agricultural and Environmental Education bulletin, VPS-30). The pathogen *P. cubensis* infects when wind-blown sporangia are introduced onto cucurbit hosts under favorable environmental conditions. *P. cubensis* is a biotroph and, with the exception of oospore production, survives only on living host tissue (Bains and Jhooty, 1976). Environmental conditions affect

overwintering capacity as well as disease development and severity. Leaf moisture is required for germination of sporangia. Rain, dew, or irrigation can easily supply enough moisture for sporangia to germinate. Under optimum temperature, infection can occur within two hours of leaf wetness (Cohen, 1977). Kehinde (2011) observed that highest incidence of downy mildew occurred during dry season. However during rainy spells also this disease was relatively high compared to other diseases such as powdery mildew, Cercospora leaf spot due to the ability of the fungus to be easily carried by rain splash, wind currents and also due to its adaptability to hot temperature followed by the spore development which is favoured by cool and moderately warm temperatures.

2.1.1. Isolation of the pathogen causing anthracnose of culinary melon

Anthracnose of cucurbits has rarely been recorded in India except for a few earlier reports of Prakash (1976); in which *C. lagenarium* was stated as the pathogen of cucumber and pumpkin respectively. Mukerji and Bhasin (1986) reported *C. capsici, C. lagenarium* and *C. orbiculare* on *C. maxima* from India. Linde (1990) stated that the acervulus was often surrounded by black, hair-like structures, known as setae that were only visible under a microscope. The conidia were oval, or pill-shaped, clear, and had no cross walls. However in studies conducted later, Sutton (1992) included cucurbits as one of the 470 recorded host plants of *C. gloeosporioides*. *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is one of the most common and widely distributed plant pathogens in the world (Sutton, 1992; Cannon *et al.,* 2000). Since its original description as *Vermicularia gloeosporioides* Penz., it has been associated with different host genera either as a primary disease-causing organism, or has been isolated from deteriorated plant parts. It is especially prevalent in the tropics, but has been recorded also from a wide range of temperate and subtropical habitats. The species is also well known as a latent pathogen causing post-harvest problems (Prusky and Plumbley, 1992). Besides,

endophytic strains of this pathogen were commonly isolated from symptomless plant parts (Cannon and Simmons, 2002). Cucumber anthracnose pathogen was identified as *C. orbiculare* based on identification of symptoms on the plant and the characters of conidia that were formed in masses known as acervuli (singular acervulus), that appeared as pink-colored, slimy masses on infected tissues (Zitter *et al.*, 1998).

According to Li and Zhang (2007), there was a heavy loss due to fruit rot caused by *C. gloeosporioides* in *Trichosanthes kirilowii* Maxim, a species within the gourd family which is cultivated in China for its edible seeds and medicinal roots. *Colletotrichum orbiculare* is recognized worldwide as the anthracnose pathogen of cucurbits (Palenchar *et al.*, 2009). Further, Rampersad (2010) isolated the pathogen *Colletotrichum gloeosporioides* from surface-sterilized tissues of symptomatic plants and reported for the first time anthracnose caused by *Colletotrichum gloeosporioides* in pumpkin in Trinidad. The limit of the *Colletotrichum gloeosporioides* species complex is defined genetically based on a strongly supported clade within the *Colletotrichum* ITS gene tree. All taxa accepted within this clade are morphologically more or less typical of the broadly defined *C. gloeosporioides*, as it has been applied in the literature for the past 50 years (Weir *et al.*, 2012).

Colletotrichum fructicola was originally reported as a pathogen of coffee berries in Thailand. This species was also known as a pathogen of *Pyrus pyrifolia* (Japan), *Persea americana* (Australia), *Malus domestica* (Brazil), *Dioscorea* (Nigeria), *Theobroma* and *Tetragastris* (Panama), *Vitis* (China), and *Mangifera indica* (Brazil). Originally reported from coffee berries from Thailand (as *C. fructicola*) and as a leaf endophyte from several plants in Central America (as *C. ignotum*), these isolates that are accepted as *C. fructicola* are biologically and geographically diverse and known to be isolated from *Coffea* from Thailand, *Pyrus pyrifolia* from Japan, *Limonium* from Israel, *Malus domestica* and *Fragaria* × *ananassa* from the USA, *Persea americana* from Australia, *Ficus* from Germany,

Malus domestica from Brazil, *Dioscorea* from Nigeria, and *Theobroma* and *Tetragastris* from Panama. It was also isolated from pear in China (Li *et al.* 2013).

Colletotrichum is one of the major plant pathogenic genera responsible for causing anthracnose, disease on a variety of hosts from trees to grasses. Different species of the pathogen are reported from a variety of plant host including cereals and grasses, legumes, vegetables, perennial crops and tree fruits in India (Gautam *et al.*, 2014).

2.1.3. Pathogenicity test

All isolates of *C. orbiculare* that originated from cucurbit hosts were pathogenic on the susceptible cucumber (Marketer) and watermelon (Black Diamond) differentials (disease ratings 5.0). H19 was resistant (disease ratings 2.5) to races 2 and 2B and Charleston Gray was resistant to races 1 and 2B of *C. orbiculare* Wasilwa *et al.*, (1993). The mean disease ratings of isolates of *C. orbiculare* from cocklebur and isolates of *C. trifoii*, *C. lindemuthianum*, and *C. malvarum* were below 0.5 and no evidence of infection was observed for the isolates examined and thus, all were considered nonpathogenic on the susceptible cucurbit hosts. Furthermore, several isolates of all of the species were tested in additional pathogenicity tests whereby inoculum concentrations were increased to 4×10^6 conidia/ ml and, again, no infections were observed on the cotyledons of any of the cucurbit differentials (Liu *et al.*, 2007).

The common inoculation methods for pathogenicity testing included drop inoculation and wound /drop inoculation (Kanchana-udomkan *et al.* 2004, Lee *et al.* 2005), micro-injection and spraying with high pressure guns (Freeman *et al.*, 1996, Lin *et al.* 2002, AVRDC, 2003, Sharma *et al.* 2005, Than *et al.* 2008b, Cai *et al.* 2009). Artificial inoculation methods *in vitro* were used to test the pathogenicity of a fungal species, as it was easy to control environmental conditions (Photita *et al.*,

2004). Ranjana (2008) identified *Colletotrichum gloeosporioides* (*Glomerella cingulate*) as the pathogen causing anthracnose of *Pluteus bombycinus* in Assam by morphological examination and pathogenicity tests. This is the first report of *C. gloeosporioides* causing anthracnose in *Pluteus bombycinus* in Assam.

A pathogenicity test was conducted by six plants (cv. Jamaican squash) for each of five isolates of *C. gloeosporioides* were spray inoculated to runoff with a conidial suspension (1.0×10^6 conidia/ml). Control plants were sprayed with sterile distilled water. In repeated tests, plants were symptomatic of infection 7 days post inoculation. There were no symptoms on control plants and Koch's postulates were fulfilled with the re-isolation of the pathogen from symptomatic leaf tissues. (Rampersad, 2010). Although anthracnose was a serious threat to cucurbit production, infection was not common in pumpkin and squash.

Fungal discs (5 mm) of the representative isolate SknCSY1 of sweet pea grown on PDA was attached to leaves and stems with and without wounding. After inoculation, black spots appeared on leaves and stems, inoculated both with and without wounding and the pathogen was re-isolated from the leaves and stems (Shoji *et al.*, 2013).

2.1.4. Morphological and molecular characterization of *Colletotrichum*

Differentiation among the *Colletotrichum* morpho-groups based on traditional methods such as conidial shape and size appeared to be reliable as the differences of conidial size, both in length and width of conidia, were statistically significant (Simmonds, 1965, Sutton, 1962, 1965, 1966, 1968, 1980 and Arx, 1981).

Morpho-taxonomic criteria such as conidial shape and size, apressoria morphology and size, setae morphology, temperature response on potato dextrose agar medium (PDA) and host specificity, as well as molecular identification

techniques, are currently in use for identification of *Colletotrichum* spp. (Sutton, 1992; Freeman *et al.*, 1998; Liu *et al.*, 2007).

Conidial morphology has been traditionally emphasized over other taxonomic criteria, although conidia of *Colletotrichum* are potentially variable. Initially recognized as "special forms" of *C. gloeosporioides*, the species *C. orbiculare*, *C. trifolii*, *C. lindemuthianum* and *C. malvarum* are distinct from *C. gloeosporioides* in that they generally have ovoid conidia (Sherriff *et al.*, 1994). Connell *et al.*, (1993) observed that there was no septum in germinated conidia and they produced appressoria of similar shapes and dimension. Thus, although these species can be distinguished from *C. gloeosporioides* based on morphology, their distinction from one another has been based largely on host origin. Conidia are formed in masses known as acervuli (singular acervulus), which appeared as pink-colored, slimy masses on infected tissue. The acervulus is often surrounded by black, hair like structures, known as setae that are only visible under a microscope. The conidia are oval, or pill-shaped, clear, and have no cross walls (Zitter *et al.*, 1998).

Baxter *et al.* (1983) defined *C. gloeosporioides* aggregate by using morphological methods and reported that conidia were cylindrical with rounded ends and less than 4.5 μm in diameter. The majority of the conidia of *C. gloeosporioides* were oblong with obtuse ends, and were generally shorter and broader (Gunnell and Gubler, 1992 and Sutton, 1992). Sutton, (1992) also recognized the species as a heterogeneous group with a great variation in morphology. The identification of species of *Colletotrichum* has relied primarily on morphological differences such as colony color, size, and shape of conidia, optimal temperature, growth rate, presence or absence of setae, and existence of the teleomorph, *Glomerella* (Freeman *et al.*, 1998). Several studies have shown that cultural morphology can be useful for

grouping isolates when they are sampled at a local or regional level (Johnston & Jones 1997, Prihastuti *et al.,* 2009).

Colletotrichum fructicola and its synonym *C. ignotum* have been isolated and described as an opportunistic pathogen from berries of *Coffea arabica* and as leaf endophyte of *Theobroma cacao*, respectively (Prihastuti *et al.,* 2009; Rojas *et al.* 2010). Colonies on PDA were at first white, becoming grey to dark grey at the centre with age, in reverse greyish green with white halo maximum of 83 mm diameter in 7 days at 28°C, growth rate 10.58-11.5 mm/day. Aerial mycelium pale grey, dense, cottony, without visible conidial masses. Sclerotia absent, acervuli absent in culture. Setae absent. Conidia 9.7-14 × 3-4.3 µm (x =11.53 ± 1.03 × 3.55 ± 0.32, n = 180), common in mycelium, one-celled, smooth-walled with a large guttule at the centre and surrounded by smaller guttules, hyaline, cylindrical with obtuse to slightly rounded ends, sometimes oblong (Prihastuti *et al.* 2009). Shoji *et al.* (2013) observed that colony of *C. fructicola* on PDA were gray, cottony, pale gray to pale orange, sometimes with dark flecking on the reverse. Conidia were colorless, sub-cylindrical, attenuated and had blunt ends when produced on synthetic nutrient-poor agar medium (SNA). The size was 14.5-20.4 × 3.6-5.7 µm. Appressoria were mediumto dark brown, obovoid to ellipsoid. Hyphae grew on potato dextrose agar (PDA) at 10-35°C (28°C optimum) with a daily growth rate of 12.0 mm at 28°C.

2.1.5. Contemporary methods of identification

Traditional identification and characterization of *Colletotrichum* species has relied primarily on differences in morphological features such as colony colour, size and shape of conidia and appressoria, growth rate, presence or absence of setae, and existence of the *Glomerella* teleomorph (Smith and Black, 1991; Gunnell and Gubler, 1992; Sutton, 1992). Based on molecular and morphological data, a close relationship between C. *orbiculare* from cucumber, *C. trifolii* from alfalfa,

C. malvarum from prickly sida, and *C. lindemuthianum* from bean were observed (Pain *et al.,* 1992).

Molecular technologies based on the analysis used to examine the relationship of *Colletotrichum* spp by Bailey *et al.* (1995). Based on spore morphology, appressorium development, and sequence similarities of the rDNA, it was proposed that *C. orbiculare*, *C. trifolii*, *C. lindemuthianum*, and *C. malvarum* should be considered a single species. Many efforts were made to distinguish *C. orbiculare* into different races based on host range. However, the isolates of *C. gloeosporioides* that are specifically pathogenic to distantly related hosts may not be genetically isolated, indicating that the population structure and dynamics of *C. gloeosporioides* is very complex (Cisar *et al.*, 1994). Detailed description on the taxonomy of the *C. gloeosporioides* was given by Singh and Prasad (1967) during the epidemiological studies of anthracnose of *Dioscorea alata.* Use of molecular markers like ribosomal DNA internal transcribed spacer (ITS) sequences in understanding the phylogeny and systematics of *C. gloeosporioides* has been carried out (Sreenivasaprasad and Talhinhas, 2005).

Traditional differentiation between *Colletotrichum* species, based on host range or origin, may not be reliable criteria for fungi of this genus since taxa, such as *C. gloeosporioides*, infect a broad range of host plants (Freeman *et al.*, 1998). A combination of molecular diagnostic tools with traditional morphological techniques is an appropriate and reliable approach for studying *Colletotrichum* species complexes (Cannon *et al.,* 2000; Abang, 2003; Than *et al.,* 2008a). DNA sequence analyses have thus been suggested by various authors to overcome the inadequacies of morphological criteria (Abang *et al.*, 2002; Moriwaki *et al.*, 2002; Peres *et al.*, 2002; Guerber *et al.*, 2003; Photita *et al.*, 2005; Du *et al.*, 2005; Whitelaw-Weckert *et al.*, 2007; Peres *et al.*, 2008; Than *et al.*, 2008a, b; Crouch *et al.*, 2009). However, due to their morphological variability, the ample range of

hosting crops, and wide variety of isolates, they are partially difficult to identify by traditional taxonomic methods, which must be complemented with molecular techniques (Whitelaw-Weckert *et al.,* 2007).

The pathogenic variability of *C. capsci* associated with chilli anthracnose carried out by Sharma *et al.* (2005) was also performed by collecting fungal pathogens from different regions of Himachal Pardesh, India. Hyde *et al.* (2009) pointed out, the identification of *Colletotrichum* species is difficult on the basis of delimited morphological characters like size and shape example from coffee (*Coffea* spp.) hosts. Studies on taxonomical description of *Colletorichum* sp. based on morphological, microscopic and molecular approach are also carried out in India. Variability in *C. gloeosporioides* isolates was also studied in detail based on morphological and microscopic characters (Kumar *et al.,* 2012). Anthracnose pathogens, previously reported to be *Colletotrichum* sp., that occur on coffee in Thailand were shown as three new species of *C. asianum*, *C. fructicola*, and *C. siamense* based on the multigene sequence analysis and morphological characteristics (Prihastuti *et al.,* 2009). Similarly, Shampatkumar *et al.* (2007) examined seven isolates of *C. gloeosporioides* collected form Agri Export Zone (AEZ) of Andhra Pradesh and from Tamil Nadu. The species was confirmed using amplification of ITS loci and the isolates were evaluated for their pathogenic variability on mango seedlings and genetic diversity with molecular techniques like Random Amplified Polymorphic DNA (RAPD) and Internal Transcribed Spacer-Restriction Fragment Length Polymorphism (ITS-RFLP).

Twenty-five isolates of *C. gloeosporioides* causing mango anthracnose were collected from different agro-climatic zones of India, evaluated for their pathogenic variability on mango seedlings, and genetically characterized using random amplified polymorphic DNA (RAPD) (Gupta *et al.,* 2010). In the past few years, anthracnose pathogens from tropical fruits, which were identified as *C. gloeosporioides* based on

their morphological characteristics, were subdivided into *C. asianum*, *C. fructicola*, *C. horii*, *C. kahawae*, and *C. gloeosporioides* by comparing of the nucleotide sequences with that of the *C. gloeosporioides* epitype (Phoulivong *et al.*, 2010).

As a result, many species of *Colletotrichum*, including *C. gloeosporioides*, have been defined, mainly based on the results of molecular phylogenetic analysis (Damm *et al.*, 2009). Although the internal transcribed spacer (ITS) sequence do not separate *C. gloeosporioides* complex, some single genes or combinations of genes, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and glutamine synthetase, can be used to reliably distinguish most taxa (Weir *et al.*, 2012).

Shoji *et al.* (2013) identified the fungus causing severe fruit rot on ripening sweet peppers (*Capsicum annuum* L. var. *grossum* Sendtner) in a greenhouse, based on morphological characteristics and molecular phylogenetic analysis. The morphological characteristics matched closely with the descriptions of *C. gloeosporioides* and *C. fructicola.* The phylogenetic analysis revealed that the isolate SknCSY1 of the pathogen was included in the same cluster with *C. fructicola* and related to *C. fructicola* with 99.2 to 99.9 per cent identities. This is the first report of *C. fructicola* as a causing anthracnose of sweet pepper in Japan,

Sharma *et al.* (2014) stated the phylogenetic relationships of the fifty two *C. gloeosporioides* isolates mainly associated with disease symptoms of chilli fruits in southern India with other species of *C. gloeosporioides* species complex. Fifty two fungal isolates were indistinguishable.

2.2. Effect of foliar fertilizer, nutrients, fungicides and bio-control agents against anthracnose leaf spot disease

2.2.1. Effect of chemicals (foliar fertilizer, nutrients and fungicides)

2.2.1.1. Foliar fertilizer and nutrient

Calcium propionate and calcium chloride were effective of several calcium salts in reducing hyphal growth *Colletotrichum* spp. (Biggs, 1999); colony area of the pathogen *Physalospora vaccinii* was greater on media amended with calcium nitrate or calcium chloride compared with the control, suggesting that this fungus metabolizes components of the added salts.

Farahat *et al.* (2012) observed in their study on the effect of 0.1M aqueous solution of K and NK fertilizers recoded significantly effect in retarding linear growth of *E. turcicum* fungus on PDA medium *i.e.* 5.90 cm and 6.06 cm in comparison with control (7.00 cm) and caused inhibition of growth by 15.71 per cent and 13.43 per cent. Other treatments were not effective in reducing linear growth of the fungus. All treatments of NPK solutions significantly reduced the sporulation of the fungus and the most effective ones were NP and NPK, which inhibited spores formation and also changed the mycelium color. K and PK solutions also recorded positive effect in reducing of sporulation while NK, P and N solutions showed the least effect.

2.2.1.2. Fungicides

Tomy (1997) reported that among six fungicides tested against *C. gloeorporioides* causing black leaf spot in mulberry. Mancozeb, carbendazim and copper oxychloride proved effective in inhibiting radial growth of the pathogen.

Sendhil Vel (2003) studied the *in vitro* efficacy of azoxystrobin against the spore germination of downy mildew of grapes and found that even at a concentration

of 100 ppm, it was able to reduce the germination up to 90 per cent, with an increasing concentration of the azoxystrobin (250, 500, 750 and 1000 ppm) complete inhibition of germination.

Nithyameenakshi *et al.* (2006) reported that *in vitro* study of spore germination azoxystrobin at 0.05 per cent arrested the spore propagules of downy mildew, powdery mildew and anthracnose of grapes.

Hussain *et al.* (2008) indicated that mancozeb which is a derivative of dithiocarbamic acid is toxic to fungi because they are metabolized to isothiocyanate radicals inside the pathogen cells, which inactivates the –SH group of aminoacids and enzymes.

Filoda (2008) indicated that only at the lowest concentration (0.05%), the fungicide azoxystrobin was not satisfactory in inhibition (68.00 per cent) compared to benzimidazole at the same concentration which resulted in (81.00 per cent) inhibition of *C. gloeosporioides* whereas a high percentage of growth inhibition was observed when azoxystrobin was used at higher concentrations of 0.1% and 0.2% (80.00 per cent and 82.00 per cent respectively).

Archana (2009) reported that the azoxystrobin (23 SC) completely inhibited the sporangial germination of *P. viticola* at 300 ppm. Similarly, azoxystrobin (23 SC) recorded per cent inhibition of conidial germination at a concentration of 250 ppm and above. Azoxystrobin provided per cent control of downy mildew in grapes, when applied 1 to 5 days before inoculation and 85 per cent mean reduction of resporulation from diseased tissue, when applied 6 days after inoculation (Wong and Wicox, 2001).

Adhikary *et al.* (2013) tested the efficacy of azoxystrobin against mango anthracnose pathogen *C. gloeosporioides* under *in vitro* conditions. Azoxystrobin

significantly reduced both mycelial growth and conidial germination on PDA media. Azoxystrobin at 25, 50 and 100 ppm slightly inhibited the mycelial growth and conidial germination whereas 200, 300 and 400 ppm completely inhibited the mycelial growth and conidial germination of *C. gloeosporioides*. In control, 63.82 cm^2 surface areas were covered with mycelial growth and 94.38% conidia were germinated.

Ahiladevi *et al.* (2013) stated that the azoxystrobin 8.3%w/w + Mancozeb 68.75% was found to be more effective than other four fungicides in inhibiting the sporangial germination of *P. viticola* at 0.36 per cent concentration as it recorded 89.26 % inhibition as compared to 88.90 and 77.39 in 0.30% and 0.24% concentrations respectively. Among other fungicides, azoxystrobin was found to show 72.06 percent inhibition followed by mancozeb which recorded 62.54%, whereas the least inhibition of 55.32 was recorded in hexaconazole. The combination of metalaxyl+ mancozeb was found to be more effective when compared to mancozeb alone as it recorded 66.85 per cent reduction as compared to 62.54 per cent in mancozeb.

Fitsum *et al.* (2014) reported that when three synthetic fungicides at different concentrations were evaluated by the poison food technique against the bean anthracnose pathogen *C. lindemuthianum,* there was least mycelia growth of the pathogen in medium amended with mancozeb at 250 ppm and there was no growth at all, of the mycelium in media amended with mancozeb at 500ppm, when compared to the other two fungicides *viz.*, mancolaxyl and folpan.

2.2.2. Effect of bio-control agents

Deshmukh and Raut (1992) reported that *Trichoderma harzianum* Rifai and *T. viride* Pers. overgrew colonies of *Colletotrichum gloeosporioides* and

T. harzianum was more aggressive than *T. viride.* Narendra Singh (1992) revealed that *T. harzianum* was a strong inhibitor of *C. falcatum* under *in vitro* condition.

Jeyalakshmi and Seetharaman (1999) reported that *T. viride* reduced the mycelial growth of *Colletotrichum* spp by growing over the pathogen, causing hyphal coiling, hyphal abnormalities, lysis of hyphae and sclerotia. Padder *et al.* (2010) reported mycelia growth inhibition of 69.21 per cent and 64.20 per cent by *T. viride* and *T. harzianum,* respectively, against a local strain of *C. lindemuthianum.*

Santhakumari *et al.,* (2001) observed that the isolates of T1 and T2 of *T. harzianum* and the isolates of A1 and A2 of *Aspergillus niger* were found effective in inhibiting the growth of *C. gloeosporioides* causing anthracnose of black pepper under *in vitro* condition. Raheja and Thakore (2002) reported that, bioagents like *T. viride* and *T. harzianum* inhibited the growth of *C. gloeosporioides* causing anthracnose of yam effectively. *T. viride* and *T. harzianum* can effectively colonize *C. gloeosporioides* and *F. oxysporum* and can therefore be used as a biocontrol agent effectively against these two fungal pathogens. Both *T. viride* and *T. harzianum* is well known biocontrol agents and used for managing various plant diseases (Butt *et al.,* 2001, Tiwari and Mukhopadhyay, 2001; Rini and Sulochana, 2007).

Parthiban and Kavitha (2014) stated that the evaluation of *Trichoderma viride* isolates against growth of *C. lindemuthianum* under *in vitro* condition all the isolates were effective in inhibiting the pathogen growth. Among the seven isolates (TVMGLT1, TV21GL, TVMNT7, *T. harzianum*, TV15, TVUV10 and TVMG5) tested, isolate TVMGLT1 recorded highest mycelial inhibition of 75.00 per cent reduction over control with the highest inhibition zone of 18.00mm. An increased of the overgrowth on the *C. lindemuthianum* was observed in TVMGLT1 (65.00 mm) and TV21GL (63.00 mm). Though all the isolates showed mycelial inhibition, the

isolate TVMGLT1 recorded greater antagonistic activity against the growth of *C. lindemuthianum.*

Ngullie *et al.* (2010) tested seven antagonists against, *Pseudomonas fluorescens* exerted the maximum inhibition (67.42 per cent) of mycelial growth of *C. gloeosporioides* followed by *T. viride* and *Bacillus subtilis* that inhibited mycelial growth of *C. gloeosporioides* by 63.34 per cent and 56.86 per cent respectively compared to the control. Out of nineteen isolates of antagonistic bacteria and twelve isolates of yeast evaluated for bio-control efficiency both under *in vitro* and *in vivo* conditions, *Pseudomonas fluorescens* (FP7) recorded the maximum inhibition of mycelial growth of *Colletotrichum musae* (Faisal *et al.,* 2013).

Fitsum *et al.,* (2014) observed that three bio agents tested showed high percentage inhibition of the mycelial growth (PIMG) of *C. lindemuthianum.* The highest PIMG (80.39 per cent) was produced by *T. viride,* followed by (75.49 per cent) by *T. harzianum* and (40.20 per cent) by *P. fluorescens.*

2.3. Effect of foliar fertilizer, nutrients, fungicides and bio-control agents for control of anthracnose leaf spot pathogen under greenhouse conditions

2.3.1. Effect of chemicals (foliar fertilizer, nutrients and fungicides)

2.3.1.1. Effect of foliar fertilizer and nutrients

In greenhouse studies, (Smith and Black, 1989; Smith, 1989) determined that strawberries grown in soils with high levels of nitrogen, especially from ammonium sources, are more susceptible to anthracnose than plants grown in soils with lower nitrogen levels or those with high levels of calcium nitrate. Anthracnose fruit rot caused by *Colletotrichum acutatum* was less severe on fruit from greenhouse grown plants receiving drench or foliar applications of calcium sulfate than on fruit from

plants receiving water, calcium chloride, or calcium nitrate treatments. Fruit from plants receiving foliar applications of $CaCl_2$ developed less fruit rot than that from plants receiving soil applications of $CaCl_2$ (Gupton and Smith, 1993).

Severity of anthracnose fruit rot (caused by *C. acutatum*) was reduced when $Ca(NO_3)_2$ was applied to greenhouse grown strawberries (Smith and Black, 1993). Similar results were also reported for control of *Fusarium* diseases of tomato and chrysanthemum (Woltz and Engelhard, 1973). There was a significant difference in foliar N levels among the strawberry clones which suggests that N applications should be tailored to each cultivar; however, the trend of reduced anthracnose severity with nitrate fertilizer was similar on the five strawberry clones in these trials which indicates that other cultivars would respond in the same way.

Utkhede and Koch, (2006) observed that the efficacy of a single post-infection foliar spray of two chemicals or seven biological treatments against powdery mildew on cucumber under near commercial greenhouse conditions. Calcium nitrate at 15g 1^{-1}, Quadra 137 at 10g 1^{-1}, or undiluted homogenised milk applied as post-infection spray, reduced the number of powdery mildew (*Podosphaera xanthii*) colonies on cucumber leaves as compared with water control. Smith (2009) stated that the source and level of N in fertilizers had a major effect on severity of anthracnose crown rot in strawberry, whereas the level of P and K in fertilizers did not. Plants that received N as $Ca(NO_3)_2$ had less severe anthracnose crown rot symptoms than plants that received N in the ammonium form. Although these trials were conducted in greenhouse grown potted plants, the results had to be tested in the typical sandy soils of Florida.

2.3.1.2. Effect of fungicides

Nithyameenakshi *et al.,* (2006) stated that the efficacies of these fungicides were tested under greenhouse condition and among the treatments, azoxystrobin and

difenoconazole recorded the maximum disease reduction of 69.71 per cent and 67.77 per cent in (*Plasmopara viticola*), 61.80 per cent and 60.38 per cent in (*Uncinula necator*) and 65.73 per cent and 59.09 per cent in (*Gloeosporium ampelophagum*) respectively over control under greenhouse conditions.

Jagtap *et al.*, (2013) evaluated fungicides against the leaf spot pathogen *Colletotrichum capsici* infecting turmeric and reported that all the fungitoxins, reduced disease when compared to control and that 25.64 per cent was recorded after the third spray with the fungicide propiconazole whereas the fungicide azoxystrobin was least effective resulting in disease incidence of 34.45 per cent.

2.3.2. Effect of bio-control agents

Fungi belonging to the genus *Trichoderma* and the bacteria such as *Psudomonas fluorescens and Bacillus subtilis* are the most promising bio-control agents which act against a wide range of plant pathogens. *Trichoderma* spp. are capable of controlling a number of diseases of plants, they control large number of foliar and soil borne diseases (Papavizas, 1985).

Application of *P. fluorescens* strains 558 significantly reduced anthracnose in mango caused by *Colletotrichum gloeosporoides* when the fruits were inoculated by the antagonist (Koomen and Jeffries, 1993) in the UK. Strain Pfcp protected banana plants from wilt disease caused *by P. solanacearum* up to 50 per cent in the greenhouse and in the field (Anuratha and Gnanamanickam, 1990) at Madras, India.

Umesha *et al.* (1998) carried out an experiment under greenhouse and field conditions in Karnataka, India, where they treated seeds of pearl millet (*Pennisetum glaucum*) with *P. fluorescens* formulated in talc powder which increased seedling vigour and inhibited sporulation of the downy mildew pathogen. *P. fluorescens*

controlled downy mildew by both seed treatment and foliar application, but efficacy was significantly higher when seed treatment was followed by a foliar application.

P. fluorescens isolate P6 and P10 were found to be antagonistic to *Verticillium* wilt pathogen and increased the yield from 117-344 per cent (P10) in greenhouse trials and 113-247 per cent (P6) in field trials (Berg *et al.*, 2000).

In sunflower, the highest sclerotium root/collar rot disease suppression was exhibited by *P. fluorescens* strain PDCAB 2. Rangeshwaran and Prasad (2000) observed that relying on the greenhouse test was better than the laboratory test for evaluation of the bio-control agent. Meena *et al.* (2000) found that foliar application of *P. fluorescens* strain Pf1 significantly controlled late leaf spot and rust (*Puccinia arachis*) of groundnut in greenhouse conditions.

Trichoderma isolates are known for their ability to control plant pathogens (Elad and Freeman, 2002). Intensive research on bio-control of which direct *T. harzianum* had been carried out under commercial conditions, and there have been some significant achievements in greenhouse crops and in vineyards (Elad and Shtienberg, 1995). The first bio-control agent (BCA) to be commercialized, registered and used in greenhouse crops and vineyards was isolate T-39 of *T. harzianum* (TRICHODEX), which effectively controlled *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Cladosporium fulvum* diseases in greenhouse grown tomato and cucumber and in vineyards (Elad, 1994; 2000a,b). *T. harzianum* isolates were also reported to control strawberry grey mould (Tronsmo and Dennis, 1977). Various isolates of *Trichoderma* that originated from a *Trichoderma* collection of 76 BCA isolates, including isolate T-39 were effective in controlling anthracnose and grey mould in strawberry under laboratory and greenhouse conditions (Freeman *et al.*, 2001; Elad and Freeman, 2002).

Freeman *et al.* (2004) *Trichoderma* isolates are known for their ability to control plant pathogens. It has been shown that various isolates of *Trichoderma*, including *T. harzianum* isolate T-39 from the commercial biological control product TRICHODEX, were effective in controlling anthracnose (*Colletotrichum acutatum*) and grey mould (*Botrytis cinerea*) in strawberry, under controlled and greenhouse conditions. Three selected *Trichoderma* strains, namely T-39, T-161 and T-166 were evaluated in large-scale experiments using different timing of application and dosage rates for reduction of strawberry anthracnose and grey mould.

Bangari *et al.* (2012) stated that the anthracnose caused by *Colletotrichum graminicola* (Ces.) Wilson, is one of the most destructive foliar diseases. Experiment was conducted to develop strategies for combating this disease. In a glass house soil drenching experiment maximum germination was observed with TH-43 and TH-38 (86.66 per cent), TH-43 recorded 17.00 per cent increase in plant height whereas maximum reduction in disease severity was observed with TH-39 (33.94 per cent). Maximum germination was obtained with TH-38 bio-primed seeds (90.00 per cent), whereas maximum increase in plant height (25.72 per cent) and reduction in disease severity (47.82 per cent) was observed with TH-39 under glass house conditions while in glass house bio-priming + drenching experiment maximum germination was in TH-39 (86.66 per cent), plant height was maximum in TH-43 (31.80 per cent), increase and reduction of disease severity was maximum in TH- 43 treatment (45.05 per cent) as compared to control.

Purohit *et al.* (2013) made attempts to developing effective bio-control system for management of zonate leaf spot caused by *Gloeocercospora sorghi*. Four isolates of *T. harzianum viz.,Th*-43, *Th*-39, *Th*-32 and *Th*-31 and three isolates of *P. fluorescens viz., Psf*-28, *Psf*-11 and *Psf*-r which to be found more effective *in vitro* were further tested in glasshouse condition. *Th*-43 was found most effective in reducing disease severity 36.44 per cent followed by *Th*-39 (33.84%),

Th-32 (32.33%) and *Th*-31 (30.42%). In case of two foliar sprays, *Th*-43 (49.30%) was most effective followed by *Th*-39 (45.76%), *Th*-32 (45.32%) and *Th*-31 (44.47%). In three foliar sprays, reduction in disease was maximum with *Th*-43 (57%) followed by *Th*-39 (53.63%), *Th*-32 (52.23%), *Th*-31(48.13%) and *Psf*-28 (45.05%). *Trichoderma* spp. is the one of the best alternatives for the management of this pathogen due to various mechanisms like mycoparasitism, antibiosis and competition for colonization. *T. harzianum* had been found effective against a range of economically important aerial and soil borne plant pathogens and is used as bio-pesticide in green house and field applications (Tondje *et al.,* 2007; Kharayat and Singh, 2012; Srivastava *et al.,* 2010; Bangari and Singh, 2011).

2.3.4. Adjuvants

Adjuvants are additives commonly applied with pesticides to improve spray performance, including persistence on foliage (Hart *et al.,* 1992), coverage (Steurbaut, 1993), absorption (Thompson, 1996), efficacy (Grayson *et al.,* 1996) and pesticide translocation (Maschoff *et al.,* 2000). Compounds used as adjuvants include petroleum and crop based oils, inorganic salts, and organic surfactants. Although fungicide utility and efficacy may be significantly enhanced by the addition of an adjuvant, research with adjuvants has predominantly focused on herbicide performance (Foy, 1993).

Percich and Nickelson, (1982) reported that mancozeb efficacy against brown spot of wild rice (*Zizania aquatica* L.) caused by *Drechslera oryzae*, *Bipolaris oryzae* and *D. sorokiniana* improved with adjuvants, with corresponding yield increases realized for all treatments that included an adjuvant. Stevens (1993) found that only 2% of publications on the use of organosilicone surfactant adjuvants were associated with fungicides or disease control.

Steurbaut (1993) also suggested azoxystrobin as a model contact protectant fungicide with certain systemic properties and a broad range of application in multiple host–pathogen systems, we demonstrated that adjuvants can significantly enhance absorption. Aero Dyne-Amic improved azoxystrobin absorption nearly fourfold in onion and potato compared with water. Bond improved azoxystrobin absorption more than four- and threefold in onion and dry bean, respectively.

According to Gent *et al.,* (2003) the successful adoption of adjuvants for fungal disease control would improve net returns to grown and simultaneously reduce the fungal pathogen pesticide load in the environment.

2.4. Effect of foliar fertilizer, nutrients and fungicides for control of anthracnose leaf spot pathogen under field trials

2.4.1. Effect of chemicals (foliar fertilizer, nutrients and fungicides)

2.4.1.1. Effect of foliar fertilizer and nutrients

In Malaysia, the use of combined spray applications of Mancozeb, the insecticide dicrotophos and foliar fertilizer gave excellent control of mango anthracnose and boosted fruit yields when used at intervals of 7-10 days from the beginning of flower bud formation (Kwee and Chong, 1985).

Foliar application cleaned the block of nutrient uptake and enriched the target organs (*viz.*, the foliage) directly with the appropriate amount of nutrients. There are previous lines of evidence that suggested that foliar application of nutrients were effective in reducing Alternaria leaf spot severity in tomato (Stevenson and Stewert, 1988 and Zitter and Wolfe, 1989).

Reuveni *et al.* (1997) reported that a single phosphate foliar application can induce high levels of systemic protection against powdery mildew caused by

Sphaerotheca fuliginea in Cucumber. A similar response was also found in maize, where foliar sprays with phosphates induced systemic protection against common maize rot caused by *Puccinia sorghi* and northern leaf blight (NLB) caused by *Exserohilum turcicum* (Farahat and Salama, 2012).

Systemic induced resistance (SIR) has been found to be induced by foliar sprays of nutrients such as phosphates, K and N. It has been hypothesized that during SIR an immunity signal released or synthesized at the induction site of the inducer leaf is systemically translocated to the challenged leaves, where it activates the mechanisms for defense (Reuveni and Reuveni, 1995).

Wiese *et al.* (2003) introduced the term chemically induced resistance (CIR), which is used to describe the systemic resistance after application of synthetic compounds. This resistance is proposed to be related to the formation of structural barriers such as lignification, induction of pathogenesis related proteins and conditioning of the plants. The ability of the N.P.K fertilizer to induce systemic resistance (SIR) could therefore be integrated with host resistance as an environment friendly alternative to reduce disease severity and also slow down the rate of development of pesticide resistant strain of pathogens.

Foliar application of phosphorus reduced potato tuber infection (Cooke and little, 2002). The severity of infection was caused by *R. solani* was reduced by the foliar application of Mikrosol U (4.0% weight N-NH_4, 2.8% Mg, 2.5% S and B, Cu, Zn, Fe, Mn, Mo), (Rebarz *et al.,* 2007) sulfate and elemental sulfur (Klikocha *et al.,* 2005).

Calcium is a vital macronutrient element for normal plant development taking part in copious physiological and biosynthesis processes (Barker and Pilbeam, 2007). With respect to calcium nitrate there are controversial reports on the impact of the element in suppressing plant diseases. Despite successful control of tip burn disease

of lettuce in the laboratory foliar application of calcium salts have not been effective in controlling the disease in the fields (Jules 1959; Corgan and Cotter, 1971). However, Walter (2008) indicated that calcium nitrate when used as a source of nitrogen in strawberry reduced the disease caused by *Botrytis cinerea*. Similarly Singh *et al.,* (2009) observed that application of calcium carbonate or calcium nitrate reduced *Phytophthora* stem rot of soyabean. Eryani-Raqeeb *et al.* (2009) indicated that calcium could be used as an alternative firming agent for fruit and that its usage decreased anthracnose disease of papaya fruits after harvesting.

Farahat and Nagwa Salama, (2012) foliar spray by solutions of 0.1 M of NPK and PK followed by K and NK fertilizers significantly reduced maize leaf blight disease severity. Nwobaga and Iwuagwu (2015) recorded higher level of percentage disease control due to application of foliar fertilizer though not better than the fungicide hexaconazole, in the management of fungal diseases of cucumber like anthracnose, powdery mildew and downy mildew.

2.4.1.2. Effect of fungicides

Emua and Fajola, (1983) evaluated five fungicides (captan, basic copper chloride, captafol, mancozeb, and Phaltan) for inhibition of conidial germination of two leaf spot diseases of cluster yam caused by *Cercospora contraria* and *Didymosphaeria donacina.* The fungicides were also evaluated after artificial infection in the greenhouse and natural infection in the field during two growing seasons. Four of the fungicides (captan, captafol, mancozeb, and Phaltan) were consistently effective in inhibiting conidial germination in the laboratory and inhibiting the diseases in the greenhouse and in the field.

Singh *et al.* (1989) when sorghum seeds were treated with vitavax (Carboxin) prior to sowing followed by six fungicidal sprays of dithane Z-78 (Zineb), topsin-M (Thiophanate methyl) and dithane M-45 (Mancozeb) to reduce the infection by *C.*

graminicola, the fungicides carboxin and zineb were found most effective followed by carboxin and topsin-M in reducing anthracnose disease. Dale *et al.*, (1999) found that Amistar (Azoxystrobin) at 125-250 mg ai/l provided longer disease protection than benomyl against anthracnose disease of chilli (*Colletotrichum capsici*).

The fungicide Manganese ethylenebisdithiocarbamate (Maneb) was recommended for anthracnose management in chilli was (Smith, 2000), although it did consistently control the severe form of anthracnose on chilli fruit. The strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio) were recently labeled for the control of anthracnose of chilli, but only preliminary reports are available on the efficacy of these fungicides against the severe form of the disease (Alexander and Waldenmaier, 2002; Lewis and Miller, 2003).

Nithyameenakshi *et al.* (2006) observe that the fungicides azoxystrobin and difenoconazole were generally non phytotoxic at or below the recommended dose for field application (2.2 µg a.i/ml) for control of *Gloeosporium ampelophagum* in grapevine, but at higher concentration, both the fungicides exhibited.

Sundaravadana *et al.* (2007) found that treating trees with azoxystrobin 8.3% w/w + mancozeb 66.7% w/w *viz.*, 1, 2 and 4 ml/l. concentrations provided 100 and more than 60 per cent reduction of panicle and leaf anthracnose compared to untreated mango trees where 27.73 and 53.68 PDI were recorded.

Cushman *et al.* (2007) reported that among spray treatments consisting of azoxystrobin/chlorothalonil, alone or in combination with potassium bicarbonate, foliar phosphite (0N–12.2P–21.6K), or foliar nitrogen (25N–0P–0K), all fungicide treatments reduced foliar diseases of pumpkin and significantly increased the yield from the crop.

Anand *et al.* (2010) reported that azoxystrobin treatment resulted in minimum chilli fruit rot incidence (3.75 per cent). The Mancozeb and Carbendazim treatments resulted in 10.72 and 12.02 per cent fruit rot incidence, respectively.

Adhikary *et al.* (2013) stated that the reduction of mango leaf anthracnose varied with the doses of azoxystrobin. At the time of final observation (*i.e.* 120 days after spraying) azoxystrobin at 25 and 50ppm concentrations slightly reduced the mango leaf anthracnose *i.e.* 50.60 per cent and 54.91 per cent disease reduction over control respectively whereas other high doses 100, 200, 300 and 400 ppm significantly suppressed the mango leaf anthracnose *i.e.* 71.26 per cent, 73.45 per cent, 74.64 per cent and 75.29 per cent disease reduction over control respectively.

Ajithkumar *et al.* (2014) stated that new combination fungicide UPF 509 (Azoxystrobin 8.3% + Mancozeb 66.7%) 75% WG was effective in reducing powdery mildew and anthracnose disease without exhibiting phytotoxic symptoms on the plants.

Ajithkumar *et al.* (2014) stated that the maximum pooled yield of 21.91 q/ha was recorded with UPF 509 (Azoxystrobin 8.3% + Mancozeb 66.7%) 75% WG (1800 g/ha) during two seasons. This management effect was mainly due to translaminar and systemic movement of azoxystrobin, inside the tissues, as azoxystrobin is widely distributed from the application site by diffusion (Vincelli and Dixon, 2002).

2.5. Effect of foliar fertilizer, nutrients and fungicides for control of anthracnose leaf spot pathogen under farmers' field trials

2.5.1. Effect of chemicals (foliar fertilizer, nutrients and fungicides)

2.5.1.1. Effect of foliar fertilizer and nutrients

A single phosphate spray induced a high level of systemic protection against powdery mildew caused by *Sphaerotheca fuliginea* in cucumbers (Reuveni *et al.,* 1993). Systemic induced resistance against powdery mildew was obtained on the leaves of 2, 3 and 4 of 5-leaf-stage cucumber plants in response to a foliar spray of phosphate on leaf 1 as early as 2 h before inoculation with conidial suspensions of S. *fuliginea.* The best protection was observed on leaf 2 and declined acropetally. Induced resistance was effective for a long time and at locations distant from the place of application. Direct or an indirect influence of the phosphate on conidial germination or development is not clear. The initiation of such signals by phosphate salts was proposed as an important factor in immunization of cucumbers against various diseases including powdery mildew (Gottstein and Kuc, 1989; Mucharromah and Kuc, 1991). A single spray of K_2HP0_4 on leaf 1, applied 2 or 4 days before inoculation, stimulated plant growth, regardless of disease (Reuveni et *al.,* 1993).

Nwogbaga and Iwuagwu, (2015) reported that the farmers use fungicides for the management of cucumber fungal diseases. Fungicide (Hexaconazole 5% SC) was applied at the rate of 1.140 liters/ha, while the N.P.K foliar fertilizer was applied at the rate of 19.181kg/ha. Each treatment was applied three times at 15 days intervals starting from three weeks after plating till the commencement of fruiting.

Nwogbaga and Iwuagwu, (2015) also observed that although NPK foliar fertilizer controlled fungal diseases of cucumber (*Cucumis sativus*) though not better

than the fungicide, Hexaconazole, the application of the foliar fertilizer gave higher marginal revenue (B:C ratio) compared to the fungicide.

2.5.1.2. Effect of fungicides

Azoxystrobin (Amistar 25 SC) possess a novel biochemical mode of action and its fungicidal activity results from the inhibition of mitochondrial respiration in fungi. This is achieved by the prevention of electron transfer between cytochrome b and cytochrome c. Because of its novel mode of action, azoxystrobin is effective against pathogens which have developed reduced sensitivity to other fungicides (Hewitt, 1998). Azoxystrobin was found effective against powdery mildew of sweet cherry at Oronda (Grover and Boal, 1998).

Azoxystrobin proved its effectiveness in checking powdery mildew and downy mildew of summer squash and muskmelon, respectively and was found effective against metalaxyl resistant strains of *Phytophthora infestans* (Mont.) de Barry. The compound appeared to be effective against *Fusarium moniliforme* Sheld. (Sheath rot of rice), *Helminthosporium oryzae* (brown leaf spot) and *Aspergillus niger* Van Tieghem (collar rot of groundnut) (Thind *et al.*, 2002).

The effectiveness of azoxystrobin against *Pythium aphanidermatum* (Eds.) Fitz. in cucumber root rot (Utkhede and Bogdanoff, 2003), *Claviceps africana* McRao in sorghum ergot (Prom and Isakeit, 2003) and *Alternaria alternata* (Fr.) Keissler in apple for moldy rot disease were also studied earlier. The fungicide azoxystrobin provided an effective control of downy mildew and powdery mildew diseases of grapevine (Schwartz and Gent, 2005).

Anand *et al.* (2010) conducted field experiments during March–June, 2004 in the farmer's holding at Kaveripattinam, Krishnagiri Tamil Nadu, India with the variety PKM-1 of tomato to study the bio-efficacy of azoxystrobin against leaf blight

and leaf spot diseases. Spraying of azoxystrobin at various doses *viz.*, 31.25, 62.50 and 125 g a.i. ha^{-1} revealed that 125 g a.i. ha^{-1} (500 ml ha^{-1}) recorded only 3.90 and 4.86 per cent disease index (PDI) of leaf blight and 0.00 and 2.42 (PDI) of leaf spot and the same treatment also recorded the higher yield of 27.60 and 26.30 tonnes ha^{-1} in the first and second season, respectively. No phytotoxic effect of azoxystrobin was observed in both the field trials of tomato even at four times the recommended doses of 125 g a.i. ha^{-1}.

Ajithkumar *et al.* (2014) In an experiment conducted for management of anthracnose leaf spot disease, two seasons pooled data revealed that minimum PDI (6.67) was noticed in treatment with the foliar sprays of UPF 509 (Azoxystrobin 8.3% + Mancozeb 66.7%) 75% WG at 1800 g/ha against chilli anthracnose disease with the maximum yield (21.91q/ha.) which was significantly superior to all other treatments.

2.6. Economic analysis

Nwogbaga and Iwuagwu, (2015) stated that the in assessment of the economic benefit of fungicide and foliar fertilizer application the gross margin (net revenue) was higher in application of N.P. K foliar fertilizer (₦ 76,200) compared to the fungicide application Hexaconazole (₦ 46,400). This was because N.P. K foliar fertilizer gave higher yield (480 kg/ha) compared to the fungicide (320kg/ha), even though the total variable cost (TVC) was almost the same. The marginal revenue (cost-benefit ratio) was equally higher in N.P.K foliar fertilizer (1:4.8) compared to the fungicide (1:3.4). This showed that for every ₦ 1.00 invested in foliar fertilizer application, a return of ₦ 4.80k could be expected, compared to fungicide application where for every ₦ 1.00 invested gave a return of ₦ 3.40k.

2.7. Microbial population of the rhizosphere and phyllosphere of the plants

Bertelsen *et al.* (2001) studied the effects of the fungicides azoxystrobin (a strobilurin) and epoxiconazole (a sterol biosynthesis inhibitor) on phyllosphere fungi, senescence and yield in winter wheat in field trials.

Diedhiou *et al.* (2004) showed that azoxystrobin application as a soil drench inhibited mycorrhizal activity and its use as foliar fungicide reduced total phylloplane yeast population considerably (Buck and Burpee, 2002). Contradictory report by Bending *et al.* (2007) indicated that use of azoxystrobin had mild effects on soil dehydrogenase activity and did not significantly affect overall fungal community structure in the soil types investigated.

Foliar fungicides applied as spray may result in high proportions of the fungicide being deposited in the soil. Up to 55 per cent of sprayed fungicides can be deposited in soil especially if applied in the early growth stages of crop cultivation and with reduced crop cover (Jensen and Spliid, 2003). Fungicides like azoxystrobin had been reported to inhibit both mycelia and spore germination of fungi in the rhizosphere (Bartlett *et al.*, 2002; Slawecki and young, 2002; Demirci *et al.*, 2003 and Ma and Michailides, 2004).

Rhizosphere microorganisms can play critical roles in suppressing soil borne diseases through a variety of mechanisms such as nutrient competition, antagonism, and parasitism. Exploring rhizosphere microbial diversity related to plant species and genotypes, therefore, is another approach to understanding soilborne disease incidence and severity (Broeckling *et al.*, 2008; Garbeva *et al.*, 2004; Yao and Wu, 2010).

Karlsson (2014) observed that fungicide-use was associated with moderate but significant changes in fungal community composition on wheat leaves and

advocated further research to identify the mechanisms behind fungicide-fungi interactions in the phyllosphere of agricultural crops. Identification of the interactions between pathogenic and saprotrophic phyllosphere fungi and management practices has the potential to guide the development of sustainable disease control strategies.

2.8. Studies on induction of defense mechanism

Systemic resistance mechanisms are induced in crop plants by treatment with chemical inducers such as isonicotinic acid (INA), benzothiadiazole (BTH), probenazole and salicylic acid (SA) (Kessman *et al,* 1994; Gorlach *et al,* 1996; Pieterse *et al,* 1998; De Meyer *et al,* 1999; Sakamoto *et al.,* 1999). Chemical fungicides often induce systemic resistance to pathogens in plants. In leaves treated with probenazole and inoculated with *Pyricularia oryza* Cav., the activity of PO, PPO, PAL, tyrosine ammonialyase and catechol-o-methyl transferase was higher than in untreated and or uninoculated leaves. Induced resistance has been well documented and has been found in many plant taxa (Karban and Baldwin, 1997).

Reuveni and Reuveni (1995) investigated possible use of foliar fertilizer NPK as agents of induction of systemic protection. A single phosphate spray induced a high level of systemic protection against powdery mildew caused by *Sphaerotheca fuligena* in cucumber (Reuveni *et al.,* 1993). The initiation of signals by phosphate salts was proposed as important factor in immunization of powdery mildew (Muchamurah and Kuc, 1991). Post-inoculation of PO_4 on the first leaf of cucumber plants induced systemic protection against powdery mildew on upper leaves when it was sprayed 4 days after inoculation. In a study by Descalzo *et al.* (1990), PO_4 was sprayed 1 and 2 weeks before challenge inoculation and was ineffective in inducing systemic resistance against powdery mildew in cucumber plants.

Kalim *et al.* (2000) reported that there was increase in activity of PPO in roots of plants raised from seeds treated with the systemic fungicide carbendazim. They

also observed greater induction of defence related compound phenols in plants raised from seeds treated with the systemic fungicide carbendazim.

Sendhil vel (2003) observed that activity of the activity of PAL, PPO, β-1, 3 glucanase, SOD and total phenols was higher in azoxystrobin treated grapevine plants. Hewitt (1988) had indicated that the disease controlling effect of azoxystrobin was caused by host mediated reaction. The accumulation of PAL in tomato leaves treated with Fosetyl-Al to control *Fusarium* wilt has also been reported (Bompeix *et al.,* 1981).

Defensive enzymes and phenolics are among the most influential and widely distributed products in the plants. Biochemical parameters, *viz.* PAL, PPO, SOD activities, phenols and sugars were reported in plants treated with various biotic and abiotic inducers. Induced systemic resistance is a phenomenon whereby resistance to infectious disease is systemically induced by localized infection or treatment with microbial components or products or by a diverse group of structurally unrelated inorganic or organic components (Tosun *et al.,* 2007). Phenylalanine ammonia lyase (PAL) is the key enzyme catalyzing the biosynthesis of phenolics and lignin from the aromatic amino acid phenylalanine (Cartea *et al.,* 2010). The involvement of phenols in plant disease resistance is based on their cytotoxicity, which is associated with their oxidation products. It has been claimed that the first stage of the defense mechanism of plants involves rapid accumulation of phenols at the infection site, which function to slow down the growth of the pathogens.

2.8.1. Phenylalanine ammonia lyase (PAL)

Phenyl propanoid metabolism starts with the conversion of L-Phenylalanine into transcinnamic acid by PAL and supplies the precursors for flavanoid pigments, lignin and phytoalexins (Massala *et al.*, 1980; Hahlbrock and Scheel, 1989). The accumulation of PAL in tomato leaves treated with Fosetyl - Al to control *Fusarium*

wilt has also been reported (Bompeix *et al.,* 1981). Increase in PAL activity subsequently increases the phenolic contents leading to disease resistance (Klessig and Malamy, 1994). PAL is one of the key enzymes in the phenylpropanoid and the flavonoid pathway where it was increased in both incompatible and compatible interactions between plants and pathogens. Also, O'Neill and Saunders (1994) demonstrated that the existence of phenolic phytoalexins in cucumbers may be produced through a PAL pathway. PAL catalyses the conversion of phenylalanine to trans-cinnamic acid, a key intermediate in the synthesis of salicylic acid (Ryals *et al.,* 1996). PAL is the first enzyme involved in the phenylpropanoid pathway which plays a significant role in regulating the accumulation of phenolics, phytoalexins, and lignin, the three key factors responsible for disease resistance (Vidhyasekaran *et al.,* 1997).

Archana *et al.* (2011) observed that application of fungicide enhanced the PAL activity in different treatments. The PAL activity was induced from first day of inoculation and reached maximum at three days after inoculation with *Plasmopara viticola.* Among the treatments, PAL activity was maximum with azoxystrobin 23SC and was almost statistically on par with *Pseudomonas fluorescence* sprayed seedlings. There were almost two fold increases in PAL activity in the above treatment over pathogen inoculated and healthy control.

2.8.2. Peroxidase (PO)

Peroxidase is one of the key enzyme involved in phenyl propanoid pathway and it is associated with disease resistance in plants (Hammerschmidt *et al.*, 1982). The products of the enzyme in the presence of hydrogen donor and hydrogen peroxide have antimicrobial activity (VanLoon and Callow, 1983). Peroxidase (PO) is a component of an early response in plants to pathogen infection and plays a major

role in the biosynthesis of lignin which limits the extent of pathogen spread (Bruce and West, 1989).

Bradley *et al.* (1992) reported that increased PO activity has been correlated with resistance in many species including barley, cucurbits, cotton, tobacco, wheat and rice and these enzymes are involved in the polymerization of proteins and lignin or suberin precursor into plant cell wall, thus constructing a physical barrier that could prevent pathogen penetration of cell walls or movement through vessels. Native PAGE analysis showed the presence of the three isoforms (PO1 to PO3) of peroxidase in all treatments except healthy control.

Vidhyasekaran *et al.,* (1997) reviewed that the peroxidase activity was more in the plants over infection by the pathogens in some plants and it has great role in inhibit the pathogen development. Peroxidase contributes to resistance by oxidation of phenolic compounds in cotton was reported by Martinez *et al.* (1996). Maximum reduction in disease was noticed in the cotton plants due to treatment with *P. fluorescens* and *T. harzianum* followed by *B. subtilis*. The disease reduction might be attributed to the suppression of the activity of pathogen in the host and soil by the antagonists through their over colonization (Cook, 1991).

Peroxidase activity is widely related to the polymerization of phenolic compounds, the deposition of lignin, and the cross-linking of phenolics to cell wall proteins. A lack of penetration by the fungus *Colletotrichum orbiculare* has been also observed in cucumber plants in which the expression of induced disease resistance to leaf infecting pathogen has been associated with the rapid modification of the outer epidermal cell wall of the host at the point of attempted infection: histochemical analysis have detected lignin deposits under the appressoria that have been not successfully penetrated (Hammerschmidt, 1999).

Archana *et al.* (2011) stated that the higher induction of peroxidase was observed in *P. fluorescens* treated followed by azoxystrobin 23 SC. The enzyme activity in azoxystrobin 23SC treated seedlings was statistically on par with the azoxystrobin 23 SC treated seedlings.

Vinothini *et al.* (2014) stated the peroxidase activity was maximum on 5th day with 0.789 absorbance in Azoxystrobin 8.3 % + Mancozeb 64.7 at 0.36 % concentration followed by Azoxystrobin 8.3 % + Mancozeb 64.7 at 0.30 percent and Azoxystrobin 8.3 % + Mancozeb 64.7 at 0.24 percent.

2.8.3. Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) usually accumulates upon wounding in plant. Biochemical approaches to understand PPO function and regulation are difficult because the quinine reaction products of PPO covalently modify and cross-link the enzyme. The increased activation of PPO could be detected in the cucumber leaf in the vicinity of lesions caused by some foliar pathogens. PPO was induced *via* Octadecanoid defense signal pathway (Constable *et al.*, 1995). Kalim *et al.* (2000) reported that there was increase in activity of PPO in roots of plants raised from seeds treated with the systemic fungicide carbendazim.

Archana *et al.* (2011) stated that the increase activity of PPO was observed in grapevine seedlings challenge inoculated with downy mildew pathogen. Application of *P. fluorescens* and azoxystrobin led to increase PPO activity up to the third day when compared to control. Induction of PPO oxidase in *P. fluorescens* treated seedlings was more and was followed by seedlings treated with azoxystrobin 23 SC. In the study it was inferred that grapevine seedlings inoculated with pathogen alone recorded comparatively less PPO activity.

Lima bean (*Phaseolus lunatus*) showed a high efficiency of PPO activity as defense against *Colletotrichum gloeosporioides* indicating a critical role of PPO in the defense response to *C. gloeosporioides* in this plant system (Ballhorn, 2011). PPOs largely stored in the thylakoid lumen until liberation from tissue damage or pathogen attack play a key role in the oxidation of intracellular phenolics to reactive quinones. These quinones are highly oxidizing and generate reactive oxygen species (ROS), serving as an effective defense to pathogens.

Vinothini *et al.* (2014) stated that native gel electrophoretic separation of crude poly phenol oxidase enzyme extracted from grapevine treated with Azoxystrobin 8.3 % w/w + Mancozeb 64.7 along with the standard fungicides, mancozeb 75% WP, azoxystrobin 23% SC, hexaconazole 2% SC and metalaxyl 8% + mancozeb 64% WP and *P. fluorescens* showed different poly phenol oxidase (PPO) isoform patterns. The intensity of PPO was more in Azoxystrobin 25 + Mancozeb at (0.36 per cent) when compared to other treatments after challenge inoculation with *P. viticola.*

Ahmed (2016) observed that the best inducer treatments in increasing the activities of PPO in cucumber plants were amistar fungicide (azoxystrobin), Potassium silicate and humic acid respectively while, the least effective treatment was propolis extract .

2.8.4. β -1,3-Glucanase activity

Peroxidase and ß-1,3-glucanase are related to the cross-linking of cell wall components, the polymerization of lignin and suberin monomers and the subsequent resistance to pathogens in several host pathogen interactions (Reuveni and Reuveni, 1995).

Jacops *et al.,* (1999) reported that grape vine contain gene encoding at least for four different chitinase, three glucanase and two thaumatin like protein showed varying response to pathogen attack and ethylene treatment.

Glucanases are lytic enzymes that hydrolyze β-1,3-glucans, one of the major components of the cell walls of fungi and bacteria (VanLoon *et al.,* 2006), and an increase in glucanase activity has been associated with apple resistance against fire blight (Brisset *et al.,* 2000) and induced resistance against bean rust (Borsato *et al.,* 2010).

Anand *et al.* (2007) stated that the effect of *P. fluorescens* and that of azoxystrobin on glucanase was similar 24 h after inoculation in cucumber. Activity peaked on the 3rd day after challenge inoculation with *Pseudoperonospora cubensis* and *Erysiphe cichoracearum.* It was higher in plants treated with *P. fluorescens*, and somewhat lower in plants treated with azoxystrobin.

Christopher *et al.* (2014) observed β-1,3-glucanase activity in the leaf samples of chilli at different days interval. Among various treatments, application of *P. fluorescens* (seed treatment @ 10g/kg + prophylactic spray @ 0.2% conc. at 25, and 75 DAT) + coir pith compost recorded the maximum induction of β-1,3-glucanase activity 76.7 µg of glucose released/ min/g of fresh tissue on 5th day after pathogen inoculation followed by 0.2% conc. of mancozeb which recorded 68.4 µg of Glucose/min/g of fresh tissue on 5th day after pathogen inoculation.

2.8.5. Super oxide dismutase (SOD)

Plant enzymes are involved in the defence reaction against plant pathogens. This include oxidative enzymes like chitinase, lipoxygenase (LPO), super oxide dismutase (SOD) and hydrogen peroxide (H_2O_2), which catalyse the formation of lignin and other phenols that contribute to the formation of defence barriers for

response of the plant cell against the entrance of foreign organisms (Garmendia *et al.*, 2006).

Elicitation of plants with elicitor molecules results in the activation of a series of defense responses, including cell wall reinforcement by deposition of lignin and induction of an array of defense enzymes (Desender *et al.*, 2007). Fang *et al.* (2012) reported increased SOD activity from ~80% to ~200% of the control during the first 72 HAI in strawberry (*Fragaria ananassa*) leaves infected with *Colletotrichum fragariae.*

Ahiladevi and Prakasam, (2013) observed that induction of systemic resistance by defence and antioxidising enzymes in chilli plants against anthracnose and powdery mildew disease by azoxystrobin 25 SC and bio-agents. The defence and antioxidizing enzyme profile may provide information to understand the level of resistance offered by the chemical.

2.8.6. Phenol

Phenolics are fungitoxic in nature and increase the physical and mechanical strength of the host cell wall. Plant phenolics and their oxidation products such as quinones are highly toxic to invading fungi thereby offering resistance against a wide range of pathogens (Cahill and Mccomb, 1992). Some phenolics may act as signal molecules or antioxidants and thus induce resistance (Malamy *et al.*, 1990).

Sundravadana *et al.*, (2007) stated that the plant contains low molecular weight antimicrobial compounds. Phenolics are one of the secondary antimicrobial compounds found in plants. Phenolics were directly toxic to the fungal pathogens, namely, *Colletotrichum falcatum* Went and *Rhizoctonia solani* Kuhn (Ramesh Sundar *et al.* 2001, Kalim *et al.* 2003).

reinforcing the cell structure (Avdiushko *et al.*, 1993). The defence enzymes degrade the fungal cell wall and causes the lysis of fungal cell. The chitin and glucan oligomers released during degradation of fungal cell act as elicitor that elicit various defence mechanism in plants. When plants are infected by pathogens and insect pests various lipids breakdown to form the products is including C6 volatiles from linoleic acid and linolenic acid by sequential steps involving lipoxygenase, hydroperoxidelyase and hydroperoxide hydralase in the so called lipoxygenase pathway (Croft *et al.*, 1993).

Superoxide dismutase catalyzes the conversion of two superoxide anions and two protons to oxygen and hydrogen peroxide. Dismutation is a reaction in which two identical molecules are converted into different substances. Superoxide dismutase plays a pivotal role in protecting against oxygen toxicity. It has been assumed that SOD has a central role in the defense against oxidative stress (Scandalias, 1993).

Superoxide is a reactive substance that can cause the modification of cellular proteins, nucleic acids and lipids in membranes, and it is therefore toxic (Roskoski, 1996). Plants have endogenous defense mechanisms that can be induced in response to attack by insects and pathogens (Bostock *et al.*, 2001; Heil, 2001). It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. However superoxide dismutase activity did not confer protection against oxidative damage in salt-stressed cowpea leaves (Cavalcanti *et al.*, 2004).

The induction of reactive oxygen species (ROS) scavenging enzymes, such as SOD and POD, is the most common mechanism for detoxifying ROS synthesis during plant-pathogen interactions. The induction of SOD can be a protective

Vinothini *et al.* (2014) stated that the plants sprayed with Azoxystrobin 8.3 % + Mancozeb 64.7 at 0.36 per cent found to have maximum phenol content (0.820 absorbance) after 15 days of inoculation followed by Azoxystrobin 8.3 % + Mancozeb 64.7 at 0.30 per cent (0.814 absorbance) which were on par.

2.9. Physiological parameters

2.9.1. Effect of nutrient content

Nutrients can affect disease resistance or tolerance. Disease resistance of the host is its ability to limit the penetration, development and reproduction of the invading pathogens (Graham and Webb, 1991).

Reuveni *et al.* (1997) stated that the effectiveness of foliar fertilization is limited by a number of factors, including nutrient-specific element type and degree of mineral uptake, or inability to supply the required amounts (Karhadkar and Kannan, 1984). Foliar application of phosphorus can induce local and systemic protection against powdery mildew in cucumber, roses, wine grapes, mango and nectarines (Reuveni and Reuveni, 1995).

The increased potassium content in mulberry leaf was due to availability of adequate K in soil as well as through the foliar spray of nutrients. Further, the increase in K content may also be attributed to the increased nitrogen content of the leaves which had a synergistic effect on the K in the leaves (Shankar *et al.,* 1994). Increased N and P content were observed by foliar application of seriboost to mulberry (Singhvi *et al.*, 2000 and Raje Gowda *et al.*, 2000). Furuya and Umemiya (2002) reported that peach (*Prunus persica* Batsch) leaf treatment with urea appeared to be more effective in increasing N content than other inorganic forms of N.

K reduced the incidence of various diseases such as bacterial leaf blight, sheath blight, stem rot, sesamum leaf spot in rice, black rust in wheat, sugary disease in sorghum, bacterial leaf blight in cotton, cercospora leaf spot in cassava, tikka leaf spot in peanut, red rust in tea, cercospora leaf spot in mungbean and seedling rot caused by *Rhizoctonia solani* (Huber and Graham, 1999; Sharma and Duveiller, 2004; Sharma *et al.,* 2005).

Foliar nutrients are beneficial due to enhancement of the efficacy of plant protection chemicals. The synergistic effect of mineral nutrients when applied in combination with plant protection products has been shown in several investigations (Dordas, 2009).

2.9.2. Effect of nutrient use efficiency

Warncke, (2007) stated that the effective and economic nutrient management begins with an understanding of the nutrient requirements of the crops being grown and the nutrient status of the soil. The nutrient requirements may vary with management practices and with type or variety, especially with pumpkins and watermelons. Muskmelon, watermelon, pumpkin, and squash generally accumulate in the vegetation and fruit 145 to 160 lbs. nitrogen (N), 30 to 45 lbs. phosphate (P_2O_5) and 160 to 180 lbs. potassium (K_2O) per acre. The actual amount varies with yield. Cucumber tends to accumulate about half those amounts. In many fields, available P and K levels have been improved by past additions. This is especially true for P because it is relatively immobile in the soil. In sandy soils P may leach out of the root zone from fall to spring, so build up may be limited.

Fixen *et al.,* (2010) stated that the partial factor productivity (PFP) is a simple production efficiency expression, calculated in units of crop yield per unit of nutrient applied. It is easily calculated for any farm that keeps records of inputs and yields. It can also be calculated at the regional and national level, provided reliable statistics on

input use and crop yields are available. However, partial factor productivity values vary among crops in different cropping systems, because crops differ in their nutrient and water needs. A comparison between crops and rotations is particularly difficult if it is based on fresh matter yields, since these differ greatly depending on crop moisture contents (e.g. potato vs. cereals).

Thimmaiah, (2015) stated that the nutrient use and agronomic use efficiency was found highest in NPK+FYM at 7.5 t ha^{-1}+PGPR at 2 kg ha^{-1}+Frond compost at 3.75 t ha^{-1} as top dress at 25 DAT. The highest available NPK and physiological use efficiency was recorded in NPK+FYM at 7.5 t ha^{-1}+PGPR at 2 kg ha^{-1}+Vermicompost at 3.75 t ha^{-1} as top dress at 25 DAT. The highest partial factor productivity was recorded in NPK+FYM at 7.5 t ha^{-1}+PGPR at 2 kg ha^{-1}. Similarly, higher net returns (Rs. 66,728 ha^{-1}) was recorded in recommended NPK + FYM at 7.5 t ha^{-1} + PGPR at 2 kg ha^{-1} + Frond compost at 3.75 t ha^{-1} as top dress at 25 DAT.

Gagandeep, (2015) stated that nutrient management practices FYM and 100% N equivalent through organics recorded significantly taller plants (74.8 cm), leaf area (942.4 cm^2 $hill^{-1}$), number of effective tillers (26.3 $hill^{-1}$) and total dry matter accumulation (83.05 g $hill^{-1}$), grain yield (3580 kg ha^{-1}) and 1000 grain weight (22.2 g). However, it was on par with application of FYM and 100 % NPK through inorganics and FYM and 50 % N equivalent through organics and 50 % NPK through inorganics as compared to application of 100 % NPK through inorganics.

Vishwanath, (2015) stated that the quantum of yield increase was 21.62 per cent as compared to recommended dose of NPK (62.13 q ha^{-1}). Nutrient uptake by the crop also registered similar trend as that of growth and yield parameters with statistically higher uptake of N (85.82 kg ha^{-1}), P_2O_5 (30.51 kg ha^{-1}) and K_2O (50.58 kg ha^{-1}).

2.9.3. Pigment status of the leaves chlorophyll a, b and total chlorophyll

Azoxystrobin application had given greener appearance of leaves with longer green leaf duration compared to leaves treated with other fungicidal compounds (Dimmock and Gooding, 2002).

Pepler *et al.* (2005) reported that the chlorophyll content was mainly related to the maintenance of the green leaf area and the reduction of the disease incidence caused by fungicides (azoxystrobin). Treatments resulting low disease incidence and long leaf life period resulted in higher chlorophyll content. Previous studies revealed a reduction of the chlorophyll loss produced by strobilurin and azole application in wheat (Grossmann and Retzlaff, 1997; Jaleel *et al.*, 2006).

Dkhil *et al.* (2011) determined a significant increase of chlorophyll *a* concentration by 15.80 per cent in response to foliar treatment with 1g KNO_3 L^{-1} foliar treatment. Fungicide azoxystrobin alleviated the decrease of chlorophyll caused by drought in the leaves of wheat plant (Baranoviya, 2014). Eleiwa *et al.* (2012) stated that foliar application of foliar fertilizer (22% N; 21% P_2O_5; 17% K_2O with microelements) significantly increased concentration of chlorophyll *a,* chlorophyll *b* and carotenoids in potato leaves compared to control.

2.9.4. Effect on relative water content

Leaf water content expresses the relative amount of water present in the plant tissues, and is a good indicator of water balance (Yamasaki and Dillenburg, 1999).

Jiang *et al.* (2003) stated that the water requirement of any crop depends on the factor such as variety, growth stage, growth duration, growing season conditions and plant population as well as soil and climate factors and crop management practices.

Jadon and Shah (2012) stated that the relative water content in diseased bell pepper leaves decreased appreciably (7.59 per cent) as compared to healthy leaves.

Han *et al.* (2012) stated that the higher relative water content was observed in red peppers treated with the standard dose of the trifloxystrobin fungicide, but to a lesser extent than that of the trifloxystrobin SC-treated red peppers.

Grimmer *et al.* (2012) stated that the relative transpiration rate decreased with increasing disease severity, with the amount of decrease, varying substantially between pathosystems. These variations were due in some part to the type of trophic relationship between pathogen and host. At low levels of infection rusts in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.), increased transpiration compared with disease-free controls. At higher rust severities transpiration was reduced, but the reduction was smaller than expected based on the proportion of leaf area affected.

Agamy *et al.* (2013) stated that the infection of tomato plants with the pathogen led to a significant reduction in leaf relative water content by 10.51 per cent as compared to healthy control.

The most significant effect on increasing water use efficiency (WUE) was found following the application of azoxystrobin both in dry and wet variants. Besides, the negative impact of drought on yield was also mitigated mainly by application of azoxystrobin (Baranyiova and Karel, 2014).

2.9.5. Effect on epicuticular wax content

Yamada *et al.* (1965) stated that the specific penetration of urea is related to the loosening of chemical bonds of the cuticular membrane. The reduced uptake of mineral nutrients along with the leaf age is related to the environmental conditions determining an increase in amounts of the epicuticular waxes (Leece, 1978).

Świetlik and Faust (1984) and Reickenberg and Pritts (1996) concluded that absorption of urea by the leaves of most crops is greater and faster than that of inorganic N forms. This phenomenon is related to the fact that the cuticular membrane is 10 to 20 times more permeable to urea than to inorganic ions (Yamada *et al.,* 1965). Thus, penetration of urea molecules through the cuticular membrane is not driven by diffusion.

In plants, epicuticular waxes are located on the outermost part of cuticle (Walton, 1990). When microbes adhered to the epicuticular waxes, the waxes showed some important changes (Schonherr and Schmidt, 1982). It has been shown that wax components acted as allele chemicals by influencing fungal development (Carver *et al.,* 1990).

Podila *et al.,* (1993) indicated that the induction of appressorium formation by avocado wax is quite specific for *C. gloeosporioides,* indicating that there may be qualitative differences that exist between various anthracnose fungal conidia in their ability to perceive the right host.

Knoche *et al.* (1994) stated that foliar application of nutrient solutions causes salt concentrations on a leaf surface to be higher than those of soil solutions. Increased tolerance of the epidermis to high spray solution concentrations is caused by the presence of the wax layer and the cuticular membrane. Since most mineral nutrients passively diffuse into the epidermal cells, absorption depends on their concentrations on the leaf surface. They also stated that there was a strong correlation between nutrient concentration on a leaf surface and the rate of its uptake by the epidermal cells. However, elevated nutrient concentrations caused leaf injury leading to the reduction in nutrient absorption. According to Marschner (1995) such absorptions by damaged leaves is limited by the destruction of ectodesmata structures. Santos *et al.,* (2013) observed that a decrease in epicuticular wax intensity

on treating soybean plants with fungicide + amino acids; they concluded that fungicides reduce wax content.

2.9.6. Effect on stomatal frequency

Fisher and Walker (1955) also reported a higher P absorption by young apple leaves than that of old ones. Lower nutrient uptake by basal leaves was attributed to a decreased metabolic activity and/or a lower amount of ectodesmata on the surface of a leaf.

According to Hull (1970) the high dynamics of nutrient absorption by the lower leaf surface results from the presence of a thin layer of the cuticular membrane and large number of stomata.

Lindsey and Gudauskas (1975) suggested that stomata within chlorotic regions of maize leaves, following infection with *Maize dwarf mosaic virus* Will. & Alex. were less functional than those in greener areas of infected leaves. It was proposed that the reduction in chlorophyll content associated with infection was responsible for decreased stomatal conductance, since chlorophyll pigments are a prerequisite for stomatal opening in the light (Virgin, 1957).

Ahmed *et al.,* (1983) systemic fungicide benomyl upon growth and related activities of sunflower *(Helianthus annuus),* cotton *(Gossypium barbadense)* and cowpea plants *(Vigna sinensis)* were followed. It was generally found that, the changes in the total number of stomata, the number of closed stomata, in either upper or lower epidermis of variously treated plants, was always increased at the expense of open ones. Consequently the mean rates of transpiration of variously treated plants were found to be considerably lowered.

Amadi (1994) showed that stomatal distribution was denser on the abaxial (lower) leaf surface than on the adaxial (upper) leaf surface. Application of azoxystrobin in soybean reduced the water conductance through stomata closure resulting in lower rates of intercellular CO2, transpiration, and net photosynthesis. However, Nason, (2004) reported that azoxystrobin reduced photosynthesis regardless of the effect on stomata.

Materials and Methods

3. MATERIALS AND METHODS

The present study on "Integrated management of foliar fungal disease of culinary melon (*Cucumis melo* L. var. *acidulus* Naudin)" was conducted during the period 2013-2016 at College of Agriculture, Vellayani. The initial study comprising of survey was conducted in Kalliyoor and Venganoor panchayats near the College of Agriculture, Vellayani. Laboratory and greenhouse studies were conducted at Department of Plant Pathology and field experiments at Instructional Farm of College of Agriculture, Vellayani, in order to make a comparative evaluation of the efficacy of foliar application of fertilizer, micronutrient, bio-control agents and newer fungicide, to be included as promising components in the management of foliar fungal disease (Colletotrichum leaf spot) affecting culinary melon. Final confirmation field trials were laid out in farmers' field of Venganoor, Vavamoola and Venjaramoodu in Thiruvananthapuram district. The materials and methods followed in this study are described below.

3.1. Survey on the occurrence of foliar diseases in different culinary melon growing areas

A preliminary survey was conducted in ten different locations including College of Agriculture, Vellayani and cultivated fields in its neighbouring areas during September 2013 to December 2013, to assess the prevalence of major diseases affecting culinary melon in the fields. In the survey conducted by random sampling, culinary melon fields were selected from ten different locations located in Kalliyoor and Venganoor panchayats. During the survey, observations on incidence and severity of the prevalent diseases were also recorded from these ten selected culinary melon growing fields.

3.1.1. Diagnosis of plant diseases affecting leaf samples

From each of the selected areas consisting of more than 50 culinary melon plants, 25 plants were chosen at random for collecting five disease leaf samples per plant, at 10 days' interval. Laboratory studies were conducted with the collected leaf samples to diagnose the diseases prevalent during the period of survey by studying the nature of symptoms in comparison with the plant disease diagnostic keys (James, 1971). Leaf specimens obtained from infected culinary melon plants collected from the surveyed locations were also examined under the microscope for identifying spores or fruiting bodies associated with the pathogens of fungal diseases.

3.1.2. Incidence and severity of diseases observed in the leaf samples collected

Incidence and severity of diseases affecting culinary melon plants in the field, as diagnosed in 3.1.1, were assessed separately, according to the type of disease affecting the plants.

3.1.2.1. Incidence of disease

The incidence of disease was recorded according to James, (1974) and Abdel-Kader *et al.,* (2012) as follows:-

$$\text{Disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants observed}} \times 100$$

3.1.2.2. Disease Index

Disease index denotes the percentage of relevant host tissue (or) organ covered by symptom (or) lesion damaged by the disease and it depends on number and size of the lesions present on the infected part (Sharma *et al.,* 2005).

Disease index (severity) was calculated for respective disease based on the score assigned to each disease.

Score chart for anthracnose leaf spot (Mayee and Dattar, 1986)

Disease scale	Percent leaf area affected
0	No visible symptoms
1	<1% leaf area affected
3	1-10% leaf area affected
5	11-25% leaf area affected
7	26- 50 % leaf area affected
9	>50% leaf area affected

Score chart for downy mildew and powdery mildew - Jamadar and Desai (1997)

Disease scale	Percent leaf area affected
0	No infection
1	0-10% leaf area affected
2	10.1-30% leaf area affected
3	30.1- 60% leaf area affected
4	60.1- 80% leaf area affected
5	80.1-100% leaf area affected

Disease index formula (McKinney, 1923).

$$\text{Per cent Disease Index} = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

3.1.3. Isolation and identification of pathogen inciting anthracnose leaf spot of culinary melon

After conducting survey and assessment of the major diseases affecting culinary melon in the field, subsequent studies were undertaken mainly on anthracnose leaf spot affecting the crop.

Leaves exhibiting typical symptoms of anthracnose leaf spot were collected from the surveyed areas and diagnosed as described in 3.1. The pathogen inciting the disease was then isolated from leaf specimens as indicated below:

Typical specimens of anthracnose leaf spot were cut into small fragments ($1cm^2$) and surface-sterilized in 0.1 percent mercuric chloride for 30 sec. They were then washed three times in sterile distilled water, plated on potato dextrose agar (PDA) medium containing streptomycin sulphate and incubated at room temperature (28±2°C) for seven days. Fungal isolates exhibiting typical characters of anthracnose pathogen that were consistently obtained during the isolation from the diagnosed disease specimens were transferred to PDA slants and stored at room temperature (28±2 °C) for conducting subsequent studies.

3.1.4. Pathogenicity test

Pathogenicity tests of the isolates obtained and maintained were conducted for proving Koch's postulates, as follows

Artificial inoculation of intact leaves of thirty days old potted plants of culinary melon (cv. Vellayani local)

Pathogenicity tests were also conducted on thirty days old potted plants of culinary melon (cv.Vellayani local). Seedlings of culinary melon were transplanted from potting mixture comprising of river sand, farm yard manure and soil in the proportion of 1:1:1. After thirty days of growth, culinary melon plants with four to five leaves, were artificially inoculated by pin prick method on adaxial side with mycelial culture of each isolate obtained from different locations. Three plants inoculated without the pathogen were maintained as control. Second and third fully opened leaves from bottom were selected for inoculating on the upper (adaxial) side of leaf after wounding. A thin layer of moist cotton was placed over the wounded area. Humidity was maintained over 90 per cent for first 5 days by spraying water.

After typical symptoms of the disease were expressed on the inoculated leaves, the pathogen was re-isolated and the morphological characters were compared with those of the respective isolate. Size of the lesions that appeared on each inoculated leaf was estimated by measuring the area which was expressed in cm^2. Virulence rating was also assessed by a scale formulated visually on the basis of the lesion size observed.

3.1.4.1. Observation of symptom development in pathogenicity test

Each inoculated leaf of potted plant of culinary melon (cv. Vellayani local) was observed for appearance of symptoms from 24 hr after inoculation and continued up to 12 days. Nature and development of symptoms of inoculated leaves were carefully studied and classified into different stages.

3.1.5. Screening of virulent isolate of the pathogen by estimation of incubation period (IP) and disease development time (DDT)

Among the typical isolates of the pathogen obtained from anthracnose leaf spot specimens, the most virulent one was screened based on incubation period (IP) and disease development time (DDT) as calculated below after artificially inoculating each isolate on four plants. This virulent isolate screened was used for conducting subsequent studies.

Incubation period (IP- time measured in days between inoculation and the appearance of first lesion and disease development time (DDT- time measured in days between inoculation and the appearance of mature lesion) were determined for each of the fungal isolate (Te beest *et al.,* 1977) and the most virulent isolate was screened based on the shortest IP and shortest DDT recorded for the isolates.

3.1.6. Morphological and cultural studies of the virulent isolate of the pathogen

The virulent isolate that was screened and further identified in the Department of Plant Pathology, College of Agriculture, Vellayani, based on the morphological and cultural characters. The culture of the virulent isolate grown on PDA was examined for morphological characters by observing the colour, septation and width of the hyphae of the pathogen. The culture was also examined for the presence of spores, and if present their colour, size, shape and septation were also observed and recorded.

Cultural characters of fungal growth on PDA were studied by observing the texture as well as colour of hyphal colonies from both upper and reverse sides of the Petri plates. Diameter of the fungal colony and time taken for the fungal colony to completely cover the surface of agar medium in the Petri plate were also recorded.

3.1.7. Molecular characterization of isolates of the pathogen by DNA sequencing using universal primers of ITS

After studying the morphological and culture characters of the virulent isolate of anthracnose leaf spot pathogen, molecular characterization was also conducted by performing ITS sequencing of the isolate, at the National Fungal Culture Collection of India, Pune, in order to confirm the identity of the pathogen. The procedure adopted for molecular characterization of the isolate was as follows:

3.1.7.1. DNA isolation using GenElute Plant Genomic DNA Miniprep Kit (Sigma)

The tissue/mycelium (about 50 mg) was transferred to a micro-centrifuge tube and ground in 350 µl of lysis solution A and 50 µl of lysis solution B using a micro pestle. The mixture was incubated at 65°C for 10 min with occasional inversion.

Precipitation solution (130 µl) was added to the mixture, mixed completely by inversion and the sample was placed on ice for 5 min.

The sample was centrifuged at 14,000 rpm (Eppendorf Centrifuge 5804 R) for 5 min. to pellet the cellular debris, proteins, and polysaccharides. The supernatant was transferred to the GenElute filtration column tube and centrifuged at 14,000 rpm for 1 min. This removed any cellular debris not removed in the previous step. The filtration column was discarded and 700 µl of binding solution was added directly to the flow through liquid and mixed thoroughly by inversion. 700µl of this mixture was added into GenElute nucleic acid binding column and centrifuged at 14,000 rpm for one min.

The flow through liquid was discarded and the collection tube was retained. The column was returned to the collection tube and the remaining sample was applied to the column. Centrifugation was repeated as above and the flow through liquid and the collection tube were discarded. The binding column was placed into a fresh 2 ml collection tube. 500 µl ethanol-added wash solution was added to the binding column and centrifuged at 14,000 rpm for one min. The flow through liquid was discarded and the collection tube was retained. The wash was repeated once more.

The binding column was transferred to a new collection tube. 30 µl of elution solution (pre-warmed to 65°C) was added to the binding column and centrifuged at 14,000 rpm for one min. The stock DNA was properly labelled and stored at 4 °C.

3.1.7.2. Agarose Gel Electrophoresis for DNA Quality check

The quality of the DNA isolated was checked using agarose gel electrophoresis. One µl of 6 X 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 0.5 X TBE (Tris-Borate-EDTA) buffer containing 0.5

µg/ml ethidium bromide. Electrophoresis was performed with 0.5 X 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 trans illuminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.1.7.3. PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1 X PCR buffer (100 mM Tris HCl , pH-8.3; 500 mM KCl), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 2.5 mM $MgCl_2$, 20 ng DNA, one unit of AmpliTaq Gold DNA polymerase enzyme, 0.1 mg/ml BSA and 4 % DMSO, 5 pM of forward and reverse primers.

3.1.7.4. 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).

3.1.7.5. PCR amplification profile

3.1.7.5.1. ITS & LSU

95 °C	-	5.00 min	
95 °C	-	0.30 min	}
58 °C	-	0.40 min	} 40 cycles
72 °C	-	1.00 min	
72 °C	-	5.00 min	
4°C	-	∞	

3.1.7.6. Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 5 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromo phenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 2-log DNA ladder (NEB). The gels were visualized in a UV trans illuminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.1.7.7. Exo SAP-IT Treatment

Exo SAP-IT (GE Healthcare) consisted of two hydrolytic enzymes, Exo nuclease 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 µl of PCR product was mixed with 2 µl of Exo SAP-IT and incubated at 37°C for 15 min followed by enzyme inactivation at 80°C for 15 min.

3.1.7.8. Sequencing using Big Dye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Bio systems) using the Big Dye Terminator v3.1 Cycle sequencing Kit (Applied Bio systems, USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (Exo SAP treated)	- 10-20 ng
Primer	- 3.2 pM (either 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 1st cycle at 96°C for two min followed by 30 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C for four min for all the primers.

3.1.7.9. Post Sequencing PCR Clean up

1. Mastermix I of 10 µl milli Q and 2 µl 125 mM EDTA per reaction were made.

2. 12 µl of master mix I was added to each reaction containing 10 µl of reaction contents and were properly mixed.
3. Master mix II of 2 µl of 3 M sodium acetate pH 4.6 and 50 µl of ethanol per reaction were made.
4. 52 µl of master mix II was added to each reaction.
5. Contents were mixed by inverting.
6. Incubated at room temperature for 30 min
7. This was centrifuged at 14,000 rpm for 30 min
8. The supernatant was decanted and added 100 µl of 70% ethanol
9. This was centrifuged at 14,000 rpm for 20 min.
10. The supernatant was decanted and 70 % ethanol wash was repeated
11. The supernatant was decanted and the pellet was air-dried.

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

3.1.7.10. Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Bio systems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al.*, 2010).

3.2. Management of anthracnose leaf spot disease of culinary melon

The identified most virulent isolate of the pathogen inciting anthracnose leaf spot was used for conducting experiments related to the management of the disease, in laboratory as well as under greenhouse conditions.

3.2.1. *In vitro* evaluation of foliar fertilizer, nutrient, bio-control agents and fungicides against anthracnose leaf spot pathogen

A few selected chemicals and bio-control agents were evaluated for their efficacies in inhibiting the growth of the most virulent isolate of pathogen screened in 3.2.

Among the chemicals, efficacies of two fungicides *viz.* azoxystrobin (0.15 ml/l) and mancozeb (0.4 per cent) and two nutrient NPK 19:19:19 (0.5 per cent) and calcium nitrate (5g/l) and two bio-control agents (fungal antagonist *Trichoderma viride* and bacterial antagonist *Pseudomonas fluorescens*) were evaluated by poisoned food technique, in the laboratory of Department of Plant Pathology, College of Agriculture, Vellayani.

3.2.1.1. a) Assay of foliar fertilizer, nutrient, bio-control agents and fungicides on growth inhibition of the pathogen by poisoned food technique (Nene and Thapliyal, 1993)

The nutrients NPK 19:19:19 (0.5 per cent) and calcium nitrate (5g/l), talc based formulations of bio-control agents *Trichoderma viride* (two per cent), *Pseudomonas fluorescens* (two per cent) and the fungicides azoxystrobin (0.15 ml/l) and mancozeb (0.4 per cent) were evaluated for inhibition of mycelial growth of the most virulent isolate of pathogen by following the poisoned food technique (Adhikary *et al.,* 2013) as described below

Details of laboratory study

Design : CRD

Replication : 3

Treatments : 8

Treatments

T1 - 0.5 per cent (19:19:19 NPK)

T2 - 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb @ 0.4 per cent)

T3 - 0.5 per cent (19:19:19 NPK) + newer fungicide (azoxystrobin @ 0.15ml/l)

T4 - 0.5 per cent (19:19:19 NPK) + calcium nitrate (5g/l)

T5 - Calcium nitrate (5g/l)

T6 - Two per cent *Pseudomonas fluorescens*

T7 - Two per cent *Trichoderma viride*

T8 - Absolute control

Molten potato dextrose agar (PDA) was separately mixed with respective concentration of fungicide *viz.*, mancozeb (0.4 per cent) and azoxystrobin (0.15 ml/ l), foliar nutrients *viz.*, NPK 19:19:19 and calcium nitrate (0.5 per cent) and bio-control agents *viz.*, talc based formulations of *Trichoderma viride* (two per cent) and *Pseudomonas fluorescens* (two per cent) and their combinations, poured into sterilized Petri plates and allowed to solidify. A disc of 5 mm diameter of most virulent isolate grown on solidified PDA medium was placed aseptically at centre of each Petri plate amended with test nutrient, fungicides and bio-control agents and incubated at room temperature for ten days. Culture discs grown under same conditions on PDA without any amendment were maintained as control. The plates were incubated at room temperature (25 ± 2°C). Mycelial growth of the pathogen in each inoculated plate was recorded by measuring the colony diameter. Per cent inhibition of the pathogen over control was calculated by adopting the formula (Vincent, 1927)

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

Where,

I = per cent inhibition.

C = growth of pathogen in unamended medium.

T = growth of pathogen in amended medium.

3.2.1.2. b) *In vitro* assay of bio-control agents on growth inhibition of the pathogen by dual culture method (Huang and Hoes, 1976); (Skidmore and Dickinson, 1976); (Mishra, 2010)

Design : CRD

Replication : 7

Treatments : 3

The fungal and bacterial antagonists *viz., Pseudomonas fluorescens* and *Trichoderma viride* were also tested for inhibitory action against the virulent isolate of anthracnose leaf spot pathogen by dual culture technique (Mishra, 2010) using the culture of antagonists obtained from Department of Microbiology, College of Agriculture, Vellayani.

For evaluating the efficacy of the fungal antagonist, a mycelial disc of *Trichoderma viride* (5 mm diameter) was placed at 2.5 cm away from periphery of the Petri plate containing PDA. An agar disc of the pathogen of the similar size (5mm) was placed opposite to this, 2.5 cm away from the periphery. The plates were incubated at 25 ± 2°C and mycelial growth of the pathogen in each inoculated plate was recorded by measuring the colony diameter. A 5mm agar disc of the pathogen

placed 2.5 cm from periphery of Petri plate without the inoculation of any bio-control agent served as control.

Antagonistic activity of *P. fluorescens* was evaluated by streaking a twenty four hour old culture of the bacteria in a two cm line 1 cm away from the periphery of the Petri plate and at three equidistant points marked on the plates containing King's B (KB) medium. A mycelial disc of the pathogen (5 mm diameter) was placed at centre of these three equidistant streaks (Georgakopoulos *et al.* 2002). Plates were incubated at room temperature (25 ± 2°C) and mycelial growth of the pathogen in each inoculated plate was recorded by measuring the colony diameter. A mycelial disc of the pathogen (5 mm diameter) placed at edge of Petri plate containing KB medium, without the inoculation of bacterial antagonist, served as control.

In the above two experiments, a five mm agar disc of the pathogen placed at the 2.5 cm from periphery of Petri plate without the inoculation of any bio-control agent served as control.

Observation on the extent of mycelial growth of the screened isolate of the pathogen was recorded 24 h after inoculation and continued up to the period when the fungal growth completely covered the surface of agar medium in the Petri dish of control (Petri plate containing PDA without any bio-control agent). The diameter of the mycelial growth of the virulent isolate of the anthracnose leaf spot pathogen was measured in each Petri plate containing the respective treatments.

Per cent inhibition of the pathogen over control was calculated by adopting the formula (Vincent, 1927)

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

Where,

I = Per cent growth of inhibition.

C = Growth in control (measured as diameter of mycelial growth in cm)

T = Growth in treatment (measured as diameter of mycelial growth in cm)

3.2.1.3. Effect of foliar fertilizer, nutrient, bio-control agents and fungicides on germination of conidia of anthracnose pathogen (Hua-Youn Gang and Hua, 2001)

An experiment was conducted for estimating inhibition of conidial germination of the anthracnose pathogen by the different chemical and non-chemical treatments tested.

Conidial suspension (10^6conidia/ml) of the virulent isolate of the pathogen was prepared and incorporated with foliar fertilizer NPK 19:19:19 (0.5 per cent) separately and also in combination with calcium nitrate (0.5 per cent), azoxystrobin (0.15 ml/l) and mancozeb (0.4 per cent). The bio-control agents *viz.*, talc based formulation of the two bio-control agents (fungal antagonist *Trichoderma viride* (2%), bacterial antagonist *P. fluorescens* (2%) were also tested separately in the spore suspension of the pathogen.

Conidial suspension (0.1 ml) incorporated with each treatment was transferred at 5 minutes interval to separate cavity slides and incubated for 24 hr. in moisture chamber at 25°C. A drop of lactophenol cotton blue was then placed over conidial suspension on each of the cavity slides. The slides were observed under the microscope for recording the inhibition percentage of conidial germination of the pathogen (Imtiaj *et al.*, 2005).

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

Where,

I = per cent inhibition.

C = germination in control.

T = germination in treatment.

3.3. Evaluation of foliar fertilizer, nutrient, bio-control agents and fungicides for control of anthracnose leaf spot pathogen under greenhouse conditions

A pot culture experiment was conducted in completely randomized design (CRD) to evaluate the efficacies of foliar fertilizer, nutrient, bio-control agents and fungicides in the management of anthracnose leaf spot disease. The experiment was initiated during 2014 at College of Agriculture, Vellayani using the culinary melon cultivar Vellayani local, for the evaluation. The experiment was conducted for two different periods *viz.*, March to June 2014 and August to October 2014. Details of the experiment are presented below

Location : Department of Plant Pathology, College of Agriculture, Vellayani

Period : March to June 2014 and August to October 2014.

Variety : cultivar Vellayani local

Design : CRD

Replications : 3

Treatments : 12

T1 - Fertilizer application as foliar spray @ 0.5 per cent (19:19:19 NPK) + adjuvant

T2 - Fertilizer application as foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb @ 0.4 per cent) + adjuvant

T3 - Fertilizer application as foliar spray @ 0.5 per cent (19:19:19 NPK) + adjuvant followed by foliar spray of fungicide (mancozeb @ 0.4 per cent)

T4 - Fertilizer application as foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (azoxystrobin @ 0.15ml/l) + adjuvant

T5- Fertilizer application as foliar spray @ 0.5 per cent (19:19:19 NPK) + adjuvant followed by foliar spray of newer fungicide (azoxystrobin @ 0.15ml/l)

T6 - Fertilizer application as foliar spray @ 0.5 per cent (19:19:19 NPK) + calcium nitrate (5g/l) + adjuvant

T7 - Foliar spray of calcium nitrate (5g/l) + adjuvant

T8 - Foliar spray of *P. fluorescens* @ two per cent + adjuvant

T9 - Foliar spray of *T. viride* @ two per cent + adjuvant

T10- Farmers' practices of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted)

T11 - Cultivation practices according to POP (KAU) (2011)

T12 - Absolute control

Conidial suspension was inoculated by pinpricking method on the leaf surface of all the treated plants. Culinary melon plants maintained without any treatment and inoculated with the pathogen served as control.

The fungicides and bio-control agents were applied at 15 days interval starting from appearance of initial infection up to the harvest of the crop.

In treatments T1-T6, fertilizer application by foliar spray was given at 15 days interval. In each of the treatments organic manure was applied according to the POP recommendation of KAU (2011).

In treatments T7-T9, fertilizer and manures were applied according to the POP recommendation of KAU (2011).

3.3.1. Observations on disease incidence (DI) and percentage disease index (PDI)

Symptoms of anthracnose leaf spot disease that appeared on the artificially inoculated plants in the above experiment were recorded at 7 days intervals and percentage of disease incidence and disease index were calculated as described in 3.1.2.1 and 3.1.2.2.

3.3.1.1. Biometric observations

Biometric observations such as plant height (cm), number of fruits/plant, number of branches/plant, number of leaves/plant were recorded at the time of harvest of the crop. Observations were recorded from two observational plants in each pot.

3.3.1.2. Plant height (cm)

Height of the plant from the base to the growing tip at 30 days after sowing were taken from two observational plants. The mean plant height was calculated and expressed in cm.

3.3.1.3. Leaf length (cm)

Length of the leaf was measured from two observational plants, the average worked out and expressed in cm.

3.3.1.4. Leaf breadth (cm)

Breadth of the leaves was measured from two observational plants, the average worked out and expressed in cm.

3.3.1.5. Number of branches/plant

The total number of branches arising from the main stem of two observational plants was counted at the peak harvest stage and average was found out.

3.3.1.6. Number of leaves/plant

Number of functional leaves were counted at the time of observation and the average value recorded.

3.3.1.7. Number of fruits/plant

Number of fruits obtained from two observational plants were recorded and the mean calculated.

3.3.1.8. Pooled analysis for two seasons

Pooled analysis of both periods with respect to plant height (cm), number of branches/plant, number of leaves/plant, number of fruits/plant, disease incidence and disease index were recorded in all the treatments.

3.4. Evaluation of effective treatments in field experiments (two seasons)

A field experiment was conducted in randomized block design (RBD) to evaluate the more effective treatments that were screened from the pot culture studies. The experiments were conducted during January to March 2015 and April to June 2015 at College of Agriculture, Vellayani (8°26'0.143'' (N) Latitude and 76°59'16.623'' (E) Longitude). Seeds of culinary melon (cv. Vellayani local) were sown and two plants/pit were maintained for evaluation of the treatments (Fig. 1.).

3.4.1. Experiment details

Location : College of Agriculture,Vellayani

Season : January to March 2015 and April to June 2015

Variety : Cultivar Vellayani local

Design : RBD

Replication : 4

Gross plot size: 8m x 8m

Net plot size : 4m x 4m

Spacing : 2m x 2m

Treatments : 7

T1-T4: Most effective treatments evaluated in the pot culture study

T5-Farmers' practices of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted)

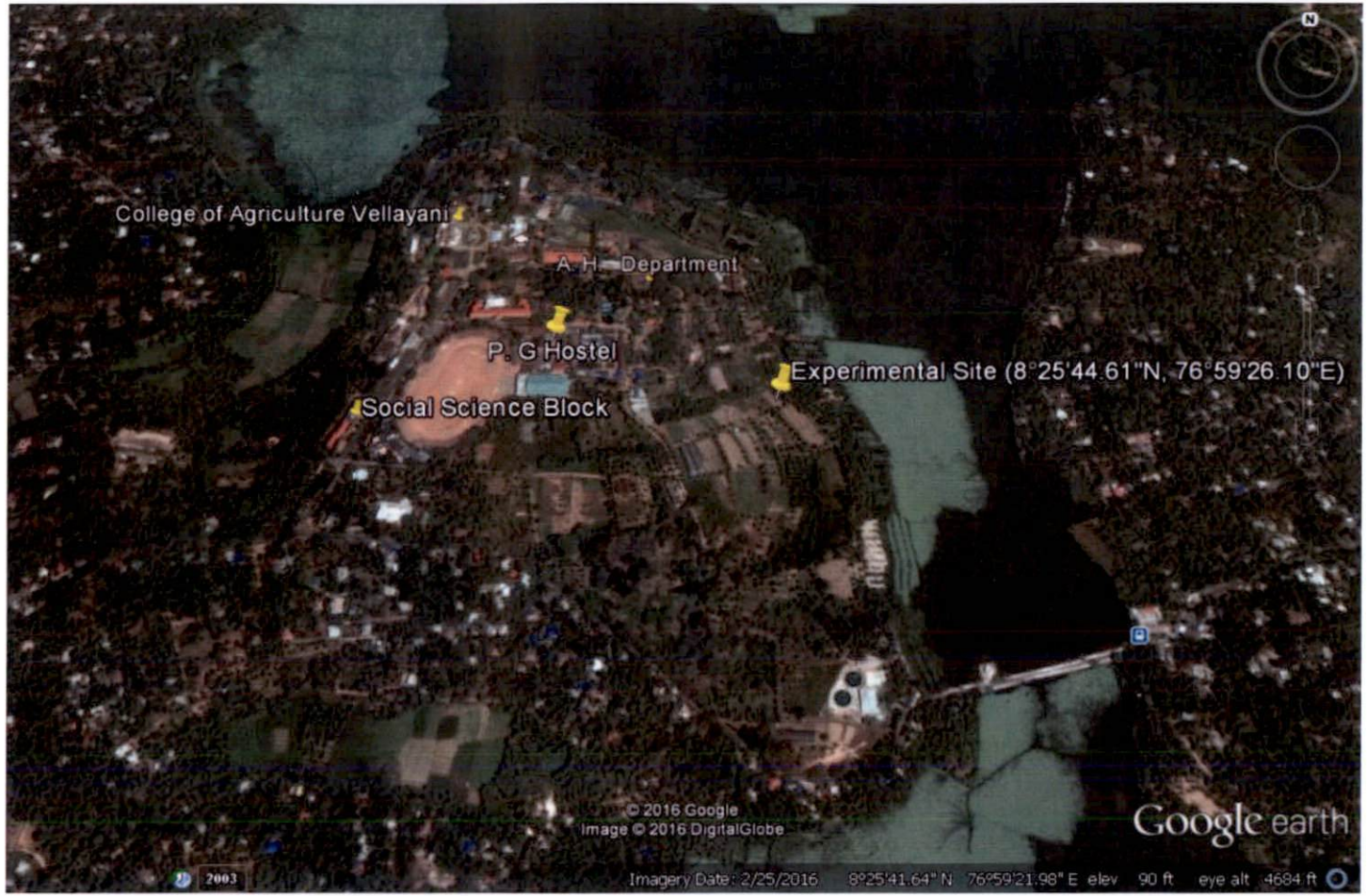


Fig. 1. GPS image of field experiment site

T6-Cultivation practices according to package of practices recommendations (POP), KAU (2011)

T7-Absolute control

3.4.2. Disease incidence (DI) and percentage disease index (PDI)

Observations were made on the natural incidence of anthracnose leaf spot disease that occurred on the plants. Symptoms of the leaf spot disease were recorded at seven days intervals and expressed as per cent disease index (PDI) and disease incidence (DI).

3.4.2.1. Biometric observations

The growth parameters *viz.*, length and girth of fruit (cm), fruit weight (g) and total yield per season were recorded in all the treatments.

3.4.2.2. Fruit Weight (g)

The weight of white and green mottled fruits obtained from two observational plants were recorded at each harvest. Mean weight of fruit was calculated and expressed in gram.

3.4.2.3. Length of fruit (cm)

Length of fruits obtained from two observational plants were recorded at each harvest. Mean length of fruit was calculated.

3.4.2.4. Breadth of fruit (cm)

Breadth of fruits obtained from two observational plants were recorded at each harvest. Mean breadth of fruit was calculated.

3.4.2.5. Total yield per season

The total weight of fruits/season obtained from two observational plants was recorded at each harvest. The total weight of fruits/ season from the harvests was worked out and the mean weight was calculated and expressed in kg.

3.4.2.6. Pooled analysis for two seasons

Pooled analysis of two seasons *viz.*, total yield per pit, disease incidence and disease index were recorded in all the treatments listed in 3.4.3.

3.5. Evaluation of effective treatments for the management of anthracnose leaf spot of culinary melon in the farmers' field trials

Two most effective treatments, screened in the two field trials conducted consecutively at Instructional Farm, College of Agriculture, Vellayani during January - March 2015 and April - June 2015, were evaluated in farmers' fields selected from three locations *viz.*, Venganoor (8°23'45.31''(N) Latitude and 77°0'12.67''(E) Longitude), Vavamoola (8°24'47.20''(N) Latitude and 76°59'46.60''(E) Longitude) and Venjaramoodu (8°41'23.03''(N) Latitude and 76°55'22.14''(E) Longitude). Seeds of culinary melon variety (cv. Vellayani local) were sown in each treatment plot maintaining four plants per plot. The following treatments were evaluated in each of the locations.

3.5.1. Details of the farmers' field trials

Design – RBD

Replication – 4

Variety – cultivar Vellayani local

Spacing – 2m x 2m

Plot size – 8m x 8m

Treatments – 5

Number of pits in field – 20

Five treatments of the experiment (including control) were as follows.

T1-T2: Most effective treatments screened from the field trials conducted at CoA, Vellayani.

T3-Farmers' practices of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted).

T4-Cultivation practices according to package of practices of (POP), KAU (2011)

T5-Absolute control

3.5.2. Disease incidence (DI) and percentage disease index (PDI)

Observations on the natural incidence of anthracnose leaf spot disease that occurred on the plants in the field trial were observed in three plants per pit. Symptoms of the leaf spot disease were recorded at seven days' interval and expressed as per cent disease index and disease incidence (Akem *et al.*, 2011).

3.5.2.1. Biometric observations

The growth parameters *viz.*, number of harvest, daily yield, average yield, total yield, and B:C ratio were recorded in all the treatments.

3.5.2.2. Number of harvests

Harvests of the fruits were made from 45 days after sowing in the field and subsequent harvests were made at seven days' interval. The fruits were picked with appearance of a whitish green colour.

3.5.2.3. Daily yield

The daily yield of fruits/pit obtained from three observational plants was recorded at each harvest. The daily yield of fruits/pit from each harvest was calculated and the mean weight expressed in kg.

3.5.2.4. Average yield

The average yield of fruits/pit obtained from three observational plants was recorded at each harvest. The average weight of fruits/pit from each harvest was recorded and the mean weight expressed in kg.

3.5.2.5. Total yield

The total yield of fruits/season obtained from three observational plants was recorded at each harvest. The total weight of fruits/ season from each harvest was recorded and the mean weight expressed in kg.

3.5.2.6. Pooled analysis of data from three locations

Pooled analysis of the following data from three locations *viz.*, total yield per pit, disease incidence and disease index recorded from the locations of the trial, were conducted.

3.6. Economic analysis

The economics of cultivation using the treatments were worked out, considering the total cost of cultivation and the prevailing market price of the produce. Net returns and benefit:cost ratio were computed as follows.

Net returns = Gross income – Cost of cultivation

$$B : C \text{ ratio} = \frac{\text{Gross income}}{\text{Cost of cultivation}}$$

3.7. Estimation of rhizosphere and phyllosphere microflora

The fungal and bacterial populations of rhizosphere and phyllosphere of treated culinary melon plants from the farmers' field trials, were estimated.

3.7.1. Estimation of fungal population in rhizosphere (Cavaglieri *et al.*, 2009)

Root samples were collected from culinary melon plants of all the treatment plots in the farmers' field trials. One gram of root samples with adhering soil were taken from each treated plant. The root samples were washed with sterile distilled water and shaken in a 250 ml flask containing 100 ml of 0.82 per cent NaCl solution at 150 rpm for 30 min. Serial dilution were prepared and one ml aliquot from the dilutions 10^{-4} was transferred to each sterilized petri dish over which 15 ml of Martin's Rose Bengal Agar medium supplemented with streptomycin (0.1%) was poured gently and incubated at room temperature (28±2°C) for 48 hr. Typical colonies of fungi which appeared on the plates were counted and population estimated.

3.7.2. Estimation of bacterial population in rhizosphere (Johnston *et al.*, 2016)

Root samples were collected from culinary melon plants of all the treatment plots in the farmers' field trials. One gram of root samples with adhering soil were taken from each treated plant. The root parts were washed with sterile distilled water and shaken in a 250 ml flask containing 100 ml of 0.82 per cent NaCl solution at 150 rpm for 30 min. Serial dilution were prepared and one ml aliquot from the dilutions $10^{-6}$was transferred to each sterilized petri dish over which 15 ml of Nutrient Agar medium was poured gently and incubated at room temperature (28±2°C) for 48 hr. Typical colonies of bacteria which appeared on the plates were counted and population estimated.

3.7.3. Estimation of fungal population in phyllosphere (Lindow and Brand, 2003)

Leaves were collected from culinary melon plants of all the treatment plots in the farmers' field trials. One gram of leaf bits were taken and serial dilutions were prepared. One ml aliquot was transferred from the dilutions 10^{-4} to each sterilized petri dish over which 15 ml of Martin's Rose Bengal Agar medium supplemented with streptomycin (0.1%) was poured gently and incubated at room temperature (28±2°C) for 48 hr. Typical colonies of fungi which appeared on the plates were counted and population estimated.

3.7.4 Estimation of bacterial population in phyllosphere (Yadav *et al.*, 2010)

Leaves were collected from culinary melon plants of all the treatment plots in the farmers' field trials. One gram of leaf bits were taken and serial dilutions were prepared. One ml aliquot was transferred from the dilutions 10^{-6} to each sterilized petri dish over which 15 ml of nutrient agar medium and incubated at room temperature (28±2°C) for 48 hr. Typical colonies of bacteria which appeared on the plates were counted and population estimated.

3.8. Detection of induced systemic resistance in culinary melon plants from farmers' field trials

Defense related enzymes and compound (phenol) were assayed by standard protocols for detecting any induction of systemic resistance in culinary melon plants due to the influence of different treatments applied in the farmers' field trials.

3.8.1. Sample collection and enzyme extraction

Leaf samples were collected from all the treatment plots at 5^{th}, 10^{th} and 15^{th} days after spraying of the plants with the treatments and they were analysed for the changes in the activities of the enzymes *viz.*, phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), β-1, 3 glucanase, super oxide dismutase (SOD) as well as defense related compound (phenol).

3.8.1.2. Assay of phenylalanine ammonia lyase (PAL) activity

PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Fresh plant leaves (1 g) were homogenized in 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. Enzyme activity was expressed in fresh weight basis as nmol trans-cinnamic acid min^{-1} mg^{-1} of sample (Dickerson *et al.,* 1984).

3.8.1.3. Assay of peroxidase activity

Fresh plant leaves (1 g) were homogenized in 3 ml of 0.1 M sodium phosphate buffer (pH 7.0) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 18000 rpm at 58°Cfor 15 minutes and supernatant was used within two to four hours which served as an enzyme source. To a spectrophotometric sample cuvette, 3 ml of buffer solution, 0.05 ml guaiacol solution, 0.1 ml enzyme extract and

0. 03 ml H2O2 solution were added and mixed well. The absorbance was recorded at 420 nm using spectrophotometer. The enzyme activity was expressed as changes in absorbance min^{-1} g^{-1} of fresh tissue (Hammerschmidt and Kuc, 1982).

3.8.1.4. Assay of polyphenol oxidase (PPO) activity

The polyphenol oxidase activity was determined as per the procedure given by Mayer *et al.* (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M Catechol was added and the activity was expressed as change in absorbance min^{-1} g^{-1} of protein.

3.8.1.5. Assay of β-1, 3-glucanase activity

The enzyme extracts was prepared by homogenizing 1 g tissue of the leaf in 5 ml of 0.05 M sodium acetate buffer (pH 5.0) at 4°C. The homogenate was centrifuged at 20000 rpm at 4°Cfor 10 min and the supernatant was used as enzyme source. The crude extract of 62.5 ml was added to 62.5 ml of laminarin (4 per cent) and then incubated at 40°Cfor 10 min and the reaction was stopped by adding 375 ml of dinitro salicylic acid and heated for 5 min in a boiling water bath. The resulting solution was diluted with 4.5 ml distilled water and the absorbance was read at 500 nm. The crude extract preparation with laminarin with zero time incubation served as blank. The activity was expressed as µg equivalent of glucose min-1 g-1 of fresh tissue (Kavitha *et al.,* 2005).

3.8.1.6. Assay of super oxide dismutase (SOD) activity

The enzyme extracts were prepared by homogenizing 1 g tissue of the leaf in 2 ml of 0.2 m citrate phosphate buffer (pH 6.5) at 4 °C. The homogenate was centrifuged at 15000 rpm at 4°Cfor 30 min. The supernatant served as enzyme source

and SOD activity was determined as its ability to inhibit the photochemical reduction of NBT (Giannospolitis and Ries, 1977). The assay mixture (3 ml) contained 50mM sodium phosphate buffer (pH 7.8, 13 mM methionine, 75μM NBT, 2 μM riboflavin, 0.1mM EDTA 100 μl of the enzyme extract and the riboflavin was added at the end. Tubes were shaken and placed under a 40-W fluorescent at 25°C. The reaction was initiated and terminated by turning the light on and off respectively. The absorbance at 560 nm was measured against identical non illuminated in parallel to the sample tubes for blank. Each extract was substracted from the blank and multiplied by 100 to obtain the percentage inhibition of NBT-photoreaction. The SOD activity was expressed in SOD units g^{-1}tissue (50 per cent NBT inhibition=1 unit) (Belid *et al.*, 1993).

3.8.1.7. Assay of phenol activity

One gram of the leaf samples was ground in a pestle and mortar in 10 ml of 80 per cent methanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was evaporated to dryness and the residue was dissolved in five ml of distilled water. From this, 0.2 ml was taken and the volume was made up to three ml with distilled water. To that 0.25 ml of (1N) Folin-Ciocalteau reagent was added. After three minutes, one ml of 20 per cent sodium carbonate was added and mixed thoroughly. Thus the tubes were placed in boiling water for one minute and cooled. The absorbance was measured at 725 nm against a reagent blank. The phenol activity was expressed as μg of catechol $g^{-1}$of plant tissue (Zieslin and Ben-Zaken, 1993).

3.9. Assessment of physiological parameters of culinary melon plants in farmers' field trials

3.9.1. Nutrient content (NPK)

Leaf samples collected from each treatment plot were analysed for nitrogen, phosphorus and potassium. The leaves were chopped, sun dried and oven dried at 70°c to a constant weight. Samples were ground to pass through a 0.5mm mesh in a Willey Mill and the required quantity of samples were digested and used for nutrient content analysis.

The nitrogen content in leaf samples was estimated by the modified microkjeldhl method (Jackson, 1973) and the uptake of nitrogen was calculated by multiplying the nitrogen content of leaf sample with the total dry weight of leaves. The phosphorus content in leaf samples was colorimetrically determined by wet digestion of the sample, developing colour by ascorbic acid method (Jackson, 1973) and read in a spectrophotometer. The uptake of phosphorus was calculated by multiplying the phosphorus content of leaf sample with the total dry weight of leaves. The potassium content in leaf sample was determined by flame photometer method and the uptake of potassium was calculated by multiplying the potassium content of leaf sample with the total dry weight of leaves. The uptake values were expressed in kg ha^{-1}.

Analytical methods followed in plant analysis

Sl.No	Parameters	Methods	Reference
1	Nitrogen	Microkjedahl distillation after digestion in H_2SO_4	Jackson (1973)
2	Phosphorus	Nitric-perchloric (9:4) digestion and colorimetry using Vanadomolybdo-	Jackson (1973)

		phosphoric yellow colour method	
3	Potassium	Nitric-perchloric (9:4) digestion and flame photometry	Jackson (1973)

3.9.2. Nutrient use efficiency (NPK)

It indicates kg crop yield per kg nutrient applied.

$$PFP = \frac{\text{Yield (kg/ha) (Y)}}{\text{Amount of nutrient applied (kg/ha) (N)}}$$

3.9.3. Estimation of chlorophyll (DMSO method) (Watada *et al.*, 1976)

A weighed quantity of leaf samples (0.5g) were taken from each treatment plot and cut into small bits. These bits were put in test tubes and incubated overnight at room temperature, after pouring 10 ml DMSO: 80% acetone mixture (1:1 v/v). The coloured solution was decanted into a measuring cylinder and made up to 25 ml with the DMSO-acetone mixture. The absorbance was measured at 663 and 645nm using a spectrophotometer. The chlorophyll content was measured by substituting the absorbance values in the given formulae.

Chl a = $(12.7 \times A_{663} - 2.69 \times A_{645}) \times V/1000 \times 1/$ Fresh weight

Chl b = $(22.9 \times A_{645} - 4.68 \times A_{663}) \times V/1000 \times 1/$ Fresh weight

Total Chl (a + b) = $(8.02 \times A_{663} + 20.2 \times A_{645}) \times V/1000 \times 1/$ Fresh weight

3.9.4. Relative water content (%) (Yamasaki and Dillenburg, 1999)

Relative water content was calculated by measuring the fresh weight, dry weight and turgid weight of known number of leaf discs collected from plants of the treatment plots. After measuring the fresh weight of the sample, it was submerged in distilled water for 3 hours and then the turgid weight was taken. The dry weight of

the sample was measured after keeping the samples in oven at 80°C for 3 consecutive days. The RWC of the treatment sample was calculated using the following formula.

RWC = (fresh weight - dry weight) / (turgid weight - dry weight) X 100

3.9.5. Epicuticular wax content (mg cm^{-2}) (Ebercon *et al.,* 1977)

Samples were collected from third fully opened leaves of plants in each treatment plot and were collected and cut into $10cm^2$ bits. Ten millilitres of chloroform was taken in beakers after noting down their initial weight. Samples of leaf bits were dipped into chloroform for 30seconds. After removing the leaf bits, the beakers were left for evaporation of chloroform. The final weight of beakers was noted after complete evaporation of chloroform. The difference between the final and initial weight of beakers was noted as the wax content and was expressed per unit leaf area.

3.9.6. Stomatal frequency (Maghsoudi *et al.,* 2008)

Stomatal count refers to the number of stomata per unit area of leaf. A thick mixture of thermocol and xylene was prepared and this was smeared on both the surfaces of leaf samples and allowed to dry. It was peeled gently after drying and the peel was observed under microscope and counted using a 40 X objective and 10 X eyepieces. The field of the microscope was measured using a stage micrometer and stomatal frequency per unit area was calculated.

Stomatal frequency = No. of stomata / Area of the microscopic field

3.10. Statistical analysis

Statistical analysis of the data recorded was done by using analysis of variance technique (ANOVA) as applied to completely randomized design and

randomized block Design (Panse and Sukhatme, 1978) and the significance was tested using F test (Sneaecor and Cochran, 1967). Wherever the F value was found significant, critical difference were worked out at five percent and one percent probability level. The significance of the treatments as compared against the control was also tested.

Experimental Results

4. EXPERIMENTAL RESULTS

The present study on the "Integrated management of foliar fungal disease of culinary melon "(*Cucumis melo* L. var. *acidulus* Naudin)," was conducted during the period 2013-2016 at the Department of Plant Pathology, College of Agriculture, Vellayani, at Instructional Farm (IF), Vellayani as well as in farmers' fields of Kalliyoor and Venganoor panchayats of Thiruvananthapuram district. The results obtained from the series of laboratory and field experiments conducted, are presented below:

4.1. Survey on occurrence of foliar diseases in different culinary melon growing areas

Surveys were conducted in culinary melon fields located at Instructional Farm, College of Agriculture, Vellayani as well as in farmers' fields near College of Agriculture, Vellayani, during September 2013 to December 2013, in order to assess the prevalence of major diseases affecting the crop. For conducting the survey, culinary melon fields having plants in the early stage of growth (not more than 10 days after sowing) were selected from ten different locations located in Kalliyoor and Venganoor panchayats (Table1a,b; Fig. 2 and Fig. 3). From each of these selected fields, observations were taken as described in 3.1 and 3.1.1 at 10 days interval, starting from 15 days after sowing up to 75 days stage of the crop.

4.1.1. Diagnosis of diseases affecting culinary melon plants in the field

Leaf samples collected from infected culinary melon plants in the surveyed fields, were diagnosed in the laboratory by studying the symptoms in comparison with plant disease diagnostic keys as well as by their microscopic examination, in order to identify the associated pathogen in case of fungal diseases.

Table 1a. Incidence of foliar diseases (DI) affecting culinary melon plants in surveyed location (September 2013 – December 2013)

Sl. No	Location	Disease Observed	Days after sowing						
			15th DI	25th DI	35th DI	45th DI	55th DI	65th DI	75th DI
1	Palapoor	Colletotrichum Leaf spot	0.00	0.00	20.00	28.00	36.00	44.00	50.00
		Downy mildew	0.00	0.00	0.00	20.00	22.00	26.00	30.00
2	Papanchani	Colletotrichum Leaf spot	0.00	0.00	22.00	30.00	34.00	40.00	50.00
		Downy mildew	0.00	0.00	0.00	24.00	28.00	32.00	36.00
3	Punjakari	Colletotrichum Leaf spot	0.00	0.00	28.00	38.00	42.00	48.00	56.00
		Downy mildew	0.00	0.00	14.00	16.00	18.00	22.00	26.00
4	Chavadinada	Colletotrichum Leaf spot	0.00	0.00	42.00	50.00	60.00	68.00	70.00
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00

5	Kakkamoola	Colletotrichum Leaf spot	0.00	0.00	0.00	24.00	30.00	34.00	40.00
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	Venganoor	Colletotrichum Leaf spot	0.00	0.00	24.00	32.00	40.00	46.00	54.00
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	Panangodu	Colletotrichum Leaf spot	0.00	26.00	34.00	38.00	48.00	54.00	60.00
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	Kalliyoor	Colletotrichum Leaf spot	0.00	0.00	0.00	22.00	26.00	34.00	40.00
		Downy mildew	0.00	0.00	0.00	22.00	26.00	30.00	34.00
9	Peringamala	Colletotrichum Leaf spot	0.00	0.00	24.00	34.00	38.00	42.00	52.00
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	IF, Vellayani	Colletotrichum Leaf spot	0.00	0.00	16.00	18.00	22.00	26.00	32.00
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 1b. Percent disease index (PDI) of foliar diseases affecting culinary melon in surveyed location (September 2013- December 2013)

Sl. No	Location	Disease Observed	Days after sowing						
			15th (PDI)	25th (PDI)	35th (PDI)	45th (PDI)	55th (PDI)	65th (PDI)	75th (PDI)
1	Palapoor	Colletotrichum Leaf spot	0.00	0.00	13.33	20.00	24.44	37.77	42.22
		Downy mildew	0.00	0.00	0.00	8.88	13.33	22.22	26.66
2	Papanchani	Colletotrichum Leaf spot	0.00	0.00	15.55	17.77	28.88	33.33	42.22
		Downy mildew	0.00	0.00	0.00	13.33	22.22	26.66	33.33
3	Punjakari	Colletotrichum Leaf spot	0.00	0.00	13.33	17.77	37.77	42.22	51.11
		Downy mildew	0.00	0.00	6.66	8.88	13.33	17.77	22.22
4	Chavadinada	Colletotrichum Leaf spot	0.00	0.00	20.00	24.44	46.66	55.55	64.44
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00

5	Kakkamoola	Colletotrichum Leaf spot	0.00	0.00	0.00	20.00	24.44	28.88	37.77
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	Venganoor	Colletotrichum Leaf spot	0.00	0.00	8.88	17.77	26.66	42.22	46.66
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	Panangodu	Colletotrichum Leaf spot	0.00	11.11	20.00	28.88	37.77	51.11	57.77
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	Kalliyoor	Colletotrichum Leaf spot	0.00	0.00	0.00	15.55	24.44	33.33	37.77
		Downy mildew	0.00	0.00	0.00	8.88	20.00	24.44	28.88
9	Peringamala	Colletotrichum Leaf spot	0.00	0.00	6.66	17.77	26.66	42.22	46.66
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	IF, Vellayani	Colletotrichum Leaf spot	0.00	0.00	4.44	13.33	15.55	17.77	28.88
		Downy mildew	0.00	0.00	0.00	0.00	0.00	0.00	0.00

PDI – Percentage disease index

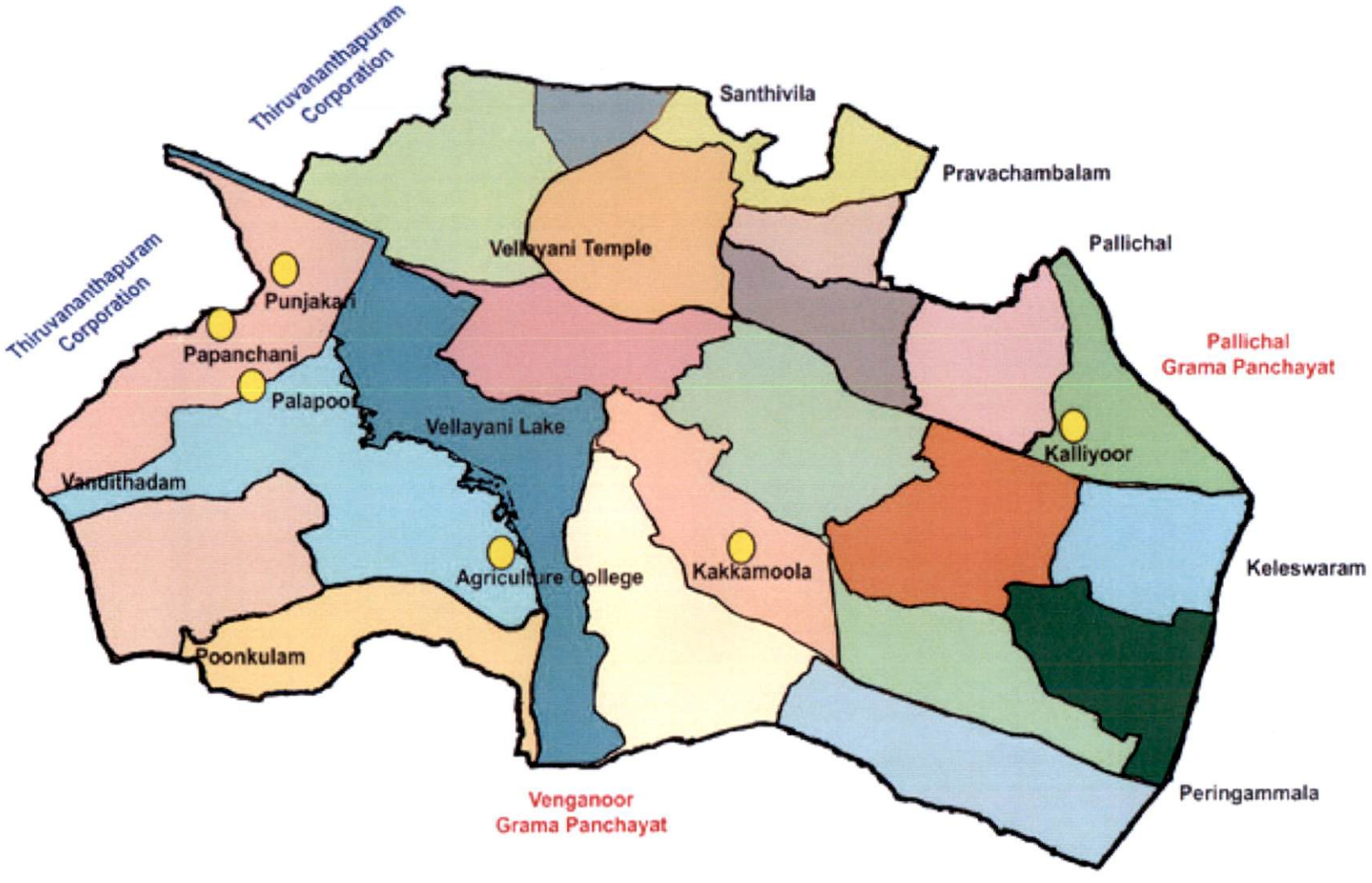


Fig. 1. Kalliyoor grama panchayat

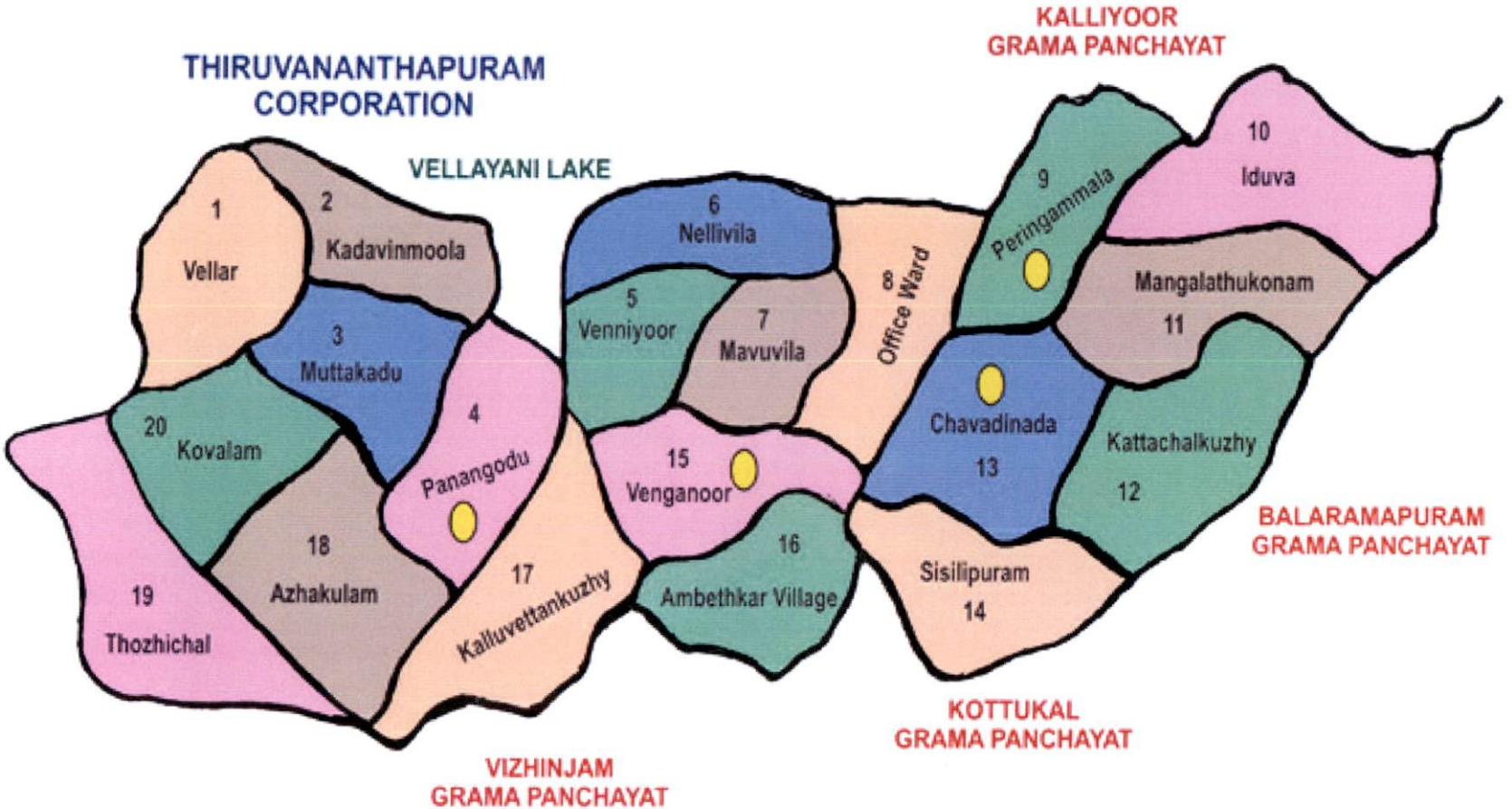


Fig. 3. Venganoor grama panchayat

The following diseases were diagnosed in the leaf samples collected from surveyed locations and their symptoms observed at different stages of growth of the crop, are described below:-

4.1.1.1. Anthracnose leaf spot

Initially, symptoms appeared on the leaves as minute, circular to irregular light brown coloured spots which later increased in size and gradually turned dark brown in colour, surrounded by a yellow halo. In the advanced stages of disease development, they coalesced together to form large, necrotic patches on the leaves, often with shot holes due to the disintegration of the necrotic tissues. Severely infected leaves dried causing excessive defoliation of the diseased plants (Plate 1). When the leaf samples exhibiting the typical symptoms of anthracnose were examined under the microscope, masses of cylindrical and hyaline, conidia with obtuse ends were observed (Plate 2).

4.1.1.2. Downy mildew

The initial symptoms appeared on the leaves as pale green patches which were angular, yellow and later deepened to a yellowish brown colour. These patches were often restricted by veins on the upper surface of the leaves. On the lower surface, opposite to these patches, a dirty grey downy growth of the pathogen was observed. The infected leaves withered quickly and in the later stages the entire plant wilted (Plate 3).

When the leaf samples were examined under the microscope, coenocytic mycelium was observed, ramifying the leaf surface. Sporangiophores were also observed that branched dichotomously at acute angles and had tapered and curved tips. Elliptical to ovoid, grey sporangia which had the characteristic papillae of the

a) Leaves become yellow with water soaked lesions

b) Leaves severely affected with greyish brown water patches

c) Leaves dried causing excessive defoliation of the plant

Plate 1. Anthracnose leaf spot symptoms occurrence on culinary melon

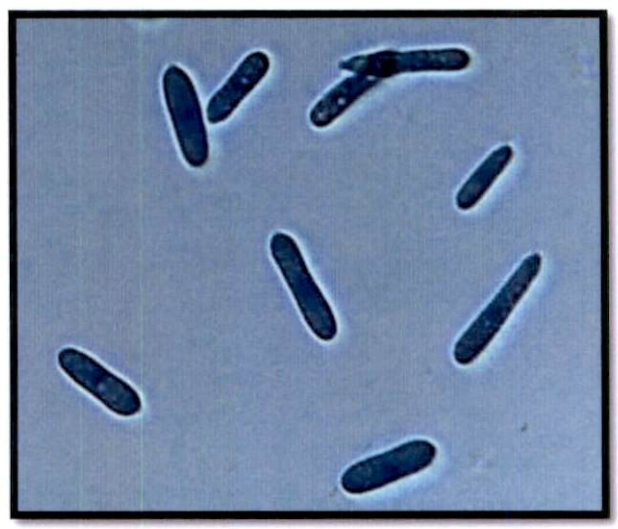


Plate 2. Conidia of anthracnose pathogen

a) Field view

b) Entire leaves infected by downy mildew

Plate 3. Downy mildew disease of culinary melon

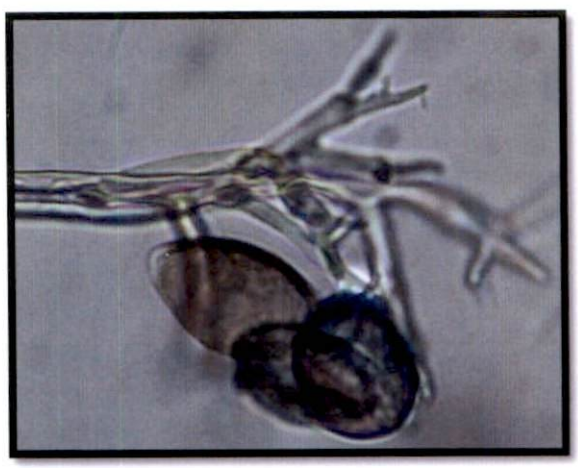


Plate 4. Dichotomously branched sporangiophores of downy mildew pathogen

downy mildew sporangium, at its distal end, were borne at the tip of these branches (Plate 4).

4.1.2. Incidence and severity of the diseases observed during the survey

Incidence and severity of the diseases observed during the survey were assessed separately as described 3.1.2.1 and 3.1.2.2 and the results are presented below

4.1.2.1. Incidence of disease

Incidence of foliar disease of culinary melon in the locations surveyed from September 2013 – December 2013 are presented in Table (1a and1c).

Culinary melon plants in all the surveyed locations were infected by anthracnose leaf spot from 35 to 45 days after sowing (DAS). Maximum incidence of the disease was recorded at 75 DAS and ranged between 32 to 70 per cent. The highest incidence (DI) of anthracnose leaf spot was recorded 75 DAS at Chavadinada (70.00 per cent) followed by that at Panangodu (60 per cent). The lowest DI of the disease was recorded at IF, Vellayani (32 per cent). The highest mean DI throughout the period of survey was recorded at Chavadinada (41.42 per cent) and the minimum mean DI was observed at IF, Vellayani (16.28 per cent) (Table 1a and Table 1c).

Incidence of downy mildew ranged between 0 to 36 per cent and the disease was recorded in four (Papanchani, Kalliyoor, Palapoor and Punjakari) out of the ten locations surveyed. The disease was observed from 35 to 45 DAS in the prevalent areas. Maximum incidence of the disease (36 per cent) was observed at Papanchani followed by Kalliyoor (34 per cent) and the DI was lowest at Punjakari (26.00 per cent). Downy mildew disease was not prevalent in Chavadinada, Kakkamoola, Venganoor, Panangodu, Peringamala and Instructional Farm (IF), Vellayani. Highest

mean DI was recorded at Papanchani (17.14 per cent) and the minimum mean DI was observed at Punjakari (13.71 per cent) (Table 1a and 1c).

4.1.2.2 Disease index/Severity of foliar diseases

Disease index of fungal diseases prevalent in the surveyed locations from September 2013 – December 2013, are presented in Table (1b and 1d).

Severity of anthracnose leaf spot disease, 75 days after sowing (DAS), ranged from (28.88 to 64.44 per cent), respectively in the different areas surveyed. The highest percent disease index (PDI) of anthracnose leaf spot were recorded at Chavadinada (64.44 per cent) and Panangodu (57.77 per cent) and least PDI was observed at Instructional Farm (IF), Vellayani (28.88 per cent) (Tables 1b and 1d). Average PDI recorded throughout the period of survey were highest Chavadinada and Panangodu (30.16 and 29.52 per cent respectively) and the average minimum PDI (11.42 per cent) was observed at IF, Vellayani (Tables 1b and 1d).

Per cent disease index (PDI) of downy mildew ranged from 0 to 33.33 per cent in the surveyed areas. PDI was maximum (33.33 per cent) at Papanchani followed by Kalliyoor (28.88 per cent) and least (22.22 per cent) at Punjakari. Downy mildew was not recorded in the culinary melon fields of Chavadinada, Kakkamoola, Venganoor, Panangodu, Peringamala and Instructional Farm (IF), Vellayani. Highest average PDI of downy mildew disease recorded during the entire period of survey were observed at Papanchani (13.65 per cent) and Kalliyoor (11.74 per cent) and minimum average PDI (9.84 per cent) was observed in the field of Punjakari (Table 1b and 1d).

Table 1c. Average incidence of foliar diseases in different culinary melon growing areas

Sl.No	Location	Mean percentage disease incidence	
		Colletotrichum leaf spot	Downy mildew
1	Palapoor	25.42	14.00
2	Papanchani	25.14	17.14
3	Punjakari	30.28	13.71
4	Chavadinada	41.42	0.00
5	Kakkamoola	18.28	0.00
6	Venganoor	28.00	0.00
7	Panangodu	37.14	0.00
8	Kalliyoor	17.43	16.86
9	Peringamala	27.14	0.00
10	IF, Vellayani	16.28	0.00

Table 1d. Average percentage disease index of foliar diseases in different culinary melon growing areas

Sl.No	Location	Mean percentage disease index	
		Colletotrichum leaf spot	Downy mildew
1	Palapoor	19.68	10.15
2	Papanchani	19.68	13.65
3	Punjakari	23.17	9.84
4	Chavadinada	30.16	0.00
5	Kakkamoola	15.87	0.00
6	Venganoor	20.31	0.00
7	Panangodu	29.52	0.00
8	Kalliyoor	15.87	11.74
9	Peringamala	19.99	0.00
10	IF, Vellayani	11.42	0.00

4.1.2.3. Details of farmers' questionnaire

The area under culinary melon crop ranged from 20 – 30 cents in the different field surveyed. In locations such as Chavadinada and IF, Vellayani culinary melon was cultivated during previous season also (Table 1e).

4.1.2.4. Details of data collected from the farmers' during the survey

In general, fungicides are not applied in any of the locations surveyed (Table 1f).

4.1.2.5. Weather data during 2013

The weather data recorded from January 2013 – December 2013 is given in (Table 1g) (Fig. 4).

4.1.3. Isolation and identification of the pathogen inciting anthracnose leaf spot of culinary melon

The pathogen associated with anthracnose leaf spot of culinary melon was isolated from infected leaves of the crop for conducting subsequent studies on the integrated management of the disease.

Four fungal isolates that were identical in cultural characters were consistently obtained from leaf samples collected from various surveyed locations, as indicated in (Plate 5). All the isolates initially produced on potato dextrose agar, white mycelial growth, that later turned grey and on examining portions of aged mycelial growth, cylindrical hyaline, conidia typical of those produced by *Colletotrichum gloeospoiriodes*, were observed.

Table 1e. Details of acerage and crops in surveyed locations

Sl.No	Location	Acerage of culinary melon crop	Crops surrounding the culinary melon fields	Crops cultivated during the previous season
1	Palapoor	25 cents	Cassava, Amaranthus, Banana, Snake gourd	Amaranthus, Bitter gourd, Snake gourd
2	Papanchani	25 cents	Amaranthus, Banana, Cassava	Banana, Amaranthus
3	Punjakari	20 cents	Bitter gourd, Amaranthus, Banana	Snake gourd, Amaranthus
4	Chavadinada	25 cents	Banana, Cassava	Culinary melon, Snake gourd, Cowpea
5	Kakkamoola	25 cents	Cowpea, Banana, Bitter gourd, Amaranthus	Banana, Bhendi, Amaranthus
6	Venganoor	20 cents	Snake gourd, Amaranthus, Cassava, Pumpkin	Bitter gourd, Amaranthus, Cowpea
7	Panangodu	20 cents	Cassava, Amaranthus	Amaranthus, Cowpea, Snake gourd
8	Kalliyoor	25 cents	Amaranthus, Cowpea	Amaranthus, Snake gourd, Bitter gourd
9	Peringamala	25 cents	Amaranthus, Cassava, Cowpea	Cowpea, Banana, Amaranthus
10	IF, Vellayani	30 cents	Banana, Amaranthus, Cowpea, Brinjal	Culinary melon, Bhendi, Cowpea, Amaranthus

Table 1f. Details of cultivation practices of farmers' in different locations surveyed

Sl.No	Cultivation details	Applications of treatments/pit	KAU Schedule
1.	Seed Sowing	Cowdung or Poultry manure – 250g	Cowdung or Poultry manure – 500g
2.	Basal application of NPK mixture	5g to 10g	28:10:10 g $N:P_2O_5:K_2O$
3.	2nd time of Poultry manure	½ kg /pit	1 kg/pit
4.	At the time of flowering (30th days) basal application of NPK mixture	25g/pit	28:10:10 g $N:P_2O_5:K_2O$
5.	45th days after fruit set	1st harvest	1st harvest
6.	Disease/pest management	Spraying Ekalux @ 1.5 ml/l at 15 days interval	Pest: Spraying Ekalux @ 1.5 ml/l at 15 days interval Disease: Mancozeb @ 0.4%
7.	Average weight per fruit	2kg	2kg
8.	Total yield per harvest	20 – 25 kg/10 cent	35 kg/10 cent

Table 1g. Weather parameters during 2013

Month	Temperature (Max)	Temperature (Min)	RH (I)	RH (II)	Total rainfall/ month (mm)
January	30.30	22.02	95.97	92.67	32.80
February	31.25	22.12	92.47	75.07	13.50
March	32.26	23.72	92.68	76.20	72.00
April	33.05	25.42	88.60	74.27	21.80
May	31.86	24.82	91.72	82.26	60.70
June	29.12	22.95	93.52	86.42	67.40
July	28.87	22.95	93.47	85.52	89.20
August	29.28	23.52	92.48	81.38	31.00
September	29.12	23.85	96.40	85.30	35.90
October	30.64	23.24	93.28	77.45	47.00
November	30.65	23.45	97.32	77.30	89.30
December	30.85	21.82	97.40	67.65	74.40

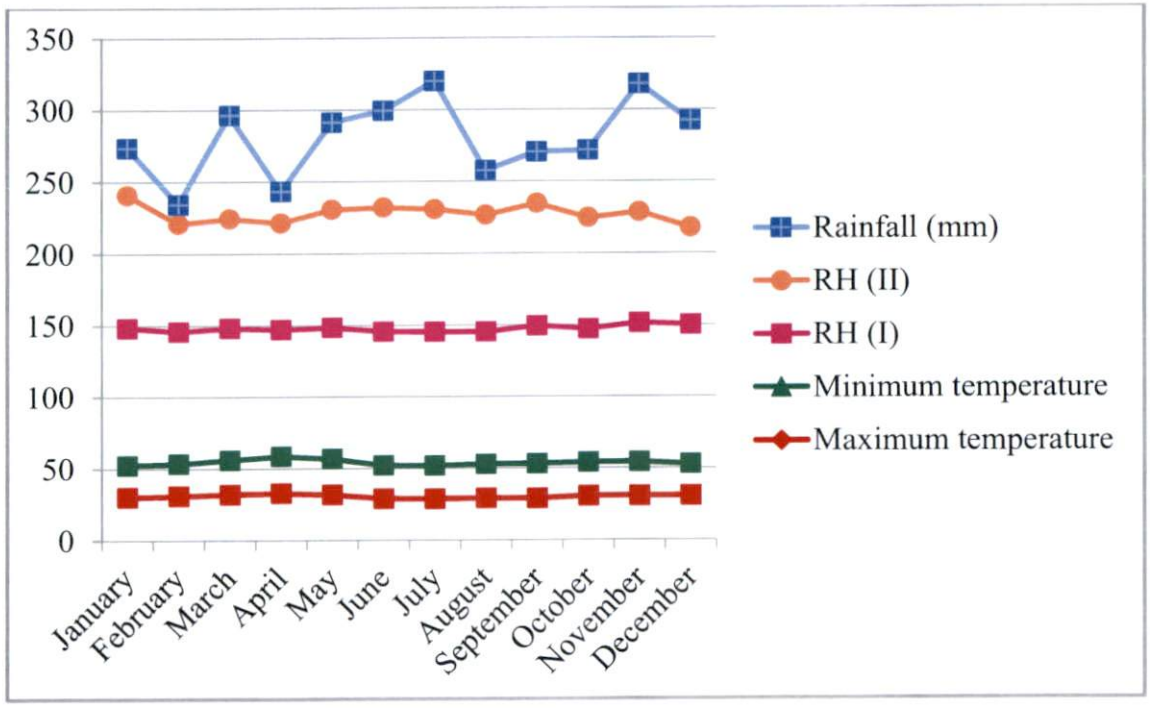
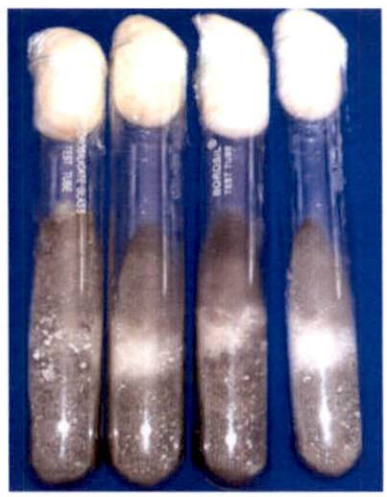


Fig. 4. Weather parameters during 2013



1 2 3 4

1. Palapoor
2. Punjakari
3. Kalliyoor
4. IF, Vellayani

Plate 5. Isolation and identification of the pathogen inciting anthracnose leaf spot of culinary melon

The isolates of the pathogen associated with anthracnose leaf spot of culinary melon were tentatively identified as, *C. gloeospoiriodes* based on the morphological and cultural characters.

4.1.4. Pathogenicity test

Pathogenicity tests were conducted by inoculating mycelia disc comprising of spores as well as spore suspension (5 x 10^5spores ml^{-1}) of each isolate obtained in (Plate 6) on leaves of thirty-days-old culinary melon plants (cv. Vellayani local). All the four isolates produced typical symptoms of anthracnose leaf spot disease of culinary melon, three to five days after inoculation.

Plants inoculated with plain agar fragments and also sprayed with distilled water were maintained as control. The morphological and cultural characters of fungal isolates that were re-isolated from artificially infected leaf tissues were similar to their original isolates.

4.1.5. Screening of virulent isolate of the pathogen by estimation of incubation period (IP) and Disease development time (DDT)

Among the four pathogenic isolates, the most virulent one was screened by estimating the Incubation period (IP) and Disease development time (DDT) for each isolate, in a separate experiment as mentioned in 3.2.2. IP ranging from 3 to 5 days were recorded for the four isolates and the shortest IP of 3 days was observed in the IF, Vellayani isolate (Table 2). DDT ranged from 7 to 10 days for the different isolates and the shortest period of 7 days was recorded for the IF, Vellayani isolate (Plate 7).

The IF, Vellayani isolate that had the shortest IP and DDT was screened as the most virulent isolate and was used in subsequent studies (Plate 8).

Plate 6. Pathogenicity test under glass house conditions

Incubation Period (IP) - It is defined as time measured in days between inoculation and appearance of the first lesion

Inoculated 

Disease development time (DDT) - It is defined as time measured in days between inoculation and appearance of the mature lesion

Inoculated 

Plate 7. Incubation Period (IP) and Disease Development Time (DDT) estimated in pathogenicity test of culinary melon plant

Description of stages	No. of. days after disease appeared	Symptoms
Stage 0: No symptom development	0	
Stage 1: Lesions initially appear as small circular areas	3	

Plate 8. Symptom development in artificially inoculated culinary melon plant

Stage 2 : Lesions enlarge and become tan to brown in colour, circular or irregular in shape	5	
Stage 3 : Lesions become dark brown and surrounded by bright yellow in colour	7	

Stage 4 : The yellowish colour becomes dark brown	9	
Stage 5 : The entire leaf become dark brown and dry up	12	

Table 2. Incubation Period (IP) and Disease Development Time (DDT) estimated in pathogenicity tests of different isolates

Sl.No	Isolate	Particulars of pathogenicity test	
		Incubation period (IP) days	Disease development time (DDT)
1	Palapoor	5	9
2	Punjakari	5	10
3	Kalliyoor	7	9
4	IF,Vellayani	3	7

4.1.6. Morphological and cultural studies of the virulent isolate of the pathogen

The morphological characters of the most virulent isolate, *viz.*,IF, Vellayani isolate were studied based on myco keys (James *et al.*,1971) and the results of which are presented below (Plate 9a).

Colonies on PDA were initially floccose white and later became grey on the upper surface, while it had a buff colour on the reverse. Hyphae were hyaline, smooth walled, septate, 1.72 – 8.72µm wide. Conidia were hyaline, cylindrical with obtuse ends, aseptate, smooth, thin walled and the average size of conidia was 31.34µm x 4.1µm (Plate 9b and Plate 9c). Based on these morphological and cultural characters, the fungus was identified as *Colletotrichum gloeosporioides*, which was confirmed by the morphological characterization conducted at the Institute of National fungal culture collections of India, Pune (accession no. 3808) (ANNEXURE-I).

4.1.7. Molecular characterization

Molecular characterization of the virulent isolate of the pathogen (IF, Vellayani isolate) was conducted by partial sequencing of internal transcribed spacer region (ITSR) of rDNA at NFCCI. In the sequence analysis of ITS_4 and ITS_5 the virulent IF, Vellayani isolate showed 100 per cent sequence similarity with *Colletotrichum fructicola.*

The ITS-rRNA region of virulent isolate of the pathogen (IF, Vellayani isolate) was sequenced for the molecular characterization and identification of the pathogen. Amplification using primers ITS_4 and ITS_5 revealed that the virulent IF, Vellayani isolate showed 100 per cent sequence similarity with *C. fructicola.* Sequence of the virulent isolate of IF, Vellayani were deposited in Genbank and used to search for similar sequences in NCBI database using BLAST program.

Whitish mycelial growth Olivaceous grey mycelial growth Reverse buff mycelial growth

Plate 9a. Growth of *Colletotrichum gloeosporioides* on PDA

Plate 9b. Hyphae of *Colletotrichum gloeosporioides*

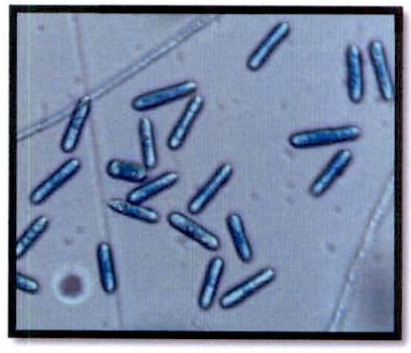


Plate 9c. Microscopic view of conidia of *Colletotrichum gloeosporioides*

5'TGGGGGGTTTTACGGCAAGAGTCCCTCCGGATCCCAGTGCGAGACGTAA
AGTTACTACGCAAAGGAGGCTCCGGGAGGGTCCGCCACTACCTTTGAGGG
CCTACATCAGCTGTAGGGCCCCAACACCAAGCAGAGCTTGAGGGTTGAAA
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ATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAA
AGTTTTGATTATTTGCTTGTACCACTCAGAAGAAACGTCGTTAAATCAGA
GTTTGGTTATCCTCCGGCGGGCGCCGACCCGCCCGGAGGCGGGAGGCCGG
GAGGGTCGCGGAGACCCTACCCGCCGAAGCAACAGTTATAGGTATGTTCA
CAAAGGGTTATAGAGCGTAAACTCAGTAATGATCCCTCCGCTGGTTCACC
AACGGAGACCTTGTTAC3'

Aligment of the sequences of representative isolates collected from surveyed location in this study with other known sequences of *Colletotrichum fructicola* obtained from Genbank revealed that an identity of 99-100 per cent existed among the sequences (Fig. 5) (ANNEXURE-II).

4.2. Management of anthracnose leaf spot disease of culinary melon

The IF Vellayani isolate that was identified as *Colletotrichum fructicola* by NFCCI was screened as the most virulent isolate inciting anthracnose leaf spot disease in culinary melon and was further used in the initial experiments conducted for the management of the disease, both under laboratory and greenhouse conditions.

4.2.1. *In vitro* evaluation of foliar fertilizer, nutrient, bio-control agents and fungicides for anthracnose leaf spot pathogen

Laboratory experiments were mainly undertaken to determine the direct impact of chemicals and bio-control agents, on the growth of the virulent isolate anthracnose leaf spot pathogen, *C. fructicola*.

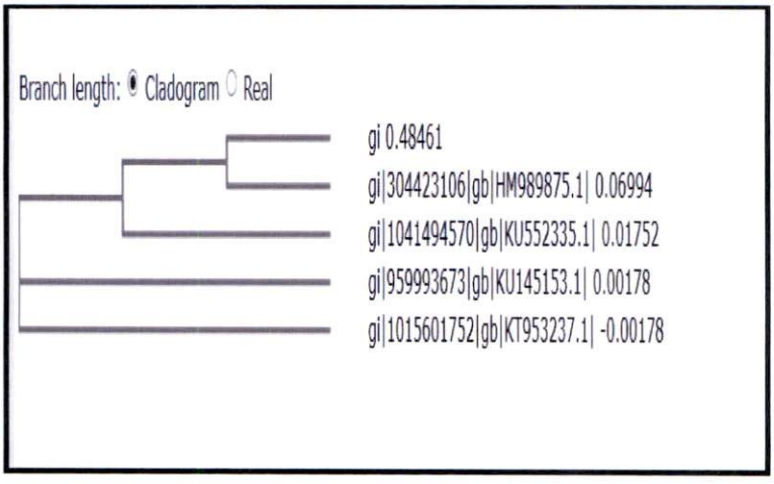


Fig. 5. Phylogenetic tree of *Colletotrichum fructicola*

4.2.1.1. Poisoned food technique - (Adhikary *et al.*, 2013)

In vitro evaluation of 8 treatments in inhibiting growth of the most virulent isolate *C. fructicola* associated with anthracnose leaf spot of culinary melon plants, was undertaken by poisoned food technique. Results of the experiment are presented in (Table 3a) (Plate 10).

All the treatments tested by the poisoned food technique significantly inhibited mycelial growth of the most virulent isolate, *C. fructicola,* at the recommended concentrations as compared to control. Among the 8 treatments, maximum inhibition of mycelia growth of *C. fructicola* (100 per cent) was observed in PDA amended with a combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and mancozeb (0.4 per cent) compared to all other treatments. It was followed by two per cent talc based formulation of *P. fluorescens* and two per cent talc based formulation of *T. viride* which gave 88.52 per cent and 86.30 per cent inhibition of the mycelial growth of the pathogen, respectively. Mixing the foliar fertilizer NPK 19:19:19 (0.5 per cent) with azoxystrobin (0.15 ml/l) resulted in 62.59 per cent inhibition of mycelia growth. Foliar fertilizer NPK 19:19:19 (0.5 per cent) produced mean colony diameter of 6.33 cm and 29.63 per cent inhibition of the growth when compared to control. This was followed by amendment of the medium with the nutrient, calcium nitrate (5g/l) in which 21.11 per cent inhibition was recorded. Maximum mean colony diameter (7.37cm) and least per cent inhibition (18.17 per cent) of the mycelia growth of the pathogen as compared to control, were recorded in PDA amended with combination of NPK 19:19:19 (0.5 per cent) and calcium nitrate (5g/l).

4.2.1.2. Dual culture technique

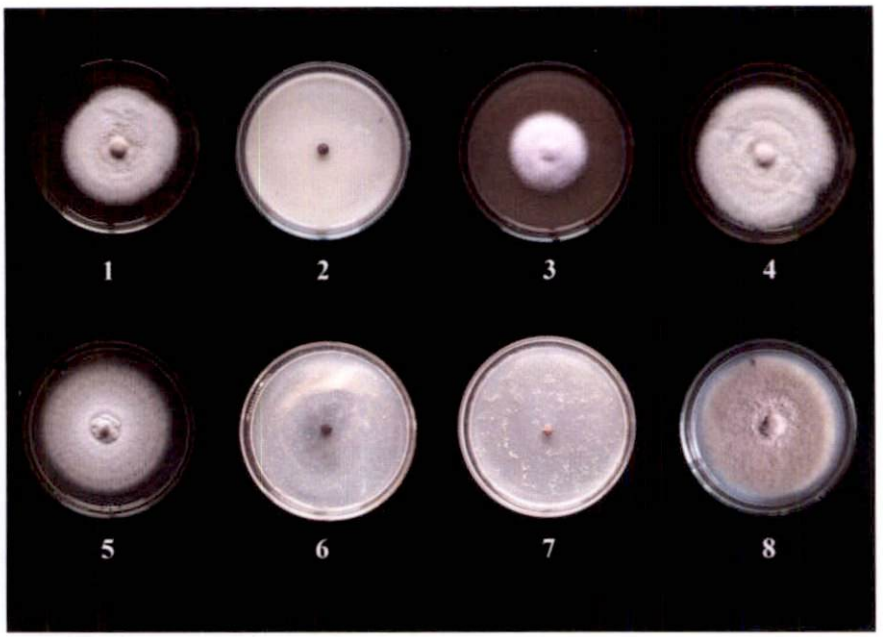
When the antagonist *P. fluorescens* and *T. viride* were assayed for inhibition of growth of the virulent isolate of the pathogen by dual culture method, simultaneous

Table 3a. Effects of foliar fertilizer, nutrients, bio-control agents and fungicides on mycelial growth of *Colletotrichum fructicola* under *in vitro* conditions

Sl. No	Treatments	Mycelial growth (cm)*	Per cent reduction over control*
1	NPK19:19:19 (0.5 %)	6.33^{d}(2.53)	29.63^{e}(23.72)
2	NPK 19:19:19 (0.5%)+ Mancozeb (0.4%)	0.00^{a}(0.00)	100.00^{a}(98.50)
3	NPK19:19:19 (0.5%) + Azoxystrobin(0.15 ml/l)	3.37^{c}(1.24)	62.59^{d}(79.21)
4	NPK 19:19:19 (0.5%) + Calcium nitrate (5g/l)	7.37^{e}(3.03)	18.17^{g}(10.23)
5	Calcium nitrate (5g/l)	7.10^{e}(2.90)	21.11^{f}(13.03)
6	Talc based formulation of *Pseudomonas fluorescens* (2%)	1.03^{b}(0.35)	88.52^{b}(95.16)
7	Talc based formulation of *Trichoderma viride* (2%)	1.23^{b} (0.43)	86.30^{c}(94.37)
8	Absolute control	9.00^{f} (3.88)	-
	CD (0.05)	0.305(1.687)	1.017(2.857)

*Mean of three replications

Values in parenthesis are arcsine transformed



1. 19:19:19 NPK (0.5%)
2. 19:19:19 NPK (0.5%) + Mancozeb (0.4%)
3. 19:19:19 NPK (0.5%) + Azoxystrobin (0.15ml /l)
4. 19:19:19 NPK (0.5%) + CaNO3 (0.5%)
5. CaNO3 (0.5%)
6. Talc based formulation *Pseudomonas fluorescens* (2%)
7. Talc based formulation *Trichoderma viride* (2%)
8. Absolute control

Plate 10. Effect of foliar fertilizer, nutrient, bio-control agents and fungicides against mycelial growth of *C. fructicola* by poisoned food technique

inoculation of culture of *P. fluorescens* resulted in (87.14 per cent) reduction of the mycelial growth of pathogen compared to control and was on par with the inhibition induced by the fungal antagonist *T. viride* (86.33 per cent) (Table 3b) (Plate 11).

4.2.1.3. Inhibition of spore germination of the pathogen

Maximum inhibition (100 per cent) of spore germination of the pathogen *C. fructicola* was induced by a combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and mancozeb (0.4 per cent) (Table 4). This was followed by two per cent talc based formulation of *P. fluorescens* and *T. viride* which resulted in 95 per cent and 93.67 per cent inhibition of spore germination of *C. fructicola.* Mixing the foliar fertilizer NPK 19:19:19 (0.5 per cent) with azoxystrobin (0.15ml/l) resulted in 91.33 per cent inhibition of spore germination. The foliar fertilizer NPK 19:19:19 (0.5 per cent) as well as its combination with calcium nitrate (5g/l), were on par resulting in 85.33 per cent and 84.00 per cent inhibition of spore germination of *C. fructicola.* Least per cent inhibition (80.67 per cent) of spore germination of the pathogen as compared to control was observed in medium amended with calcium nitrate (5g/l) (Table 4).

4.3. Evaluation of foliar fertilizer, nutrient, bio-control agents and fungicides against anthracnose leaf spot pathogen under greenhouse conditions

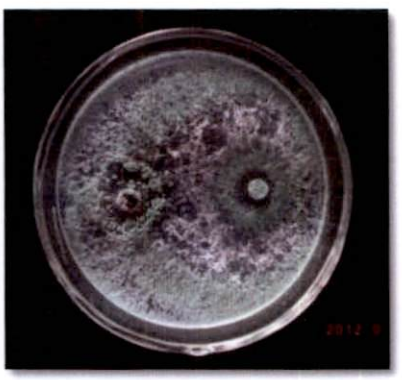
Two pot culture experiments were conducted in CRD with three replications to assess the efficacy of 12 treatments for the evaluation of foliar fertilizer, nutrient, bio-control agents and fungicides against anthracnose leaf spot disease of culinary melon. Observations on disease incidence (DI), percentage disease index (PDI), and biometric characters such as plant height, leaf length, leaf breadth, number of branches, number of leaves and number of fruits were recorded (Table 5a, 5b, 5c) (Plate 12, 13,14).

Table 3b. *In vitro* antagonism of *Trichoderma viride* and *Pseudomonas fluorescens* against *Colletotrichum fructicola*

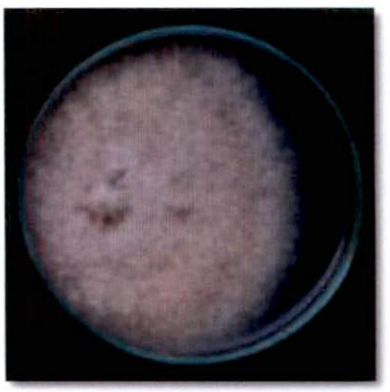
Sl No	Antagonists	Mycelial growth (cm)*	Per cent reduction over control*
1	*Trichoderma viride*	1:23[a] (0.18)	(86.33)[a] (96.50)
2	*Pseudomonas fluorescens*	1.16[a](0.17)	(87.14)[a] (96.64)
3	Absolute control	9.00[b](1.66)	-
	CD (0.05)	0.121 (0.915)	0.496 (1.235)

*Mean of seven replications

Values in parenthesis are arcsine transformed



Trichoderma viride



Absolute control

Pseudomonas fluorescens

Absolute control

Plate 11. Effect of bio-control agents against the mycelial growth of *C. fructicola* by dual culture technique

Table 4. Effects of foliar fertilizer, nutrients, bio-control agents and fungicides on spore germination of *Colletotrichum fructicola* under *in vitro* conditions

Sl No	Treatments	Spore germination (%)*	Per cent reduction over control*
1	NPK19:19:19 (0.5 %)	14.67^{e}(7.44)	85.33^{e}(94.01)
2	NPK 19:19:19 (0.5%) + mancozeb (0.4%)	0.00^{a}(0.00)	100.00^{a}(98.50)
3	NPK19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l)	8.00^{d}(3.34)	91.33^{d}(96.11)
4	NPK 19:19:19 (0.5%) + Calcium nitrate (5g/l)	19.33^{g}(11.29)	80.67^{g}(93.51)
5	Calcium nitrate (5g/l)	16.00^{f}(8.44)	84.00^{f}(92.14)
6	Talc based formulation of *Pseudomonas fluorescens* (2%)	5.00^{b}(1.91)	95.00^{b}(97.23)
7	Talc based formulation of *Trichoderma viride* (2%)	6.33^{c}(2.53)	93.67^{c}(96.83)
8	Absolute control	100^{h}(98.50)	-
	CD (0.05)	1.162(2.225)	1.219(2.384)

* Mean of three replications

Values in parenthesis are arcsine transformed

4.3.1. Disease incidence

Minimum disease incidence (DI) was recorded in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (23.33 per cent) and was on par with that of (T4) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (23.33 per cent) in first evaluation. In second evaluation, DI of plants sprayed with (T2) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (16.67 per cent) was significantly superior to all other treatments. Plants sprayed with (T1) NPK 19:19:19 (0.5 per cent) + adjuvant and plants sprayed with (T6) NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant recorded DI of (33.33 per cent) in first evaluation and were on par. In second evaluation DI of plants sprayed with (T4) NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l + adjuvant (20.00 per cent) was significantly superior to that of (T6) plants sprayed with NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant (26.67 per cent) and (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + adjuvant (30.00 per cent). In first evaluation DI of (T3) plants sprayed with NPK 19:19:19 (0.5 per cent) + adjuvant followed by foliar spray of mancozeb (0.4 per cent) and (T7) plants sprayed with calcium nitrate (5g/l) + adjuvant (40.00 per cent) were on par and were significantly superior to that of (T5) plants sprayed with NPK 19:19:19 (0.5 per cent) + adjuvant followed by foliar spray of fungicide azoxystrobin (0.15ml/l) (43.33 per cent) and that of plants sprayed with (T8) *P. fluorescens* @ 2 per cent + adjuvant and (T9) talc based formulations of *T. viride* @ 2 per cent + adjuvant (43.33 per cent, 43.33 per cent, 43.33 per cent respectively). In second evaluation DI of (T8) and (T9) (40.00 per cent) were on par and were significantly superior to DI of (T10) farmers' management practices and (T11) package of practices recommendations (POP) (KAU) (43.33 per cent) which were on par. In first evaluation (T10) and (T11) (46.67 per cent) were on par and were significantly superior to that of (T12) control (100.00 per cent). In first and second evaluation, the

maximum DI was recorded in plants of control (T12) (100.00 and 85.00 per cent) respectively (Table 5a, 5b).

Pooled data of DI ranged from 20.00 to 92.50 per cent. Minimum DI (20.00 per cent) was recorded in plants sprayed with (T2) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant as compared to all other treatments. DI recorded for plants (T4) (21.67 per cent), (T6) (30.00 per cent), (T1) (31.67 per cent), (T3) (36.67 per cent) were significantly superior to DI of (T5) plants sprayed with NPK 19:19:19 (0.5 per cent) + adjuvant followed by foliar spray of azoxystrobin (0.15ml/l) (38.33 per cent) which was on par with DI of (T7) (36.67 per cent). DI of plants sprayed with (T7) (36.67 per cent) was significantly superior to that of (T8) (41.67 per cent) which in turn was on par with DI of (T9) (41.67 per cent). DI recorded in (T10) and (T11) (45.00 per cent) were on par and significantly superior to that of (T12) (92.50 per cent). Maximum DI was recorded in plants of control (T12) (92.50 per cent) (Table 5c).

4.3.1.1. Percentage Disease Index (PDI)

Minimum percent disease index (PDI) was recorded in plants that received treatment of foliar spray of NPK 19:19:19 (0.5 per cent) + fungicide mancozeb (0.4 per cent) + adjuvant (T2) in first and second evaluations (12.59 and 11.85 per cent respectively) and it was significantly superior to all other treatments. This was followed by the treatment (T4) foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant which recorded PDI of (14.07 per cent) and (15.92 per cent) in both evaluations and was significantly superior to the remaining treatments. Treatments plants sprayed with (T6) NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant (17.40 per cent, 18.14 per cent), (T1) NPK 19:19:19 (0.5 per cent) + adjuvant (19.63 per cent, 20.74 per cent), (T3) NPK 19:19:19 (0.5 per cent) + adjuvant + mancozeb (0.4 per cent) (20.74 per cent, 22.59 per cent) and (T5)

Plate 12. General view of pot culture experiment

Table 5a. Efficacy of foliar fertilizer, nutrients, fungicides and bio-control agents on anthracnose leaf spot disease and growth parameters of culinary melon plants in greenhouse study I (March 2014 – June 2014)

Sl.No	Treatments	Pl.Ht (cm)	Leaf length (cm)	Leaf breadth (cm)	No. of branches	No.of.leaves	No. of fruits	Per cent disease index*	Per cent disease incidence*
1	T1	36.13^{b}	9.83^{d}	9.70^{d}	4.33^{b}	23.00^{d}	6.00^{b}	19.63^{d}(11.56)	33.33^{b}(29.48)
2	T2	42.90^{a}	11.10^{a}	10.8^{a}	6.00^{a}	32.00^{a}	8.33^{a}	12.59^{a}(6.00)	23.33^{a}(15.27)
3	T3	32.93^{c}	9.57^{e}	9.57^{d}	3.67^{c}	21.00^{d}	5.33^{b}	20.74^{e}(12.66)	40.00^{c}(41.64)
4	T4	36.90^{b}	10.80^{b}	10.5^{b}	5.33^{a}	29.00^{b}	8.00^{a}	14.07^{b}(7.01)	23.33^{a}(15.27)
5	T5	31.40^{d}	8.80^{g}	8.30^{g}	3.33^{c}	19.67^{d}	5.00^{b}	22.59^{f}(14.61)	43.33^{d}(47.98)
6	T6	36.17^{b}	10.50^{c}	10.10^{c}	4.67^{b}	25.33^{c}	7.00^{a}	17.40^{c}(9.58)	33.33^{b}(29.48)
7	T7	29.40^{e}	8.10^{e}	8.03^{h}	2.33^{d}	20.33^{d}	4.33^{c}	25.92^{g}(18.58)	40.00^{c}(41.64)
8	T8	33.37^{c}	9.50^{e}	9.13^{e}	4.00^{b}	20.67^{d}	4.66^{c}	26.29^{g}(19.06)	43.33^{d}(47.98)
9	T9	32.03^{d}	9.30^{f}	8.70^{f}	3.00^{d}	21.33^{d}	3.66^{c}	27.03^{g}(20.04)	43.33^{d}(47.98)
10	T10	28.17^{f}	7.80^{i}	7.73^{i}	2.00^{e}	20.00^{d}	3.33^{d}	29.25^{h}(23.17)	46.67^{e}(54.36)
11	T11	29.67^{e}	8.60^{g}	8.13^{g}	2.66^{d}	18.33^{e}	3.00^{d}	29.63^{h}(23.72)	46.67^{e}(54.36)
12	T12	20.20^{g}	5.53^{j}	5.20^{j}	1.33^{e}	10.00^{f}	1.33^{e}	50.00^{i}(60.55)	100.00^{f}(98.50)
	CD (0.05)	1.050	0.194	0.223	0.930	1.942	1.221	1.028(2.735)	2.900(2.366)

*Mean of three replications Values in parenthesis are arcsine transformed

T1 - Fertilizer application by foliar spray @ 0.5per cent (19:19:19 NPK) + adjuvant

T2 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (Mancozeb 0.4 per cent) + adjuvant

T3 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + adjuvant followed by foliar spray of fungicide (Mancozeb @ 0.4 per cent)

T4 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T5 - Fertilizer application @ 0.5 per cent (19:19:19 NPK) + adjuvant followed by foliar spray of newer fungicide (Azoxystrobin @ 0.15ml/l)

T6 - Fertilizer application @ 0.5 per cent (19:19:19 NPK) + Calcium nitrate (5g/l) + adjuvant

T7 - Foliar spray of Calcium nitrate (5g/l) + adjuvant

T8 - Foliar spray of *Pseudomonas fluorescens* @ 2 per cent + adjuvant

T9 - Foliar spray of *Trichoderma viride* @ 2 per cent + adjuvant

T10 - Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted).

T11 - Cultivation practices according to POP (KAU)

T12 - Absolute control

T2 - 0.5% 19:19:19 NPK +
0.4% Mancozeb + adjuvant

T4 - 0.5% 19:19:19 NPK +
Azoxystrobin 0.15 ml/lit +adjuvant

T6 - 0.5% 19:19:19 NPK +
$CaNO_3$ (5g/lit) + adjuvant

T1- 0.5% 19:19:19 NPK +
adjuvant

Plate 13. Evaluation of foliar fertilizer, nutrient, bio-control agents and fungicides against anthracnose leaf spot disease (March 2014 – June 2014) in pot culture experiment I

Table 5b. Efficacy of foliar fertilizer, nutrients, fungicides and bio-control agents onanthracnose leaf spot disease and growth parameters of culinary melon plants in greenhouse study II (August 2014 - October 2014)

Sl.No	Treatments	Pl.Ht (cm)	Leaf length (cm)	Leaf breadth(cm)	No. of branches	No.of.leaves	No. of fruits	Per cent disease index*	Per cent disease incidence*
1	T1	45.57^{c}	11.37^{b}	11.30^{b}	4.67^{c}	31.00^{d}	6.67^{c}	20.74^{d} (12.66)	30.00^{d} (24.29)
2	T2	50.10^{a}	12.40^{a}	12.27^{a}	6.67^{a}	38.00^{a}	9.33^{a}	11.85^{a} (5.53)	16.67^{a} (11.94)
3	T3	42.23^{d}	11.00^{b}	11.17^{b}	4.33^{c}	28.33^{d}	5.67^{d}	22.59^{e} (14.61)	33.33^{e} (41.64)
4	T4	47.20^{b}	11.90^{a}	12.17^{a}	6.00^{a}	34.67^{b}	8.33^{a}	15.92^{b} (8.37)	20.00^{b} (11.94)
5	T5	40.03^{e}	10.47^{c}	10.10^{c}	4.66^{c}	29.33^{d}	6.00^{c}	24.44^{f} (16.74)	33.33^{e} (41.64)
6	T6	46.33^{b}	11.50^{b}	11.33^{b}	5.67^{b}	33.00^{c}	7.33^{b}	18.14^{c} (10.22)	26.67^{c} (11.94)
7	T7	38.93^{f}	10.60^{c}	10.43^{c}	3.00^{e}	28.00^{d}	5.00^{e}	25.92^{f} (18.58)	33.33^{e} (41.64)
8	T8	43.10^{d}	10.03^{c}	9.93^{c}	4.00^{c}	27.00^{e}	5.33^{e}	27.40^{g} (20.54)	40.00^{f} (41.64)
9	T9	40.43^{e}	9.97^{c}	10.40^{c}	3.67^{d}	24.33^{f}	4.67^{f}	28.51^{h} (22.09)	40.00^{f} (41.64)
10	T10	39.03^{f}	9.83^{d}	9.83^{c}	2.67^{f}	24.67^{f}	3.67^{f}	30.00^{i} (24.27)	43.33^{g} (60.55)
11	T11	40.53^{e}	10.33^{c}	9.70^{d}	2.33^{f}	21.67^{g}	3.33^{g}	30.37^{i} (24.84)	43.33^{g} (60.55)
12	T12	22.03^{g}	5.27^{e}	5.17^{e}	1.33^{g}	11.00^{h}	1.67^{h}	48.51^{j} (57.85)	85.00^{h} (98.50)
	CD(0.05)	1.219	0.652	0.656	0.971	1.414	1.121	1.049(2.692)	2.793(2.522)

*Mean of three replications

Values in parenthesis are arcsine transformed

T1 - Fertilizer application by foliar spray @ 0.5per cent (19:19:19 NPK) + adjuvant

T2 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant

T3 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + adjuvant followed by foliar spray of fungicide (mancozeb @ 0.4 per cent)

T4 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T5 - Fertilizer application @ 0.5 per cent (19:19:19 NPK) + adjuvant followed by foliar spray of newer fungicide (Azoxystrobin @ 0.15ml/l)

T6 - Fertilizer application @ 0.5 per cent (19:19:19 NPK) + Calcium nitrate (5g/l) + adjuvant

T7 - Foliar spray of Calcium nitrate (5g/l) + adjuvant

T8 - Foliar spray of *Pseudomonas fluorescens* @ 2 per cent + adjuvant

T9 - Foliar spray of *Trichoderma viride* @ 2 per cent + adjuvant

T10 - Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted).

T11 - Cultivation practices according to POP (KAU)

T12 - Absolute control

T2 - 0.5% 19:19:19 NPK +
0.4% Mancozeb + adjuvant

T4 - 0.5% 19:19:19 NPK +
Azoxystrobin 0.15 ml/lit + adjuvant

T6 - 0.5% 19:19:19 NPK +
$CaNO_3$ (5g/lit) + adjuvant

T1-0.5% 19:19:19 NPK + adjuvant

Plate 14. Evaluation of foliar fertilizer, nutrient, bio-control agents and fungicides against anthracnose leaf spot disease (August 2014 - October 2014) in pot culture experiment II

0.5 per cent (19:19:19 NPK) + adjuvant + azoxystrobin (0.15ml/l) (22.59 per cent, 24.44 per cent) were significantly superior to each other in the respective ascending order of PDI in first and second evaluations. In first evaluation plants sprayed with (T5) fertilizer application NPK 19:19:19 (0.5 per cent) + adjuvant followed by foliar spray of fungicide azoxystrobin (0.15ml/l) was significantly superior to that of (T7) calcium nitrate (5g/l) + adjuvant (25.92 per cent) and the latter treatment was on par with (T8) *P. fluorescens* @ 2 per cent + adjuvant (26.29 per cent) and (T9) *T. viride* @ 2 per cent + adjuvant (27.03 per cent).

In second evaluation (T5) NPK 19:19:19 (0.5 per cent) + adjuvant followed by fungicide spray azoxystrobin (0.15ml/l) (24.44 per cent) was on par with (T7) calcium nitrate (5g/l) + adjuvant (25.92 per cent) and was significantly superior to (T8) *P. fluorescens* @ 2 per cent + adjuvant (27.40 per cent), (T9) *T. viride* @ 2 per cent + adjuvant (28.51 per cent). In first and second evaluation (T10) farmers' management practices (29.25 per cent, 30.00 per cent) and (T11) package of practices recommendations (POP) (KAU) (29.63 per cent, 30.37 per cent) were on par superior to (T12) control (50.00 per cent, 48.51 per cent). In both evaluation, the maximum PDI was recorded in control plants (50.00 and 48.51 per cent respectively) (Table 5a, 5b).

Pooled data of PDI ranged from (12.22 to 49.25 per cent). Minimum PDI (12.22 per cent) was recorded in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + fungicide mancozeb (0.4 per cent) + adjuvant as compared to all other treatments. This was followed by the treatment (T4) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant which recorded PDI of (14.99 per cent) and was significantly superior to the remaining treatments. Treatments (T6) foliar spray of NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant (17.77 per cent), (T1) foliar spray of NPK 19:19:19 (0.5 per cent) + adjuvant (20.18 per cent), (T3) foliar spray of 0.5 per cent (19:19:19 NPK) + adjuvant +

fungicide mancozeb (0.4 per cent) (21.66 per cent) and (T5) foliar spray of NPK 19:19:19 (0.5 per cent) + adjuvant + azoxystrobin (0.15ml/l) (23.51 per cent) were significantly superior to each other in the respective ascending order of PDI. (T7) foliar sprayed with calcium nitrate (5g/l) + adjuvant (25.92 per cent), (T8) foliar sprayed with *P. fluorescens* @ two per cent + adjuvant (26.84 per cent) and (T9) foliar sprayed with *T. viride* @ two per cent + adjuvant (27.77 per cent) were on par with each other and superior to that of (T10) plants raised by farmers' management practices (29.62 per cent) and were on par with (T11) plants raised by package of practices recommendations (POP) (KAU) (30.00 per cent). Maximum PDI was recorded in plants of control (T12) (49.25 per cent) (Table 5c).

4.3.2. Biometric observations

4.3.2.1. Plant height (cm)

Plants sprayed with (T2) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) recorded maximum plant height (42.90 cm) in first evaluation and (50.10 cm) in second evaluation and was significantly superior to the remaining treatments in both evaluations. This was followed by (T4) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant which recorded plant height of (36.90 cm) in first evaluation and (47.20 cm) in second evaluation and were on par with (T6) plants sprayed with NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant (46.33 cm) in second evaluation. In first evaluation (T4) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant was on par with (T6) foliar spray with NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant which recorded the plant height of (36.17 cm). In the same evaluation (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + adjuvant (36.13 cm) was on par with (T6) plants sprayed with NPK 19:19:19 (0.5 per cent) + calcium nitrate + adjuvant (36.17 cm) and in second evaluation (T1) recorded (45.57 cm). Plant height recorded

Table 5c. Pooled analysis of data of greenhouse studies I and II

Sl.No	Treatments	Pl.Ht (cm)	Leaf length (cm)	Leaf breadth (cm)	No. of branches	No.of. leaves	No. of fruits	Per cent disease index*	Per cent disease incidence*
1	T1	40.85^{b}	10.60^{a}	10.50^{a}	4.50^{c}	27.00^{b}	6.33^{c}	20.18^{d} (12.11)	31.67^{b} (26.88)
2	T2	46.50^{a}	11.75^{a}	11.53^{a}	6.33^{a}	35.00^{a}	8.83^{a}	12.22^{a} (5.76)	20.00^{a} (13.60)
3	T3	37.58^{c}	10.28^{b}	10.37^{a}	4.00^{d}	24.67^{c}	5.50^{d}	21.66^{e} (13.65)	36.67^{c} (41.63)
4	T4	42.05^{b}	11.35^{a}	11.33^{a}	5.67^{a}	31.83^{b}	8.17^{a}	14.99^{b} (7.69)	21.67^{a} (13.60)
5	T5	37.22^{c}	9.63^{b}	9.20^{b}	4.00^{d}	24.50^{c}	5.50^{d}	23.51^{f} (15.67)	38.33^{c} (44.81)
6	T6	41.25^{b}	11.00^{a}	10.73^{a}	5.17^{b}	29.17^{b}	7.17^{b}	17.77^{c} (9.90)	30.00^{b} (20.71)
7	T7	34.17^{d}	9.35^{b}	9.23^{b}	2.67^{f}	24.17^{d}	4.67^{e}	25.92^{g} (18.58)	36.67^{c} (41.64)
8	T8	38.23^{c}	9.77^{b}	9.53^{b}	4.00^{d}	23.83^{d}	5.00^{d}	26.84^{g} (19.80)	41.67^{d} (44.81)
9	T9	36.23^{c}	9.63^{b}	9.55^{b}	3.33^{e}	22.83^{d}	4.17^{e}	27.77^{g} (21.07)	41.67^{d} (44.81)
10	T10	33.60^{d}	8.82^{c}	8.78^{b}	2.33^{g}	22.33^{d}	3.50^{f}	29.62^{h} (23.72)	45.00^{e} (57.46)
11	T11	35.10^{c}	9.47^{b}	8.92^{b}	2.50^{f}	20.00^{e}	3.17^{g}	30.00^{h} (24.29)	45.00^{e} (57.46)
12	T12	21.12^{e}	5.40^{d}	5.18^{c}	1.33^{h}	10.50^{f}	1.50^{h}	49.25^{i} (59.20)	92.50^{f} (98.50)
	CD(0.05)	3.349	1.313	1.381	0.904	2.742	0.876	1.229(1.290)	2.938(2.217)

*Mean of three replications

Values in parenthesis are arcsine transformed

T1 - Fertilizer application by foliar spray @ 0.5per cent (19:19:19 NPK) + adjuvant

T2 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant

T3 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + adjuvant followed by foliar spray of fungicide (mancozeb @ 0.4 per cent)

T4 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T5 - Fertilizer application @ 0.5 per cent (19:19:19 NPK) + adjuvant followed by foliar spray of newer fungicide (Azoxystrobin @ 0.15ml/l)

T6 - Fertilizer application @ 0.5 per cent (19:19:19 NPK) + Calcium nitrate (5g/l) + adjuvant

T7 - Foliar spray of Calcium nitrate (5g/l) + adjuvant

T8 - Foliar spray of *Pseudomonas fluorescens* @ 2 per cent + adjuvant

T9 - Foliar spray of *Trichoderma viride* @ 2 per cent + adjuvant

T10 - Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted).

T11 - Cultivation practices according to POP (KAU)

T12 - Absolute control

in (T8) foliar sprayed with two per cent *P. fluorescens* and (T3) NPK 19:19:19 (0.5 per cent) + adjuvant + mancozeb (0.4 per cent) were on par in first evaluation (33.37cm and 32.93 cm) and also in second evaluation (43.10 cm and 42.23 cm) respectively. Application of (T9) two per cent *T. viride* recorded the plant height of (32.03 cm) in first evaluation and was significantly superior to the remaining treatment *viz.*, (T5) NPK 19:19:19 (0.5 per cent) + adjuvant (31.40 cm), (T11) (29.67 cm), (T7) (29.40 cm), (T10) (28.17 cm) and (T12) (20.20 cm). In second evaluation (T9) (40.43 cm) was on par with (T11) package of practices recommendations (POP) (KAU) (40.53 cm), (T5) NPK 19:19:19 (0.5 per cent) + adjuvant + azoxystrobin (0.15ml/l) (40.03 cm). In first evaluation (T5) recorded plant height of (31.40 cm) and was significantly superior to (T11) (29.67 cm) and (T7) calcium nitrate (5g/l) + adjuvant (29.40 cm). In second evaluation, (T10) farmers' management practices and (T7) were on par (39.03 cm and 38.93 cm). In first evaluation (T10) recorded lower plant height of (28.17 cm). In both first and second evaluation, the minimum plant height was recorded in control plants (20.20 cm and 22.03 cm) respectively (Table 5a, 5b).

Pooled data of plant height ranged from (21.12 cm to 46.50 cm). Maximum plant height (46.50 cm) was recorded in (T2) as compared to control. Plant height recorded for (T4) (42.05 cm), (T6) (41.25 cm), (T1) (40.85 cm) closely followed (T2) and were on par. Plant height of remaining treatments (T8) (38.23 cm), (T3) (37.58 cm), (T5) (37.22 cm), (T9) (36.23 cm), (T11) (35.10 cm) ranged from (35.10 cm to 38.23 cm) and were on par. Plant height recorded in (T7) (34.17cm) and (T10) (33.60 cm) were lower and on par (Table 5c).

4.3.2.2. Leaf length (cm)

(T2) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant recorded maximum leaf length (11.10 cm) in first evaluation and (12.40 cm) in

second evaluation and was significantly superior to all other treatments in first evaluation. In second evaluation (T4) NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (11.90 cm) was on par with (T2) (12.40 cm). In the first and second evaluations (T4) (10.80 cm, 11.90 cm) was significantly superior to the rest of the treatments. This was followed by (T6) NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant (10.50 cm, 11.50 cm) in first and second evaluation and was superior to the remaining treatments. In first evaluation (T6) (10.50 cm) was significantly superior to (T1) NPK 19:19:19 (0.5 per cent) + adjuvant (9.83 cm) and (T3) NPK 19:19:19 (0.5 per cent) + adjuvant + mancozeb (0.4 per cent) (9.57 cm) whereas in second evaluation (T6) (11.50 cm) was on par with two later treatments. Leaf length of (T8) 2 per cent *P. fluorescens* (9.50 cm) was significantly superior to (T9) *T. viride* @ 2 per cent + adjuvant (9.30 cm) which closely followed it. In second evaluation (T7) calcium nitrate (5g/l) + adjuvant (10.60 cm), (T5) (10.47 cm), (T11) package of practices recommendations (POP) (KAU) (10.33 cm), (T8) (10.03 cm), (T9) (9.97 cm) were on par. In first evaluation (T5) NPK 19:19:19 (0.5 per cent) + adjuvant + azoxystrobin (0.15ml/l) (8.80 cm) and (T11) (8.60 cm) were on par and significantly superior to (T7) calcium nitrate (5g/l) + adjuvant (8.10 cm) and (T10) farmers' management practices (7.80 cm). In second evaluation (T10) (9.83 cm) recorded lower leaf length compared to the above mention treatments. In both first and second evaluation, the minimum leaf length was recorded in control (5.53 cm and 5.27 cm respectively) (Table 5a, 5b).

Pooled data of leaf length ranged from (5.40 cm to11.75 cm). Maximum leaf length (11.75) cm was recorded in (T2). Leaf length recorded in (T4) (11.35 cm), (T6) (11.00 cm) and (T1) (10.60 cm) were on par. Leaf length of remaining treatments *viz.*, (T3) (0.28 cm), (T8) (9.77 cm), (T5) (9.63 cm), (T9) (9.63 cm), (T11) (9.47 cm), (T7) (9.35 cm) ranged from (9.35 cm to 10.28 cm) and were on par. Leaf length recorded in (T10) (8.82 cm) was significantly superior to (T12) (5.40 cm) which had the minimum length of (5.40 cm) (Table 5c).

4.3.2.3. Leaf breadth

(T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant recorded maximum leaf breadth (10.80 cm) in first evaluation and (12.27 cm) in second evaluation and was significantly superior to all other treatments in first evaluation. In second evaluation (T4) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (12.17 cm) was on par with (T2) (12.27 cm). In the first and second evaluations (T4) (10.50 cm, 12.17 cm respectively) was significantly superior to the rest of the treatments. This was followed by (T6) plants sprayed with NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant (10.13 cm, 11.33 cm) in first and second evaluation and was superior to the remaining treatments. In first evaluation (T6) plants sprayed with (10.13 cm) was significantly superior to (T1) NPK 19:19:19 (0.5 per cent) + adjuvant (9.70 cm) and (T3) NPK 19:19:19 (0.5 per cent) + adjuvant + mancozeb (0.4 per cent) (9.57 cm) whereas in second evaluation (T6) (11.33 cm) was on par with two later treatments. Leaf breadth recorded in (T8) two per cent *P. fluorescens* (9.13 cm) was significantly superior to (T9) two per cent *T. viride* (8.70 cm) which closely followed it. In second evaluation (T7) calcium nitrate (5g/l) + adjuvant (10.43 cm), (T9) (10.40 cm), (T5) (10.10 cm), (T8) (9.93 cm), (T10) plants which received farmers' management practices (9.83 cm) were on par. In first evaluation (T5) 0.5 per cent (19:19:19 NPK) + adjuvant followed by azoxystrobin (0.15ml/l) (8.30 cm) and (T11) plants which received package of practices recommendations (POP) (KAU) (8.13 cm) were on par and significantly superior to (T7) (8.03 cm) and (T10) (7.73 cm). In second evaluation (T11) (9.70 cm) was significantly superior to (T12) control (5.27 cm) which recorded the lowest leaf breadth in both evaluation (Table 5a, 5b).

Pooled data of leaf breadth ranged from (5.18 cm to 11.53 cm). Maximum leaf breadth was (11.33 cm) recorded in (T2). Leaf breadth recorded in (T4)

(11.33 cm), (T6) (10.73 cm), (T1) (10.50 cm) and (T3) (10.37) were on par. Leaf breadth of remaining treatments *viz.*, (T9) (9.55 cm), (T8) (9.53 cm), (T7) (9.23 cm), (T5) (9.20 cm), (T11) (8.92 cm), (T10) (8.78 cm) were on par. Leaf breadth recorded in treatment (T10) farmers' management practices (8.78 cm) was significantly superior to (T12) (5.18 cm) which had the minimum breadth of (5.18 cm) (Table 5c).

4.3.2.4. Number of branches

(T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant recorded maximum number of branches (6.00) in first evaluation and (6.67) in second evaluation and was on par with (T4) those of plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (5.33, 6.00) in both evaluation. This was followed by (T6) plants sprayed with NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant which recorded (4.67) branches and was on par with (T1) NPK 19:19:19 (0.5 per cent) + adjuvant (4.33), (T8) (4.00). In second evaluation number of branches recorded (T6) (5.67) was significantly superior to those recorded in (T1) (4.67), (T5) NPK 19:19:19 (0.5 per cent) + adjuvant + azoxystrobin (0.15ml/l) (4.66), (T3) NPK 19:19:19 (0.5 per cent) + adjuvant + mancozeb (0.4 per cent) (4.33), (T8) two per cent *P. fluorescens* (4.00). This was followed by (T9) *T. viride* @ two per cent + adjuvant (3.00, 3.67) in both evaluations. In first evaluation (T9) (3.00) were on par with (T11) package of practices recommendations (POP) (KAU) (2.66). In second evaluation (T9 (3.67) was significantly superior to (T7) calcium nitrate (5g/l) + adjuvant (3.00), (T10) farmers' management practices (2.67), (T11) (2.33). In first evaluation average number of branches in (T10) (2.00) on par with (T7) (2.33) and in second evaluation it was on par with (T11) (2.33). In both evaluations, the minimum number of branches was recorded in (T12) control (1.33 cm) (Table 5a, 5b).

Pooled data of number of branches ranged from (1.33 to 6.33). Maximum number of branches (6.33) was recorded in (T2) which was on par with (T4) (5.67). This was followed by (T6) (5.17) significantly superior to all other treatments. (T1) recorded average of (4.50) branches and was significantly superior to that of (T5) (4.00), (T3) (4.00), (T8) (4.00) which were equal and on par. Average number of branches (T9) (3.33) was significantly superior to that of (T7) (2.67) and (T11) (2.50) and the latter two treatments were on par. Treatment (T10) farmers' management practices produced comparatively lower number of branches (2.33) and was significantly superior only to the control plants (1.33) in (T12) (Table 5c).

4.3.2.5. Number of leaves

(T2) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant recorded maximum number of leaves (32.00) in first evaluation and (38.00) in second evaluation and was significantly superior to the remaining treatment. This was followed by (T4) NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant which recorded an average of 29.00 leaves in first evaluation and 34.67 in second evaluation. (T4) (29.00, 34.67) was significantly superior to (T6) NPK 19:19:19 (0.5 per cent) + calcium nitrate (0.15 ml/l) + adjuvant (25.33, 33.00) and (T1) NPK 19:19:19 (0.5 per cent) + adjuvant (23.00, 31.00) in both evaluations. In first evaluation (T1) NPK 19:19:19 (0.5 per cent) + adjuvant (23.00) was on par with (T9) *T. viride* @ two per cent + adjuvant (21.33), (T3) NPK 19:19:19 (0.5 per cent) + adjuvant + mancozeb (0.4 per cent) (21.00), (T8) two per cent *P. fluorescens* (20.67), (T7) calcium nitrate (5g/l) + adjuvant (20.33), (T10) (20.00), (T5) (19.67) and in second evaluation it was on par with (T5) 0.5 per cent (19:19:19 NPK) + adjuvant + azoxystrobin (0.15ml/l) (29.33), (T3) (28.33), (T7) (28.00). In first evaluation (T11) package of practices recommendations (POP) (KAU) (18.33) was significantly superior to (T12) (10.00). In second evaluation (T8) two per cent *P. fluorescens* (27.00) was significantly superior to (T10) farmers' management practices (24.67)

and were on par with (T9) (24.33). In second evaluation (T9) (24.33) was significantly superior to (T11) (21.67) and (T12) control (11.00). In first and second evaluation, the minimum number of leaves was recorded in control plants (10.00 and 11.00 respectively) (Table 5a, 5b).

Pooled data of number of leaves ranged from (10.50 to 35.00). Maximum number of leaves (35.00) was recorded in (T2) as compared to control. Number of leaves was recorded for (T4) (31.83), (T6) (29.17), (T1) (27.00) were on par. This was followed by (T3) (24.67) was on par with (T5) (24.50) and was significantly superior to (T7) (24.17). Number of leaves was recorded for the remaining treatments (T7) (24.17 cm), (T8) (23.83 cm), (T9) (22.83 cm), (T10) (22.33) were on par. Number of leaves (T11) (20.00) was significantly superior to (T12) (10.50). The minimum number of leaves was recorded in control plants (10.50) (Table 5c).

4.3.2.6. Number of fruits

(T2) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant recorded maximum number of fruits (8.33) in first evaluation and (9.33) in second evaluation and was on par with (T4) NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (8.00, 8.33) in both evaluations. This was followed by treatment T6 NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant which recorded average of 7.00 fruits and was on par with (T1) NPK 19:19:19 (0.5 per cent)) + adjuvant (6.00) in first evaluation. In second evaluation number of fruits recorded (T6) (7.33) was significantly superior to those recorded in (T1) (6.67) which was on par with (T5) NPK 19:19:19 (0.5 per cent) + adjuvant + azoxystrobin (0.15ml/l) (6.00). In first evaluation (T3) NPK 19:19:19 (0.5 per cent) + adjuvant + mancozeb (0.4 per cent) recorded average number of fruits of (5.33) which was on par with (T5) (5.00), (T8) two per cent *P. fluorescens* (4.66), (T7) (4.33) and was significantly superior to those of (T9) *T. viride* @ two per cent + adjuvant (3.66)

which was on par with (T10) (3.33). In second evaluation (T5) (6.00) was significantly superior to (T3) (5.67) and (T8) (5.33) was on par with (T7) (5.00). In first and second evaluation (T9) (3.66, 4.67) was on par with (T10) farmers' management practices (3.33, 3.67) and was significantly superior to that of (T11) package of practices recommendations (POP) (KAU) (3.00 and 3.33 respectively) and (T12) control plants (1.33, 1.67 respectively). The lowest number of fruits was recorded in control plants (1.33 and 1.67 respectively) (Table 5a, 5b).

Pooled data of number of fruits ranged from (1.50 to 8.83). Maximum number of fruits (8.83) was recorded in (T2) as compared to control. This was followed by (T4) (8.17) was on par with (T2) (8.83) and was significantly superior to that of (T6) (7.17) and (T1) (6.38). Number of fruits recorded in (T3) (5.50), (T5) (5.50), (T8) (5.00) were on par and was significantly superior to (T7) (4.67) and in the same treatment (T7 was on par with (T9) (4.17). Number of fruits was recorded for (T10) (3.50), (T11) (3.17), (T12) (1.50) was significantly superior to the other treatments. The lowest number of fruits noticed in control plants (1.50) (Table 5c).

4.4. Evaluation of effective treatments in field experiments (Two seasons)

Based on the results of greenhouse experiments, four most effective treatments were screened and tested during the period of (March 2014 – June 2014) and (August 2014 – October 2014) at Instructional Farm, Vellayani, in field trial conducted at CoA, Vellayani. The performance of these treatments were compared with treated check (package of practices recommendations (POP) KAU, farmers' management practices and absolute control) and evaluated for their efficacy controlling anthracnose leaf spot disease. Observations on disease index, incidence and growth parameters such as fruit weight, fruit length, fruit breadth and total yield were recorded (Table 6a, 6b, 6c) (Plate 15, 16, 17, 18).

4.4.1. Disease incidence (DI) and Percent disease index (PDI)

In first trial DI and PDI of (T1), plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant was minimum (37.50 and 13.05 respectively) and was significantly superior to all other treatments. This was closely followed by (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant which recorded DI and PDI of (41.25 and 14.16 respectively) were significantly superior to the remaining treatments. In second trial DI and PDI of (T2) (38.75 and 11.94 respectively) was significantly superior to the remaining treatments. This was closely followed by (T1) which recorded DI and PDI of (42.50 and 13.33 respectively) which were on par with (T4) plants sprayed with NPK 19:19:19 (0.5 per cent) + adjuvant (43.75 and 13.88 respectively). In first trial DI and PDI of (T3) plants sprayed with NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant (50.00 and 16.38) was significantly superior to the remaining treatments. This was followed by (T4) which recorded DI and PDI of (52.50 and 17.49 respectively) and was on par with (T6) plants raised by cultivation and plant protection measures of package of practices recommendations (POP) (KAU) (52.50 and 17.77 respectively). In second trial DI and PDI of (T6) (51.25 and 16.10 respectively) was significantly superior to the other treatments. This was followed by DI and PDI of (T3) (55.00 and 20.27 respectively), (T5) farmers' practice of crop management and plant protection in culinary melon (58.75 and 24.72 respectively) was significantly superior to the other treatments. DI and PDI recorded in (T7) control plots were (87.50 and 50.00) in first trial and (85.00 and 48.05) respectively in second trial (Table 6a, 6b).

Pooled analysis revealed that lowest DI and PDI were recorded in (T1) (40.00 and 13.47 respectively) and (T2) (40.00 and 13.05 respectively) and the 2 treatments were on par for both parameters and significantly superior to other treatments. This was closely followed by PDI of (T4) (15.41) were on par with (T6) (16.94). DI of

Plate 15. Management of anthracnose leaf spot disease in culinary melon – field view of the experiment I (January 2015– March 2015)

Table 6a. Efficacies of foliar fertilizer, nutrients and fungicides against anthracnose leaf spot disease and on yield parameters of culinary melon plants in field trial I (January 2015 – March 2015)

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Total yield (kg)/pit	Per cent disease index*	Per cent disease incidence*
T1	1010.95[a]	27.96[a]	25.40[a]	3.03[a]	13.05[a] (6.25)	37.50[a] (38.47)
T2	988.53[b]	27.31[a]	25.17[a]	2.96[b]	14.16[b] (7.01)	41.25[b] (44.80)
T3	955.59[c]	26.93[b]	24.25[b]	2.86[c]	16.38[c] (8.37)	50.00[c] (60.55)
T4	938.45[c]	26.17[c]	24.35[a]	2.81[d]	17.49[d] (9.27)	52.50[d] (66.31)
T5	934.48[d]	25.95[c]	23.27[c]	2.80[d]	19.99[e] (12.28)	57.50[e] (71.53)
T6	924.85[d]	25.23[d]	22.82[d]	2.78[d]	17.77[d] (9.58)	52.50[d] (66.31)
T7	610.80[e]	22.52[e]	18.40[e]	1.83[e]	50.00[f] (59.88)	87.50[f] (95.14)
CD (0.05)	19.536	0.665	1.054	0.056	0.984(2.309)	2.135(2.243)

*Mean of four replications Values in parenthesis are arcsine transformed

T1- Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant

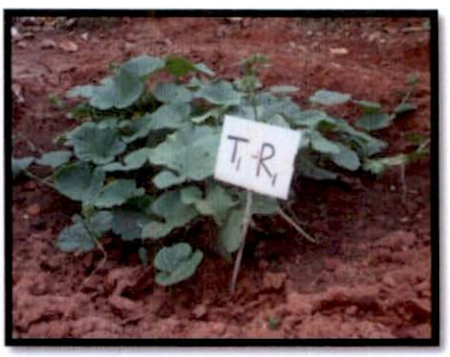
T2- Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T3- Fertilizer application @ 0.5 per cent (19:19:19 NPK) + Calcium nitrate (5g/l) + adjuvant

T4- Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + adjuvant

T5- Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted)

T6- Cultivation practices according to POP (KAU), T7- Absolute control



0.5 % 19:19:19 NPK +
Mancozeb 0.4 % + adjuvant

0.5 % 19:19:19 NPK +
Azoxystrobin 0.15ml/l + adjuvant

Absolute control

Plate 16. Evaluation of nutrient and fungicides on the management of culinary melon anthracnose leaf spot disease under field condition
(January 2015 – March 2015)

Plate 17. Management of anthracnose leaf spot disease in culinary melon – field view of the experiment II (April 2015– June 2015)

Table 6b. Efficacies of foliar fertilizer, nutrients and fungicides against anthracnose leaf spot disease and on yield parameters of culinary melon plantsin field trial II (April 2015 – June 2015)

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Total yield (kg)/pit	Per cent disease index*	Per cent disease incidence*
T1	1085^{c}	26.82^{a}	25.31^{b}	3.25^{b}	13.33^{b} (6.75)	42.50^{b} (48.01)
T2	2052^{a}	27.35^{a}	26.70^{a}	6.16^{a}	11.94^{a} (5.53)	38.75^{a} (41.57)
T3	856^{e}	28.23^{a}	26.14^{a}	2.56^{c}	20.27^{d} (12.66)	55.00^{d} (71.55)
T4	1125^{b}	28.35^{a}	27.36^{a}	3.37^{b}	13.88^{b} (6.75)	43.75^{b} (48.01)
T5	799^{f}	26.91^{a}	25.96^{a}	2.40^{d}	24.72^{e} (17.18)	58.75^{e} (73.90)
T6	934^{d}	27.69^{a}	26.62^{a}	2.80^{c}	16.10^{c} (8.37)	51.25^{c} (63.49)
T7	463^{g}	21.41^{b}	20.97^{c}	1.39^{e}	48.05^{f} (55.78)	85.00^{f} (94.62)
CD (0.05)	5.453	1.698	1.608	0.299	1.128 (2.174)	2.462 (2.196)

*Mean of four replications Values in parenthesis are arcsine transformed

T1- Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant

T2- Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T3- Fertilizer application @ 0.5 per cent (19:19:19 NPK) + Calcium nitrate (5g/l) + adjuvant

T4- Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + adjuvant

T5- Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted)

T6- Cultivation practices according to POP (KAU), T7- Absolute control

0.5 %19:19:19 NPK+ Azoxystrobin @ 0.15ml/l + adjuvant

0.5 % 19:19:19 NPK + 0.4% Mancozeb + adjuvant

Absolute control

Plate 18. Evaluation of nutrient and fungicides on the management of culinary melon anthracnose leaf spot disease under field condition
(April 2015 – June 2015)

(T4) (48.12) which was significantly superior to other treatments. This was followed by DI and PDI of (T3) (52.50 and 18.33 respectively) which were on par with DI of (T6) (51.87). DI and PDI of (T5) (58.12 and 22.36 respectively) was significantly superior to other treatments. The highest DI of (86.25) and PDI of (49.02) were recommended in absolute control plots (Table 6c).

4.4.2. Biometric observations

4.4.2.1. Fruit weight

In first trial, fruit weight was highest in (1010.95 g) (T1), plants treated with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant and was significantly superior to that of plants treated with (T2) NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant which recorded fruit weight of (988.53 g). In second trial the later treatment recorded highest fruit weight of (2052 g) which was significantly superior to the remaining treatments. (T4) NPK 19:19:19 (0.5 per cent) + adjuvant followed by which fruit weight of (T2) (1125 g) and was significantly superior to (T1) (1085 g). Fruit weight of (T3) NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant (955.59 g) and (T4) (938.45 g) were on par in first trial. (T5) farmers' management practices (934.48 g) were on par with (T6) package of practices recommendations (POP) (KAU) which recorded fruit weight of (924.85 g). In second trial recorded fruit weight of (T6) (934 g) and was significantly superior to (T3) in which the fruit weight was (856 g). In first trial fruit weight recorded (T5) (934.48 g) and (T6) (924.85 g) were on par and in second trial fruit weight in control (T7) (463 g) was significantly lower than, the remaining treatments. The lowest fruit weight was recorded in both trial (610.80 g and 463 g respectively) (Table 6a, 6b).

Table 6c. Pooled analysis of data of field trials I and II

Treatments	Total yield (kg)/pit	Per cent disease index*	Per cent disease incidence*
T1	3.14[b]	13.47[a] (6.51)	40.00[a] (43.24)
T2	4.56[a]	13.05[a] (6.27)	40.00[a] (43.18)
T3	2.71[b]	18.33[c] (10.52)	52.50[c] (66.05)
T4	3.09[b]	15.41[b] (8.01)	48.12[b] (57.16)
T5	2.60[b]	22.36[d] (14.73)	58.12[d] (72.71)
T6	2.79[b]	16.94[b] (8.97)	51.87[c] (64.90)
T7	1.61[c]	49.02[e] (57.83)	86.25[e] (94.87)
CD (0.05)	1.251	2.018(2.037)	2.428 (3.449)

*Mean of four replications

Values in parenthesis are arcsine transformed

T1- Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant

T2- Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T3- Fertilizer application @ 0.5 per cent (19:19:19 NPK) + Calcium nitrate (5g/l) + adjuvant

T4- Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + adjuvant

T5- Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted)

T6- Cultivation practices according to POP (KAU), T7- Absolute control

4.4.2.2. Fruit length

In first trial, fruit length (T1) plants treated with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant and (T2) plants treated with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant were on par (27.96 cm and 27.31 cm) respectively and were significantly superior to other treatments. (T3) plants treated with NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant recorded fruit length of (26.93 cm) and was significantly superior to other treatments. Fruit length in plants treated with (T4) NPK 19:19:19 (0.5 per cent) + adjuvant (26.17 cm) and (T5) plants raised by farmers' management practices (25.95 cm) were on par and significantly superior to (T6) that observed in plants raised by package of practices recommendations (POP) (KAU) (25.23 cm). Lowest fruit length was recorded in (22.52 cm). In second trial, fruit length with treatments from T1 to T6 ranged from (25.31 cm to 27.36 cm) and were on par with each and significantly superior to those in control (T7) (21.41 cm) (Table 6a, 6b).

4.4.2.3. Fruit breadth

Fruit breadth recorded in plants treated with (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (25.40 cm) and (T2) NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (25.17 cm) were significantly superior to the remaining treatments in the first trial. Plants treated with (T4) NPK 19:19:19 (0.5 per cent) + adjuvant, (T3) NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant, (T5) plants raised according to farmers' management practices recorded fruit breadth of 24.35 cm, 24.25 cm, 23.27 cm respectively and were on par and significantly superior to (T6) package of practices recommendations (POP) (KAU) (22.82 cm). Average fruit breadth recorded in control plot was (18.40 cm) in first trial. In second trial average fruit breadth ranged from

(27.36 to 25.31 cm) in plants applied with treatments T1 to T6. Lowest fruit breadth of control plants recorded (T7) (20.97 cm) (Table 6a, 6b).

4.4.2.4. Total yield

Highest total yield of (3.03 kg) was recorded in plants treated with (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant which was significantly superior to all other treatments in the first trial. Plants treated with (T2) NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant recorded total yield of (2.96 kg) which closely followed (T1) (2.86 kg). In second trial (T2) recorded highest total weight of (6.16 kg) which was significantly superior to remaining treatments. Plants treated with (T1) and (T4) NPK 19:19:19 (0.5 per cent) + adjuvant recorded total yield of (3.25 kg and 3.37 kg) and were on par. In first trial (T3) plants treated with NPK 19:19:19 (0.5 per cent) + calcium nitrate (5g/l) + adjuvant and (T4) recorded total yield of (2.86 kg and 2.81 kg) respectively and were on par. (T5) plants raised according to farmers' management practices recorded total yield of (2.80 kg) which was on par with that of (T4) (2.81 kg) and (T6) plants raised according to package of practices recommendations (POP) (KAU) (2.78 kg). In second trial (T6) recorded total weight of (2.80 kg) which was on par with (T3) (2.56 kg) and (T5) (2.40 kg). Total yield was lowest for control (T7) in first and second trial (1.83 and 1.39 respectively) (Table 6a, 6b).

Highest total yield/pit was recorded in (T2) (4.56 kg/pit) which were significantly superior to all other treatments. Treatments (T1) (3.14 kg/pit), (T4) (3.09 kg/pit), (T6) (2.79 kg/pit), (T3) (2.71 kg/pit) and (T5) (2.60 kg/pit) were on par and significantly superior to control plants which recorded lowest total yield of (1.61 kg/pit) (Table 6c).

4.5. Evaluation of effective treatments for the management of anthracnose leaf spot of culinary melon in the farmers' fields

Confirmation trials of the better treatments screened in field trials conducted previously during (January 2015-March 2015) and (April 2015-June 2015) at CoA, Vellayani, were laid out in farmers' field at three locations *viz.,* Venganoor, Vavamoola and Venjaramoodu. The comparative performance of these treatments with cultivation and plant protection practices according to (POP) (KAU), farmers' management practices and absolute control, in controlling anthracnose leaf spot disease, were evaluated. Yield parameters, physiological parameters as well as microbial population of rhizosphere and phyllosphere of the plants applied with different treatments were also assessed and the results are presented below (Table 7, 8, 9) (Plate 19, 20, 21) (Fig. 6).

4.5.1. Assessment of percent disease index (PDI) and disease incidence (DI)

4.5.1.1. Location1-Venganoor

Percent disease index (PDI) (11.11 per cent) and disease incidence (DI) (30.00 per cent) were lowest in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant which was significantly superior to those of the remaining treatments of the trial. PDI recorded in (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant was 14.44 per cent and DI 34.00 per cent which were significantly superior to the remaining treatments. PDI and DI of (T4) plants sprayed with (17.21 and 41.00 per cent respectively) were significantly superior to those of other treatments. PDI and DI of (T3) plants raised by farmers' management practices (21.66 and 52.00 per cent respectively) were significantly superior to those of plants in (T5) control (59.44 and 88.75 per cent respectively). Highest PDI (59.44 per cent) and DI (88.75 per cent) were recorded in control (T5) (Table 7) (Plate 19a).

Plate 19. Evaluation of nutrient and fungicides on the management of culinary melon anthracnose leaf spot disease under farmers' field condition – Venganoor

Table 7. Efficacies of foliar fertilizer and fungicides against anthracnose leaf spot disease and on yield parameters of culinary melon plants in farmers' field trial– Venganoor (July 2015 – October 2015)

Treatments	No. of. Harvest	Daily yield (kg)	Average yield (kg)	Total yield (kg)	Percent disease index*	Percent disease incidence*
T1	6.31 b	4.47 a	17.90 a	53.61 b	14.44 b (7.00)	34.00 b (31.86)
T2	7.69 a	4.73 a	18.94 a	56.82 a	11.11 a (5.04)	30.00 a (25.28)
T3	6.06 b	3.36 b	13.46 b	40.24 c	21.66 d (13.41)	52.00 d (64.07)
T4	6.37 b	3.53 b	14.13 b	42.40 c	17.21 c (9.27)	41.00 c (44.17)
T5	3.87 c	0.90 c	3.59 c	10.78 d	59.44 e (76.10)	88.75 e (95.79)
CD (0.05)	1.041	0.843	1.686	2.926	1.572 (2.930)	2.218 (2.471)

*Mean of four replications

Values in parenthesis are arcsine transformed

T1 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant
T2 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant
T3-Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted)
T4 - Cultivation practices according to POP (KAU)
T5- Absolute control

T2 - Foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T1 - Foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant

Absolute control

Plate 19a. Two most effective treatments - Venganoor

4.5.1.2. Biometric observations

4.5.1.2.1. Number of harvests

The number of harvests recorded upto 75 days after sowing was highest (7.69) in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant which was significantly superior to those of remaining treatments. Number of harvests recorded in plants raised according to package of practices recommendations (POP) (KAU) (T4) (6.37), (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (6.31) and (T3) farmers' management practices (6.06) were on par and was significantly to that from (T5) (3.87). Lowest number of harvests was recorded in control (3.87) (Table 7).

4.5.1.2.2. Daily yield

Daily yield of culinary melon was highest in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (4.73 kg) which was on par with (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (4.47 kg). Plants raised according to package of practices recommendations (POP) (KAU) (T4) (3.53 kg) which were on par with (T3) farmers' management practices (3.36 kg). Daily yield recorded from plants of control plot (T5) was significantly lower than all other treatments (0.90 kg) (Table 7).

4.5.1.2.3. Average yield

Average yield of fruits was highest in (T2) plants sprayed with (18.94 kg) which was on par with (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (17.90 kg) and was significantly superior to (T4) plants raised according to package of practices recommendations (POP) (KAU). Average yield recorded from (T3) plants raised according to farmers' management practices (13.46 kg) were on par

with (T4) and was significantly superior to that recorded in plants of control plot (T5) (3.59 kg) (Table 7).

4.5.1.2.4. Total yield

Total yield of fruits recorded up to 75 days after sowing was highest from (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (56.82 kg) and was significantly superior to the remaining treatments (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (53.61 kg). Total yield of fruits (T4) plants raised according to package of practices recommendations (POP) (KAU) (42.40 kg) were on par with (T3) plants raised according to farmers' management practices (40.24 kg). Total yield was lowest in plants of control plot (T5) (10.78 kg) (Table 7).

4.5.2. Assessment of percent disease index (PDI) and disease incidence (DI)

4.5.2.1. Location 2-Vavamoola

Percent disease index (PDI) (12.77 per cent) and disease incidence (DI) (25.50 per cent) were lowest in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant which was significantly superior to the other treatments of the trial. PDI recorded in (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (14.44 per cent) and DI (32.25 per cent) were significantly superior to the remaining treatments. PDI and DI recorded in (T4) (17.21 and 38.00 per cent respectively), (T3) plants raised according to farmers' management practices (18.88 and 48.00 per cent respectively) were significantly superior to each other. Highest PDI (58.60 per cent) and DI (86.25 per cent) were recorded in plants of the control plots (T5) (Table 8) (Plate 20a).

Plate 20. Evaluation of foliar fertilizer and fungicides on the management of culinary melon leaf spot disease under farmers' field condition – Vavamoola

Table 8. Efficacies of foliar fertilizer and fungicides against anthracnose leaf spot disease and on yield parameters of culinary melon in farmers' field trial – Vavamoola (July 2015 – October 2015)

Treatments	No. of. harvest	Daily yield (kg)	Average yield (kg)	Total yield (kg)	Percent disease index*	Percent disease incidence*
T1	8.50[a]	2.58[a]	10.31[b]	41.23[b]	14.44[b] (7.53)	32.25[b] (27.40)
T2	8.62[a]	3.05[a]	12.22[a]	48.87[a]	12.77[a] (6.00)	25.50[a] (19.05)
T3	7.56[a]	1.88[a]	7.51[c]	30.04[c]	18.88[d] (10.54)	48.00[d] (58.13)
T4	8.19[a]	2.47[a]	9.90[b]	39.61[b]	17.21[c] (9.27)	38.00[c] (36.63)
T5	5.06[b]	0.49[b]	1.98[d]	7.92[d]	58.60[e] (74.16)	86.25[e] (94.43)
CD (0.05)	1.508	0.678	1.357	2.714	1.645 (2.914)	1.939 (2.497)

*Mean of four replications

Values in parenthesis are arcsine transformed

T1 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant
T2 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant
T3-Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted)
T4 - Cultivation practices according to POP (KAU)
T5- Absolute control

T2 - Foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T1 - Foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant

Absolute control

Plate 20a. Two most effective treatments - Vavamoola

4.5.2.2. Biometric observations

4.5.2.2.1. Number of harvests

Number of harvests recorded up to 75 days after sowing was highest (8.62) from (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant and was on par with treatments (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (8.50) and that of plants raised according to package of practices recommendations (POP) (KAU) (T4) (8.19), plants raised according to farmers' management practices (T3) (7.56). Number of harvests was significantly lower from the plants in the control (T5) (5.06) (Table 8).

4.5.2.2.2. Daily yield

Daily yield of culinary melon was highest in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (3.05 kg) which was on par with that of. plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (T1) (2.58 kg), plants raised according to package of practices recommendations (POP) (KAU) (T4) (2.47 kg), plants raised according to farmers' management practices (T3) (1.88 kg). Daily yield recorded from control (T5) was significantly lower (0.49 kg) than all the other treatment (Table 8).

4.5.2.2.3. Average yield

Average yield of fruits was highest in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (12.22 kg) and was significantly superior to the remaining treatments. Average yields of (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (10.31 kg) were on par with (T4) plants raised according to package of practices recommendations (POP) (KAU) (9.90 kg) and significantly superior to that from plants raised according

to farmers' management practices (T3) (7.51 kg). Lowest average yield was recorded in plants of control plot (T5) (1.98 kg) (Table 8).

4.5.2.2.4. Total yield

Total yield of fruits was highest from (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (48.87 kg) and was significantly superior to the remaining treatments. Total yield from plants sprayed with (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (41.23 kg) were on par with (T4) plants raised according to package of practices recommendations (POP) (KAU) (39.61 kg) and significantly superior to (T3) plants raised according to farmers' management practices (30.04 kg). Total yield was lowest in plants of control plot (T5) (7.92 kg) (Table 8).

4.5.3. Assessment of percent disease index (PDI) and disease incidence (DI)

4.5.3.1. Location 3-Venjaramoodu

PDI (12.77 per cent) and DI (30.00 per cent) recorded from (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant were lowest and significantly superior to those of other treatments. PDI and DI of (T1) plants sprayed with 0.5 per cent (19:19:19 NPK) + mancozeb (0.4 per cent) + adjuvant (17.77 and 36.00 per cent, respectively), plants raised according to package of practices recommendations (POP) (KAU) (T4) (20.00 and 38.00 per cent respectively) were significantly superior lower than that of (T3) plants raised according to farmers' management practices (24.44 and 50.00 per cent, respectively). Highest PDI (55.55 per cent) and DI (90.00 per cent) were recorded in plants of control plot (T5) (Table 9) (Plate 21a).

4.5.3.2. Biometric observations

4.5.3.2.1. Number of harvests

The number of harvests recorded was highest in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (2.02) which was on par with (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2.33), (T3) plants raised according to farmers' management practices (2.08) and (T4) plants raised according to package of practices recommendations (POP) (KAU) (2.08). Least number of harvests were recorded in plants of control plot (T5) (1.92) (Table 9).

4.5.3.2.2 Daily yield

The daily yield recorded was highest in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (1.31 kg) and was on par with that of (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.12 kg), (T3) plants raised according to farmers' management practices (0.85 kg) and (T4) plants raised according to package of practices recommendations (POP) (KAU) (0.82 kg). Lowest daily yield was recorded in plants of control plot (T5) (0.35 kg) (Table 9).

4.5.3.2.3. Average yield

The average yield recorded was highest in (T2) plants sprayed with (3.92 kg) and was on par with (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (3.37 kg), (T3) plants raised according to farmers' management practices (2.56 kg) and (T4) plants raised according to package of practices recommendations (POP) (KAU) (2.47 kg). Lowest average yield was recorded in plants of control plot (T5) (1.05 kg) (Table 9).

Plate 21. Evaluation of foliar fertilizer and fungicides on the management of culinary melon anthracnose leaf spot disease under farmers' field condition –Venjaramoodu

Table 9. Efficacies of foliar fertilizer and fungicides against anthracnose leaf spot disease and on yield parameters of culinary melon in farmers' field trial – Venjaramoodu (July 2015 – October 2015)

Treatments	No. of. harvest	Daily yield (kg)	Average yield (kg)	Total yield (kg)	Percent disease index*	Percent disease incidence*
T1	2.33[a]	1.12[a]	3.37[a]	10.12[b]	17.77[b] (9.27)	36.00[b] (34.18)
T2	2.50[a]	1.31[a]	3.92[a]	11.77[a]	12.77[a] (5.96)	30.00[a] (23.27)
T3	2.08[a]	0.85[a]	2.56[a]	7.68[c]	24.44[d] (16.71)	50.00[d] (59.35)
T4	2.08[a]	0.82[a]	2.47[a]	8.17[c]	20.00[c] (11.90)	38.00[c] (39.11)
T5	1.92[b]	0.35[b]	1.05[b]	3.15[d]	55.55[e] (70.97)	90.00[e] (95.91)
CD (0.05)	0.579	0.380	0.659	1.185	1.869 (2.890)	2.048 (2.337)

*Mean of four replications

Values in parenthesis are arcsine transformed

T1 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant
T2 - Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant
T3-Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted)
T4 - Cultivation practices according to POP (KAU)
T5- Absolute control

T2 - Foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T1 - Foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant

Absolute control

Plate 21a. Two most effective treatments - Venjaramoodu

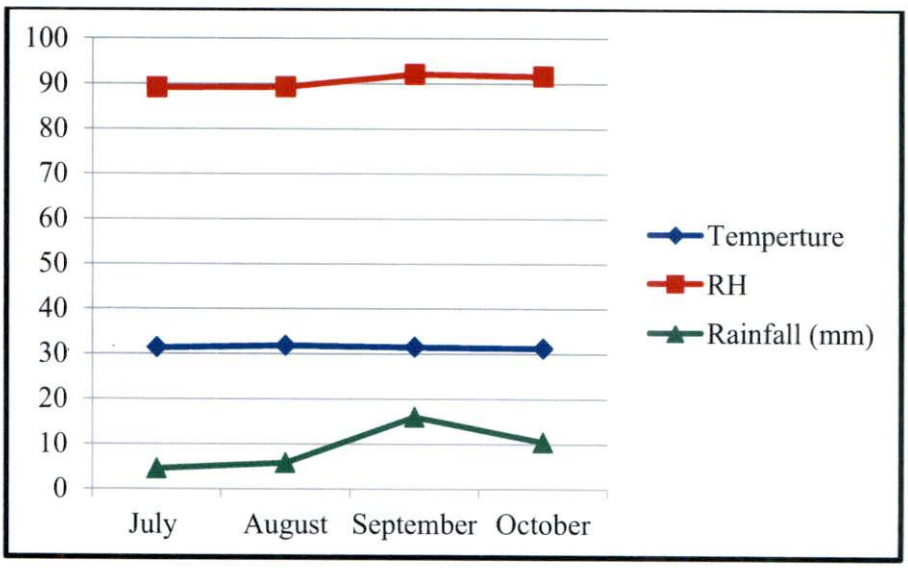


Fig. 6.Weather parameters of farmers' field trials
(Venganoor, Vavamoola and Venjaramoodu) during 2015

4.5.3.2.4. Total yield

Total yield of fruits were recorded was highest in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (11.77 kg) which was significantly superior to those of all other treatments. Total yield from (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (10.12 kg) and was significantly superior to those of (T4) plants raised according to package of practices recommendations (POP) (KAU) (8.17 kg). Plants sprayed with (T4) were on par with (T3) plants raised according to farmers' management practices (7.58 kg). Lowest total yield was recorded in plants of control plot (T5) (3.15 kg) (Table 9).

4.5.4. Pooled analysis of efficacies of foliar fertilizer and fungicides in the management of anthracnose leaf spot disease in farmers' field trials

Pooled analysis of the observations on efficacies of foliar fertilizer and fungicides in suppressing an is of culinary melon and increasing the yield from the crop, in farmers field trials were undertaken for find out the most effective treatment from among those tested in the three locations.

4.5.4.1. Assessment of Percent disease index (PDI) and Disease incidence (DI)

PDI (12.22 per cent) and DI (28.50 per cent) recorded from (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant were lowest and significantly superior to all other treatments. PDI of (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (15.55 per cent) and DI (34.08 per cent) were significantly superior to that of (T2). PDI and DI of (T4) plants raised according to package of practices recommendations (POP) (KAU) (18.14 and 39.00 per cent respectively) were significantly lower that of (T3) plants raised according to farmers' management practices (21.66 and 50.00

respectively). Highest PDI (57.86 per cent) and DI (88.33 per cent) were recorded in plants of control plot (T5) (Table 10).

4.5.4.2. Total yield

Total yield of fruits recorded was highest in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (39.15 kg) and was significantly superior to all other treatments. Total yield from (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (34.99 kg) and was significantly lower that of (T4) plants raised according to package of practices recommendations (POP) (KAU) (30.06 kg) and (T3) plants raised according to farmers' management practices (25.99 kg). Lowest total yield was recorded in plants of control plots (T5) (7.28 kg) (Table 10).

4.6. Economic analysis

Analysis of the data on benefit - cost ratio (Table 11, 12, 13) of the different treatments tested in the trials conducted at Venganoor, Vavamoola and Venjaramoodu revealed that the highest returns was recorded from treatment (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant in all three locations of the trials being Rs. 2.98, 2.57 and 0.62 for every rupee spent in each of the three locations, respectively. The lowest returns were obtained from treatment (T6) plants of control plot which were Rs. 0.75, 0.55 and 0.22 respectively in each of the three locations.

4.7. Estimation of rhizosphere and phyllosphere microflora

Fungal and bacterial population present in the rhizosphere and phyllosphere of plants after applied with of the different treatment were analysed and the results are presented below

Table 10. Pooled analysis of data of three farmers' field trials

Treatments	Total yield (kg)/pit	Percent disease index*	Percent disease incidence*
T1	34.99[b]	15.55[b] (7.93)	34.08[b] (31.15)
T2	39.15[a]	12.22[a] (5.67)	28.50[a] (22.53)
T3	25.99[d]	21.66[d] (13.56)	50.00[d] (60.52)
T4	30.06[c]	18.14[c] (10.14)	39.00[c] (39.97)
T5	7.28[e]	57.86[e] (73.74)	88.33[e] (35.39)
CD (0.05)	3.653	1.776 (1.847)	1.412 (1.741)

*Mean of four replications

Values in parenthesis are arcsine transformed

T1-Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + fungicide (mancozeb 0.4 per cent) + adjuvant

T2-Fertilizer application by foliar spray @ 0.5 per cent (19:19:19 NPK) + newer fungicide (Azoxystrobin @ 0.15ml/l) + adjuvant

T3-Farmers' practice of crop management and plant protection in culinary melon (based on data collected during the preliminary survey conducted)

T4-Cultivation practices according to POP (KAU)

T5-Absolute control

Table 1-1. Economic analysis of the experiment - Venganoor

Sl.no	Treatment	Cost of cultivation (ha^{-1})	Gross income	Net income	B:C ratio
1	T1-NPK 19:19:19 (0.5 %) +Mancozeb (0.4%) + adjuvant	72,034	177,520	105,486	2.46
2	T2- NPK 19:19:19 (0.5 %) +Azoxystrobin (0.15 ml/l)+adjuvant	76,117	227,280	151,163	2.98
3	T3-Farmers' management practices	72,178	160,960	88,782	2.23
4	T4-POP (KAU)	72,563	169,600	97,037	2.34
5	T5- Absolute control	57,750	43,120	-14,630	0.75

Table 12. Economic analysis of the experiment – Vavamoola

Sl.no	Treatment	Cost of cultivation (ha^{-1})	Gross income	Net income	B:C ratio
1	T1-NPK 19:19:19 (0.5 %) +Mancozeb (0.4%) + adjuvant	72,034	156,840	84,806	2.18
2	T2- NPK 19:19:19 (0.5 %) +Azoxystrobin (0.15 ml/l)+adjuvant	76,117	195,480	119,363	2.57
3	T3-Farmers' management practices	72,178	120,160	47,982	1.66
4	T4-POP (KAU)	72,563	158,440	85,877	2.18
5	T5- Absolute control	57,750	31,640	-26,110	0.55

Table 13. Economic analysis of the experiment – Venjaramoodu

Sl.no	Treatment	Cost of cultivation (ha^{-1})	Gross income	Net income	B:C ratio
1	T1-NPK 19:19:19 (0.5 %) +Mancozeb (0.4%) + adjuvant	72,034	37,320	-34,714	0.52
2	T2- NPK 19:19:19 (0.5 %) +Azoxystrobin (0.15 ml/l)+adjuvant	76,117	47,080	-29,037	0.62
3	T3-Farmers' management practices	72,178	30,720	-41,458	0.42
4	T4-POP (KAU)	72,563	32,680	-39,883	0.45
5	T5- Absolute control	57,750	12,600	-45,150	0.22

4.7.1. Estimation of fungal population in rhizosphere

4.7.1.1. Venganoor

Highest fungal population was recorded in the rhizosphere of plant samples obtained from (T4) plants raised according to package of practices recommendations (POP) (KAU) (15.50 cfu/g of soil) which was significantly superior to all other treatments. This was followed by (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (3.75 cfu/g of soil) which was higher and significantly superior to that of the rhizosphere samples from (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.75 cfu/g of soil), (T3) plants raised according to farmers' management practices (1.75 cfu/g of soil) and (T5) plants of the control plants (1.25 cfu/g of soil) which were on par. The lowest population was recorded from the rhizosphere of plant samples obtained from plants of control plot (T5) (1.25 cfu/g of soil) (Table 14) (Plate 22a) (Fig. 7).

4.7.1.2. Vavamoola

In the trial conducted at Vavamoola, the highest fungal population was recorded in the rhizosphere of the plant samples obtained from plants of control plot (T5) (57.75 cfu/g of soil) which was significantly higher compared to all other treatments. The fungal population in rhizosphere from (T4) plants raised according to package of practices recommendations (POP) (KAU) (52.25 cfu/g of soil) closely followed it and was superior to the remaining treatments. Rhizosphere population of fungi in (T3) plants raised according to farmers' management practices (44.50 cfu/g of soil) and (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (43.50 cfu/g of soil) were on par and significantly superior to that of (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (20.50 cfu/g of soil) which was significantly lower compared

to the populations recorded in plants of all other treatments in the trial (Table 14) (Plate 22b) (Fig. 7).

4.7.1.3. Venjaramoodu

The highest rhizosphere population of fungi estimated in the trial, was observed in (T5) plants of control plot (35.25 cfu/g of soil) which was significantly higher compared to all other treatments. This was followed by fungal population of the plant rhizosphere samples obtained in (T3) plants raised according to farmers' management practices (23.25 cfu/g of soil) which was significantly higher than that of plants raised according to (T4) package of practices recommendations (POP) (KAU) (20.75 cfu/g of soil) and (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (20.50 cfu/g of soil) both of which were on par. The lowest fungal population was recorded from rhizosphere of (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (18.75 cfu/g of soil) (Table 14) (Plate 22c) (Fig.7).

4.7.2. Estimation of bacterial population in rhizosphere

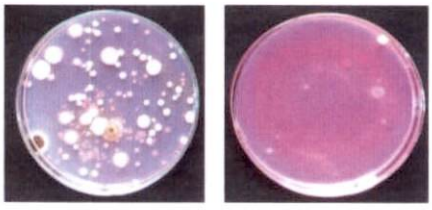
4.7.2.1. Venganoor

In the field trial conducted at Venganoor, the bacterial population estimated was highest in rhizosphere of plants of (T5) control plot (78.50 cfu/g of soil) which was significantly superior to all other treatments. This was closely followed by the rhizosphere population in (T4) plants raised according to package of practices recommendations (POP) (KAU) (67.00 cfu/g of soil) which was significantly higher to those of the remaining treatments. The bacterial population of the rhizosphere of (T3) plants raised according to farmers' management practices (49.75 cfu/g of soil) were equal and on par with the population in plants treated with (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (48.50 cfu/g of soil). Lowest

Table 14. Average population of rhizosphere fungi of culinary melon plants in farmers' fields – Venganoor, Vavamoola and Venjaramoodu

Sl.No	Treatments	Average population of fungi obtained from rhizosphere (cfu/g of soil) x 10^{-4}		
		Venganoor	Vavamoola	Venjaramoodu
1	T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	1.75	43.50	20.50
2	T2- NPK 19:19:19 (0.5%) + Azoxystrobin(0.15 ml/l) + adjuvant	3.75	20.50	18.75
3	T3- Farmers' management practices	1.75	44.50	23.25
4	T4- POP (KAU)	15.50	52.25	20.75
5	T5- Absolute control	1.25	57.75	35.25
	CD (0.05)	1.690	2.013	1.764

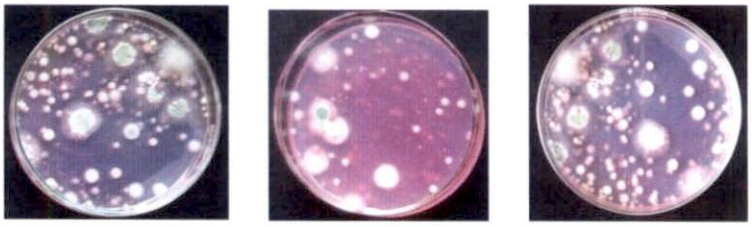
T 1 T2 T 3



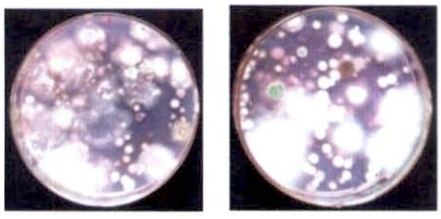
T4 T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 22a. Average population of rhizosphere fungi of culinary melon plants in farmers' field – Venganoor



T1 T2 T3



T4 T5

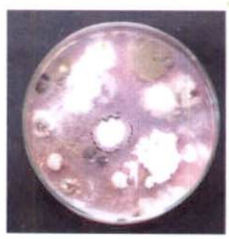
T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobi (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 22b. Average population of rhizosphere fungi of culinary melon plants in farmers' field – Vavamoola

T1

T 2

T 3

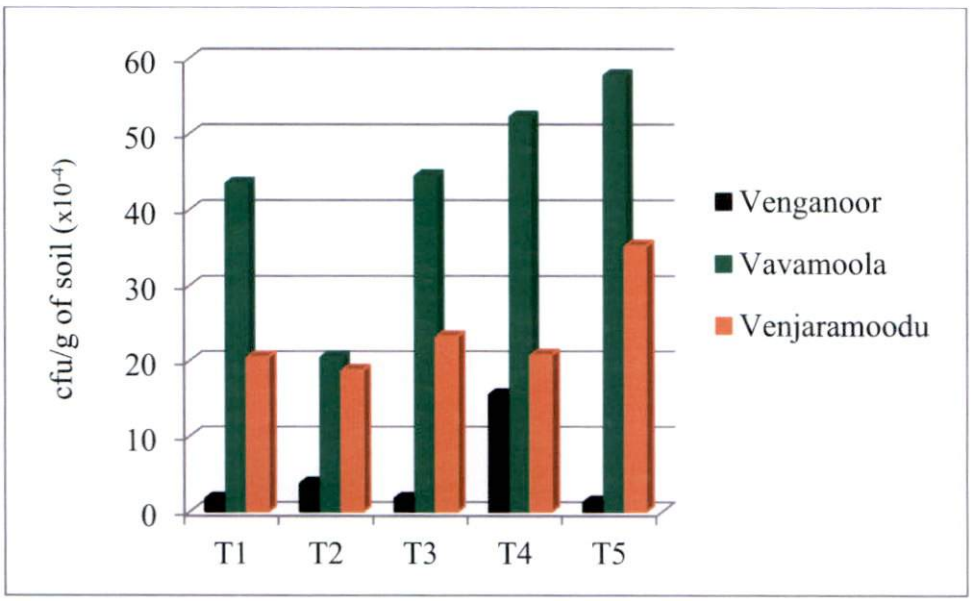


T4

T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 22c. Average population of rhizosphere fungi of culinary melon plants in farmers' field – Venjaramoodu



Treatments

T1 -19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant

T2 - 19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant

T3 - Farmers' management practices

T4 - POP (KAU)

T5 - Absolute control

Fig. 7. Average population of rhizosphere fungi of culinary melon plants in farmers' field – Venganoor, Vavamoola and Venjaramoodu

bacterial population was estimated in the plant rhizosphere of culinary melon plants sprayed with (T2) NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (38.75 cfu/g of soil) (Table 15) (Plate 23a) (Fig. 8).

4.7.2.2. Vavamoola

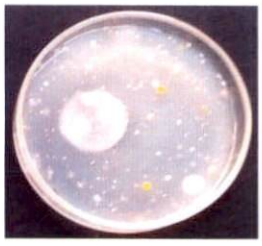
In the field trial conducted at Vavamoola, the bacterial population estimated was highest in rhizosphere of (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (34.50 cfu/g of soil) which was significantly higher to those of remaining treatments. The bacterial population in the rhizosphere of (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (24.00 cfu/g of soil) and (T3) plants raised according to farmers' management practices (23.25 cfu/g of soil) were on par. The bacterial population recorded in plants of (T5) control plot (21.25 cfu/g of soil) was on par with (T4) that of plants raised according to package of practices recommendations (POP) (KAU) (20.75 cfu/g of soil). Lowest bacterial population was estimated in the plant rhizosphere of culinary melon plants raised according to (T4) package of practices recommendations (POP) (KAU) (20.75 cfu/g of soil) (Table 15) (Plate 23b) (Fig. 8).

4.7.2.3. Venjaramoodu

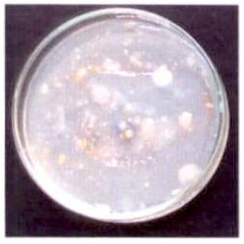
In the field trial conducted at Venjaramoodu, the bacterial population was highest in rhizosphere of (T4) plants raised according to package of practices recommendations (POP) (KAU) (23.75 cfu/g of soil) which was significantly superior to those of all other treatments. This was closely followed by the rhizosphere population in (T3) plants raised according to farmers' management practices (7.25 cfu/g of soil) and (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (6.50 cfu/g of soil) which were on par. The bacterial population recorded in (T5) plants of control plot (4.50 cfu/g of soil) was on par with (T2) that of plants sprayed with NPK 19:19:19 (0.5 per cent) +

Table 15. Average population of rhizosphere bacteria of culinary melon plants in farmers' fields – Venganoor, Vavamoola and Venjaramoodu

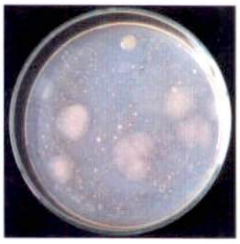
Sl.No	Treatments	Average population of bacteria obtained from rhizosphere (cfu/g of soil) x 10^{-6}		
		Venganoor	Vavamoola	Venjaramoodu
1	T1- NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant	48.50	24.00	6.50
2	T2- NPK 19:19:19 (0.5%) +Azoxystrobin (0.15 ml/l) + adjuvant	38.75	34.50	4.25
3	T3- Farmers' management practices	49.75	23.25	7.25
4	T4- POP (KAU)	67.00	20.75	23.75
5	T5- Absolute control	78.50	21.25	4.50
	CD (0.05)	3.014	1.759	1.643



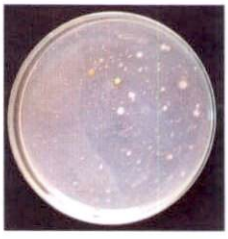
T1



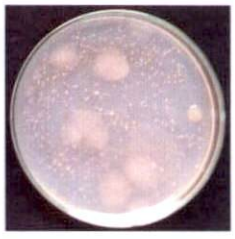
T2



T3



T4



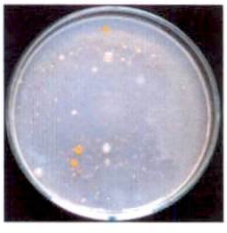
T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

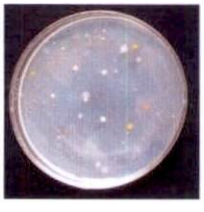
Plate 23a. Average population of rhizosphere bacteria of culinary melon plants in farmers' field – Venganoor

T1

T2



T3

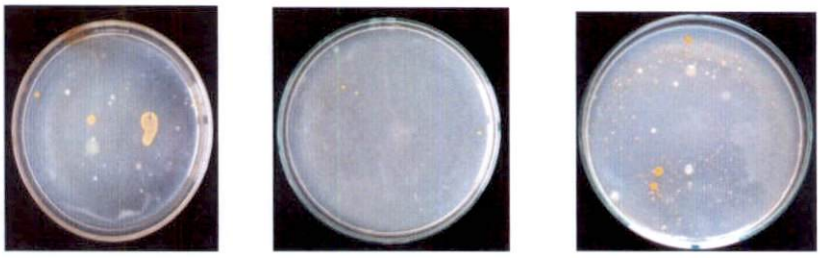


T4

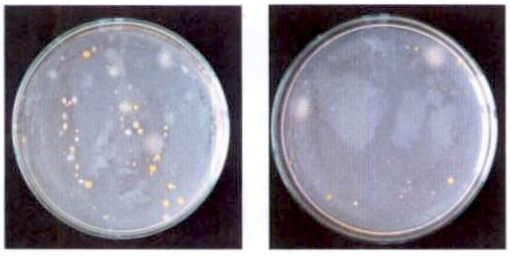
T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 23b. Average population of rhizosphere bacteria of culinary melon plants in farmers' field – Vavamoola



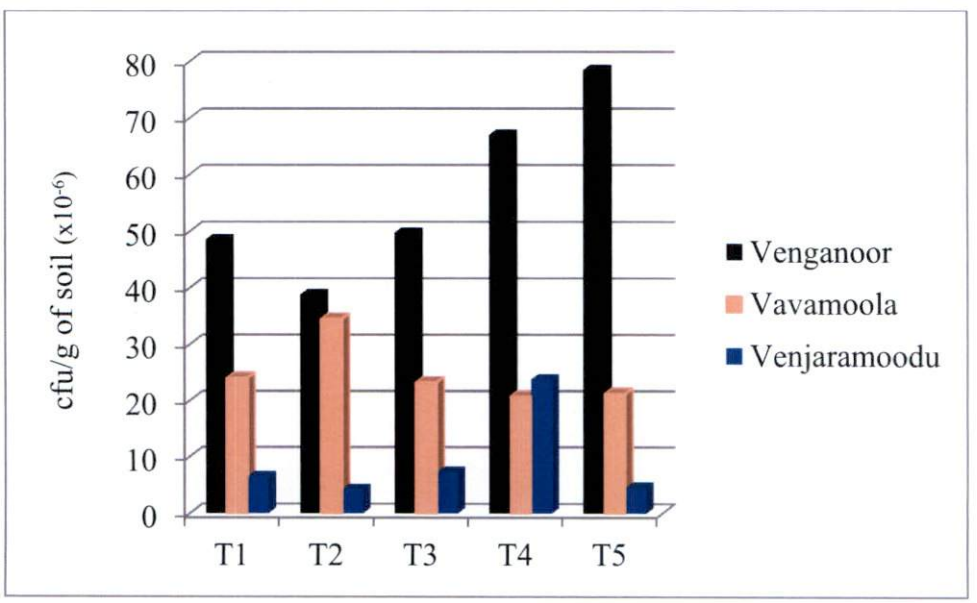
T1 T2 T3



T4 T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 23c. Average population of rhizosphere bacteria of culinary melon plants in farmers' field – Venjaramoodu



Treatments

T1 -19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant

T2 - 19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant

T3 - Farmers' management practices

T4 - POP (KAU)

T5 - Absolute control

Fig. 8. Average population of rhizosphere bacteria of culinary melon plants in farmers' field – Venganoor, Vavamoola and Venjaramoodu

azoxystrobin (0.15 ml/l) + adjuvant (4.25 cfu/g of soil) and the lowest bacterial population was estimated in (T1) (4.25 cfu/g of soil) (Table 15) (Plate 23c) (Fig. 8).

4.7.3. Estimation of fungal population in phyllosphere

4.7.3.1. Venganoor

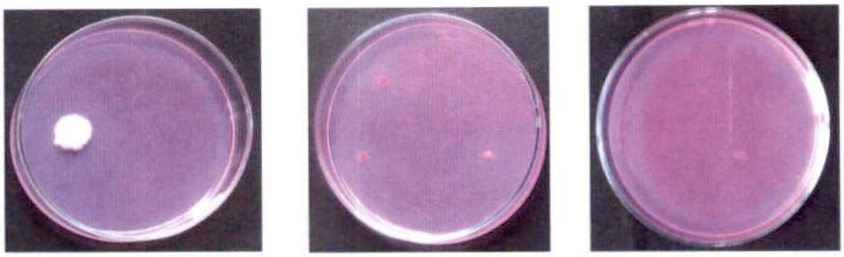
The average population of fungi in phyllosphere was highest in plant samples obtained from control plot (T5) (8.50 cfu/g of soil) which was significantly superior to those in all other treatments. This was followed by the population recorded in phyllosphere of plants sprayed with (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.25 cfu/g of soil), (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (1.00 cfu/g of soil) and (T3) plants raised according to farmers' management practices (1.00 cfu/g of soil) which were on par. Fungal population was not detected in phyllosphere of plant samples obtained from (T4) plants according to package of practices recommendations (POP) (KAU) (0.00 cfu/g of soil) (Table 16) (Plate 24a) (Fig. 9).

4.7.3.2. Vavamoola

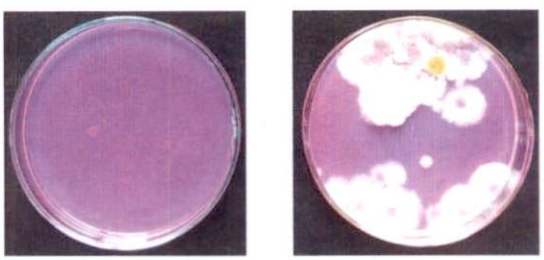
In the trial conducted at Vavamoola, the average popuation of fungi in phyllosphere was highest in samples obtained from (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (10.75 cfu/g of soil) which was significantly higher compared that in all other treatments. This was followed by fungal population in phyllosphere of sampes obtained from (T5) plants of control plot (8.50 cfu/g of soil) which was significantly higher than that of the treatments. Phyllosphere fungal population in samples of (T4) plants raised according to package of practices recommendations (POP) (KAU) (5.00 cfu/g of soil) was on par and significantly higher than those of (T3) plants raised according to farmers' management practices (3.25 cfu/g of soil) and (T1) plants sprayed with NPK

Table 16. Average population of phyllosphere fungi of culinary melon plants in farmers' fields – Venganoor, Vavamoola and Venjaramoodu

Sl. No	Treatments	Average population of fungi obtained from phyllosphere (cfu/g of leaf) x 10^{-4}		
		Venganoor	Vavamoola	Venjaramoodu
1	T1- NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant	1.25	2.00	29.25
2	T2- NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) +adjuvant	1.00	10.75	8.00
3	T3- Farmers' management practices	1.00	3.25	36.50
4	T4- POP (KAU)	0.00	5.00	35.75
5	T5- Absolute control	8.50	8.50	37.00
	CD (0.05)	0.966	1.293	1.710



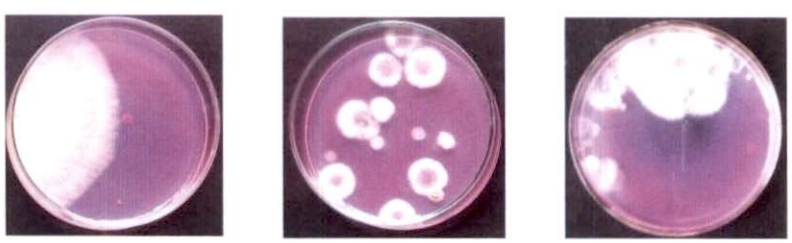
T1 T2 T3



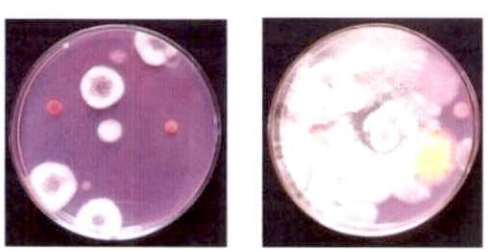
T4 T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 24a. Average population of phyllosphere fungi of culinary melon plants in farmers' field – Venganoor



T1 T2 T3

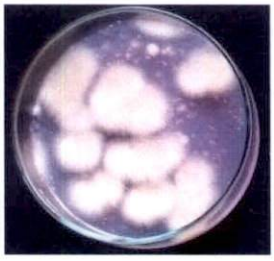


T4 T 5

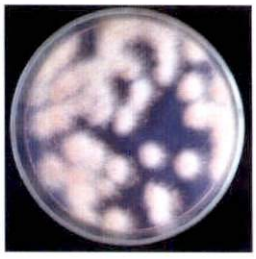
T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 24b. Average population of phyllosphere fungi of culinary melon plants in farmers' field – Vavamoola

T1

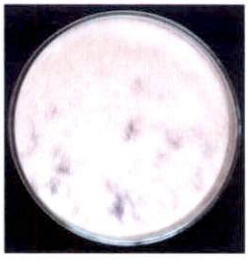


T2



T 3

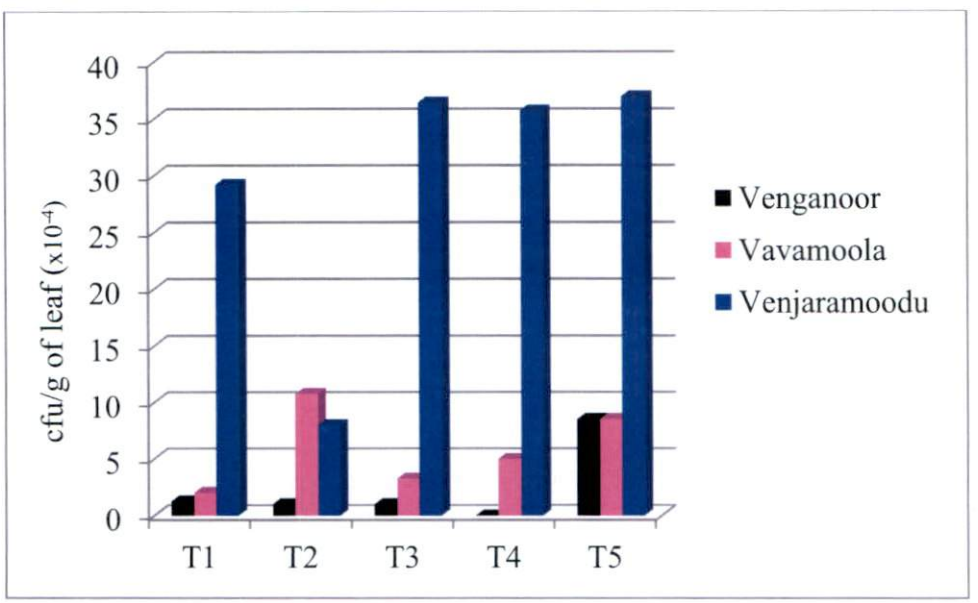
T4



T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plat 24c. Average population of phyllosphere fungi of culinary melon plants in farmers' field – Venjaramoodu



Treatments

T1 -19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant

T2 - 19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l)+ adjuvant

T3 - Farmers' management practices

T4 - POP (KAU)

T5 - Absolute control

Fig. 9. Average population of phyllosphere fungi of culinary melon plants in farmers' field – Venganoor, Vavamoola and Venjaramoodu

19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2.00 cfu/g of soil) which were on par (Table 16) (Plate 24b) (Fig. 9).

4.7.3.3. Venjaramoodu

Fungal population in phyllosphere samples obtained from (T5) plants of control plot (37.00 cfu/g of soil), (T3) plants raised according to farmers' management practices (36.50 cfu/g of soil) and (T4) plants raised according to package of practices recommendations (POP) (KAU) (35.75 cfu/g of soil) were on par and significantly superior to those of remaining treatments. This was followed by fungal population in phyllosphere samples obtained from (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (29.25 cfu/g of soil) which was significantly superior to the remaining treatment. Lowest fungal population was recorded in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (8.00 cfu/g of soil) (Table 16) (Plate 24c) (Fig. 9).

4.7.4. Estimation of bacterial population in phyllosphere

4.7.4.1. Venganoor

The highest bacterial population was estimated in phyllosphere samples of (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (22.50 cfu/g of soil) which was significantly superior to all other treatments. This was followed by the average population of bacteria in phyllosphere samples obtained from plants treated with (T4) package of practices recommendations (POP) (KAU) (8.75 cfu/g of soil) which was significantly superior to those of (T5) control (6.75 cfu/g of soil) and (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (6.50 cfu/g of soil) that were on par. Lowest population was recorded in

Table 17. Average population of phyllosphere bacteria of culinary melon plants in farmers' fields – Venganoor, Vavamoola and Venjaramoodu

Sl.No	Treatments	Average population of bacteria obtained from phyllosphere (cfu/g of leaf) x 10^{-6}		
		Venganoor	Vavamoola	Venjaramoodu
1	T1- NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant	6.50	8.50	17.25
2	T2- NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) +adjuvant	22.50	20.25	12.00
3	T3- Farmers' management practices	4.25	10.25	16.00
4	T4- POP (KAU)	8.75	9.25	12.75
5	T5- Absolute control	6.75	6.75	29.50
	CD (0.05)	1.765	1.408	1.725

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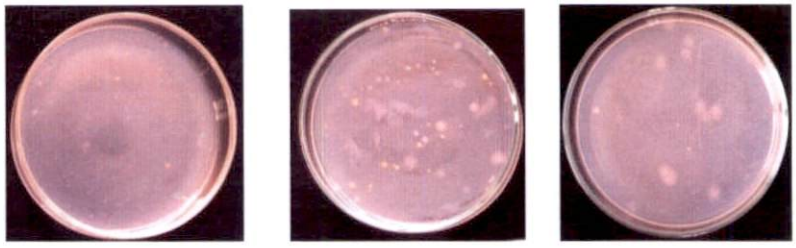
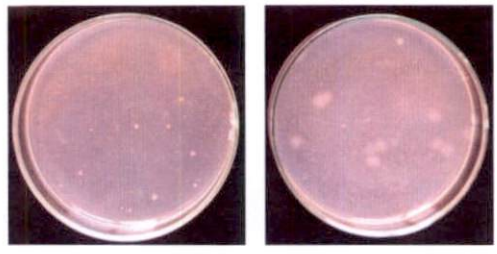


T1 T2 T3

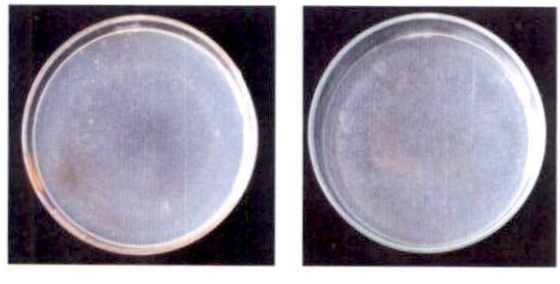


T4 T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 25a. Average population of phyllosphere bacteria of culinary melon plants in farmers' field – Venganoor

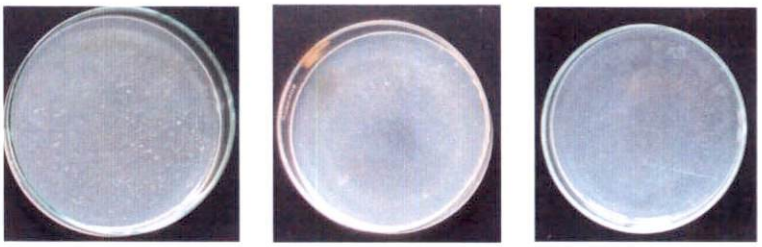
T1 T2 T3



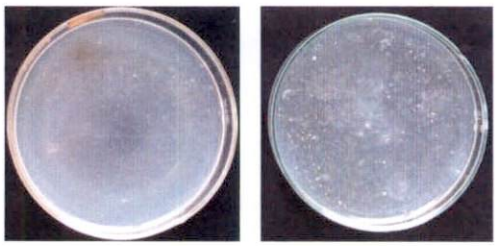
T4 T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 25b. Average population of phyllosphere bacteria of culinary melon plants in farmers' field – Vavamoola



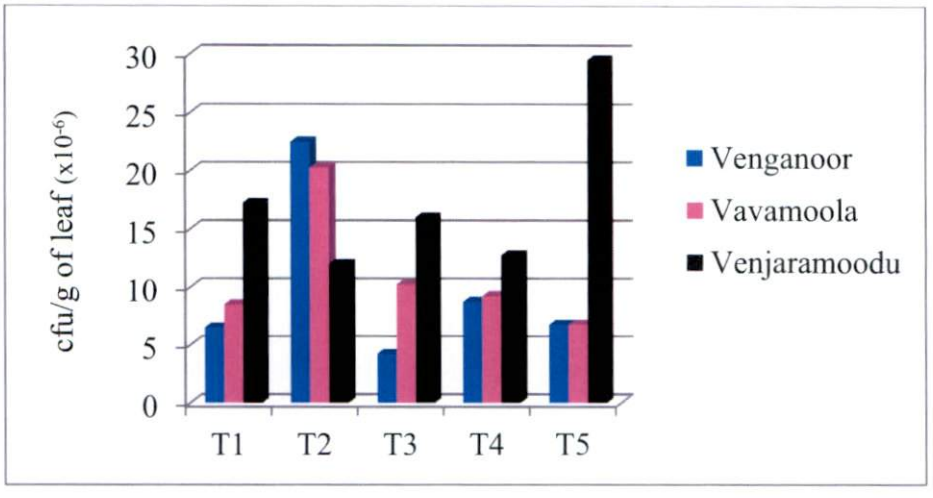
T1 T2 T3



T4 T5

T1 - 19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant
T2 -19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3 - Farmers' management practices
T4 - POP (KAU)
T5 - Absolute control

Plate 25c. Average population of phyllosphere bacteria of culinary melon plants in farmers' field – Venjaramoodu



Treatments

T1 -19:19:19 NPK (0.5%) + Mancozeb (0.4%) + adjuvant

T2 - 19:19:19 NPK (0.5%) + Azoxystrobin (0.15 ml/l)+ adjuvant

T3 - Farmers' management practices

T4 - POP (KAU)

T5 - Absolute control

Fig. 10. Average population of phyllosphere bacteria of culinary melon plants in farmers' field – Venganoor, Vavamoola and Venjaramoodu

phyllosphere samples of (T3) plants raised according to farmers' management practices (4.25 cfu/g of soil) (Table 17) (Plate 25a) (Fig. 10).

4.7.4.2. Vavamoola

The highest bacterial population was estimated in phyllosphere samples of (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (20.25 cfu/g of soil) which was significantly superior to the all other treatments. This was followed by the average population of bacteria in phyllosphere of samples of (T3) plants raised according to farmers' management practices (10.25 cfu/g of soil), (T4) plants raised according to package of practices recommendations (POP) (KAU) (9.25 cfu/g of soil) and (T1) plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (8.50 cfu/g of soil) which were on par and significantly higher compared to that of plants in (T5) control plot (6.75 cfu/g of soil) which recorded the lowest bacterial population (Table 17) (Plate 25b) (Fig. 10).

4.7.4.3. Venjaramoodu

The highest bacterial population was estimated in phyllosphere samples of plants of (T5) control plot (29.50 cfu/g of soil) which was significantly superior to the all other treatments. This was followed by the average population of bacteria in phyllosphere of samples obtained from plants sprayed with (T1) NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (17.25 cfu/g of soil) was on par with (T3) plants raised according to farmers' management practices (16.00 cfu/g of soil) and significantly higher than that of (T4) plants raised according to package of practices recommendations (POP) (KAU) (12.75 cfu/g of soil). Population in T4 was on par with that of (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (12.00 cfu/g of soil) and the latter treatment

recorded the lowest bacterial population among the treatments of the trial (Table 17) (Plate 25c) (Fig. 10).

4.8. Detection of induced systemic resistance in culinary melon plants from farmers' field trials

4.8.1. Changes in the phenylalanine ammonia lyase (PAL) activity in culinary melon plants treated with nutrients and fungicides

In all three locations namely (Venganoor, Vavamoola and Venjaramoodu), phenylalanine ammonia lyase (PAL) activity gradually increased in culinary melon leaves following treatments with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (1.233, 2.081 n mol of transcinnamic acid $min^{-1}g^{-1}$ of fresh tissue) and foliar spray of NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.183, 1.986 n mol of transcinnamic acid $min^{-1}g^{-1}$ of fresh tissue) which reached maximum level on 10^{th} day after treatment when compared to control. The enzyme activity subsequently declined on 15^{th} day. Plants treated with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant recorded maximum PAL activity (1.616 n mol of transcinnamic acid $min^{-1}g^{-1}$ of fresh tissue) followed by that in plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.578 n mol of transcinnamic acid $min^{-1}g^{-1}$ of fresh tissue) on 15^{th} day after treatment of the plants in three location respectively. Lower PAL activity was recorded in the plants of control plot (0.894, 1.254, 1.112 n mol of transcinnamic acid $min^{-1}g^{-1}$ of fresh tissue) on 5^{th}, 10^{th} and 15^{th} days after treatment of the plants (Table 18) (Fig. 11a, 11b, 11c).

Table 18. Activity of phenylalanine ammonia lyase (PAL) in culinary melon plants in farmers' field trials – Venganoor, Vavamoola and Venjaramoodu (n mol of transcinnamic acid min^{-1} g^{-1} of fresh tissue)

Treatments	Venganoor			Vavamoola			Venjaramoodu		
	5th	10th	15th	5th	10th	15th	5th	10th	15th
T1- NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant	0.985	1.090	1.183	1.411	1.686	1.986	1.285	1.386	1.578
T2- NPK 19:19:19 (0.5%) +Azoxystrobin (0.15 ml/l) +adjuvant	1.052	1.143	1.233	1.469	1.742	2.081	1.336	1.426	1.616
T3- Farmers' management practices	0.703	0.816	0.955	1.141	1.387	1.660	1.019	1.122	1.259
T4- POP (KAU)	0.813	0.949	1.012	1.269	1.552	1.830	1.123	1.204	1.328
T5- Absolute control	0.625	0.723	0.894	0.977	1.135	1.254	0.968	1.051	1.112
CD (0.05)	0.075	0.067	0.069	0.199	0.251	0.195	0.075	0.073	0.077

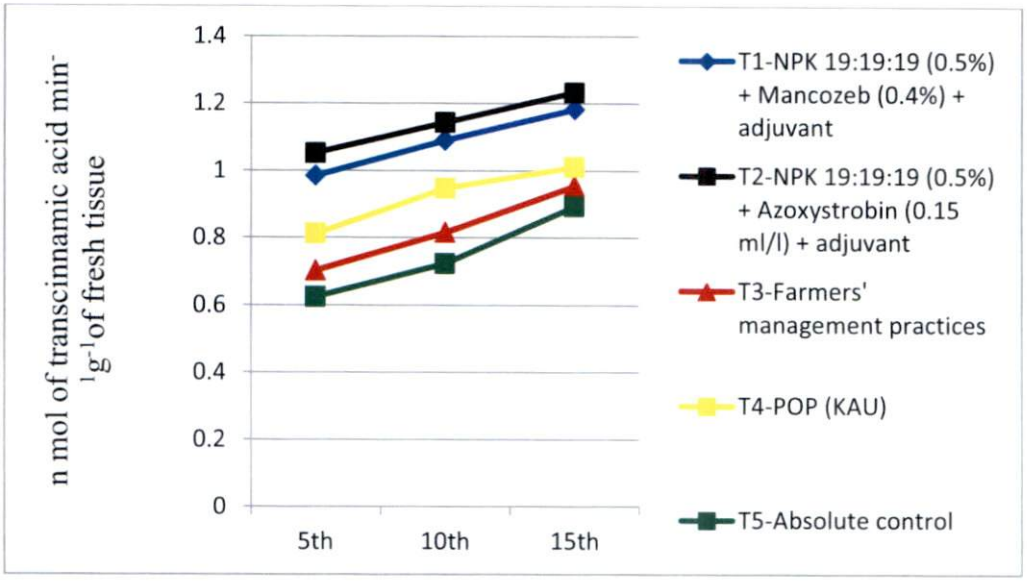


Fig. 11a. Activity of phenylalanine ammonia lyase (PAL) activity in culinary melon plants – Venganoor

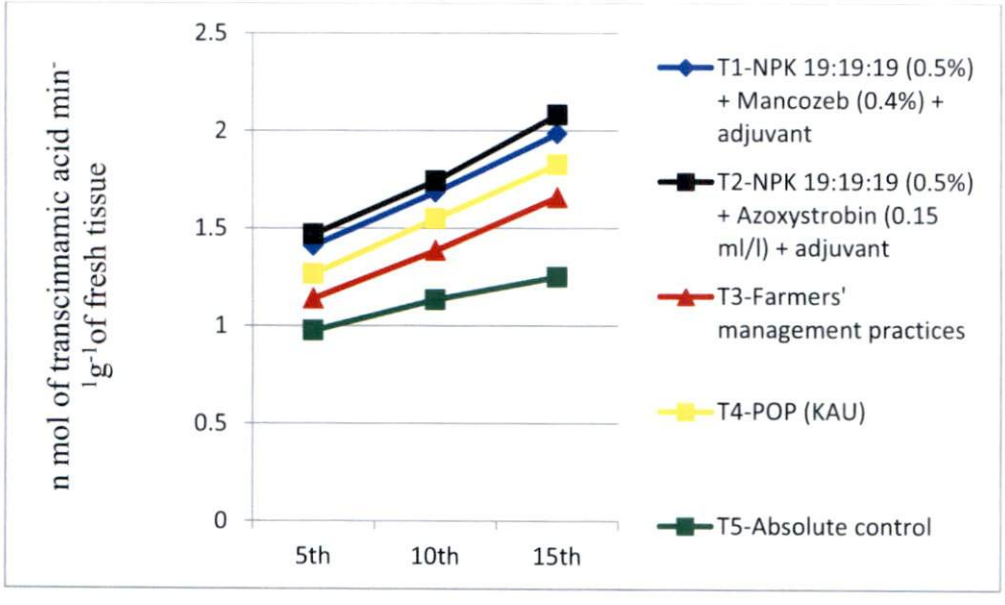


Fig. 11b. Activity of phenylalanine ammonia lyase (PAL) activity in culinary melon plants – Vavamoola

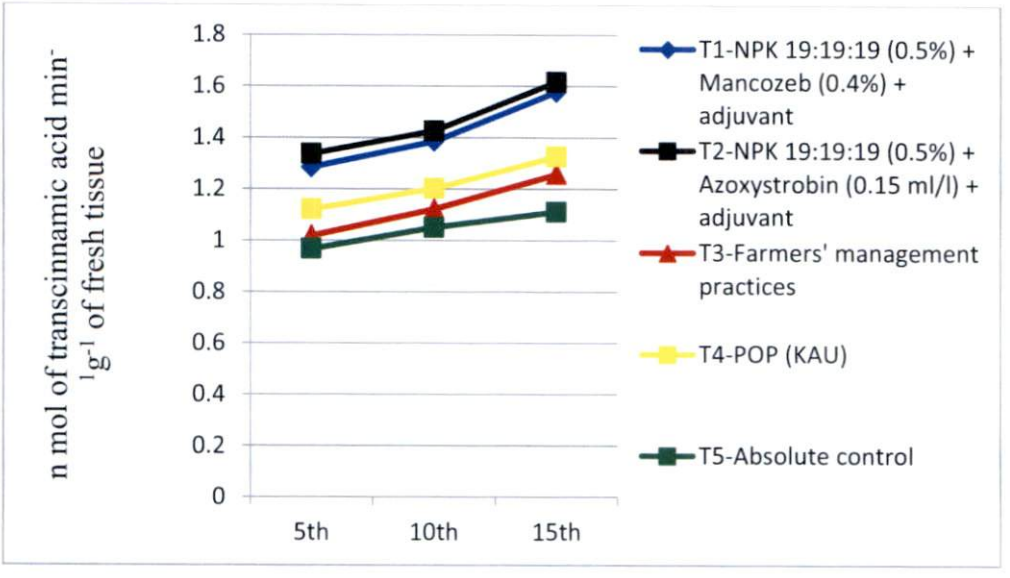


Fig. 11c. Activity of phenylalanine ammonia lyase (PAL) activity in culinary melon plants – Venjaramoodu

4.8.2. Changes in the Peroxidase (PO) activity in culinary melon plants treated with nutrients and fungicides

In all three locations namely (Venganoor, Vavamoola and Venjaramoodu), peroxidase (PO) activity gradually increased in culinary melon leaves following treatment of plants with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (1.245, 1.039 changes in A_{420} $min^{-1}g^{-1}$ of fresh tissue) and foliar spray of NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.187, 0.989 changes in A_{420} $min^{-1}g^{-1}$ of fresh tissue) which reached maximum level on 15th day after treatment when compared to that of plants in control plot. The enzyme activity subsequently declined on 15th day. Plants treated with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant recorded maximum PO activity (0.989 changes in A_{420} $min^{-1}g^{-1}$ of fresh tissue) followed by that in plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (0.917 changes in A_{420} $min^{-1}g^{-1}$ of fresh tissue) on 15th day after treatment of the plants in three location respectively. Lower PO activity was recorded in plants of control plot (0.705, 0.567, 0.517 changes in A_{420} $min^{-1}g^{-1}$ of fresh tissue) on 5th, 10th and 15th days after treatment of the plants (Table 19) (Fig. 12a, 12b, 12c).

4.8.3. Changes in the polyphenol oxidase (PPO) activity in culinary melon plants treated with nutrients and fungicides

In all three locations namely (Venganoor, Vavamoola and Venjaramoodu), polyphenoloxidase (PPO) activity gradually increased in culinary melon leaves following treatments with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (1.114, 1.661 changes in A_{490} $min^{-1}g^{-1}$ of fresh tissue) and foliar spray of NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.092, 1.585 changes in A_{490} $min^{-1}g^{-1}$ of fresh tissue) which reached maximum level at 10th day after treatment when compared to control. The enzyme activity subsequently

Table 19. Activity of peroxidase in culinary melon plants of farmers' field trials – Venganoor, Vavamoola and Venjaramoodu (changes in A_{420} min^{-1} g^{-1} of fresh tissue)

Treatments	Venganoor			Vavamoola			Venjaramoodu		
	5th	10th	15th	5th	10th	15th	5th	10th	15th
T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	0.914	1.079	1.187	0.611	0.884	0.989	0.519	0.681	0.917
T2- NPK 19:19:19 (0.5%) +Azoxystrobin (0.15 ml/l) +adjuvant	0.968	1.107	1.245	0.665	0.977	1.039	0.568	0.726	0.989
T3- Farmers' management practices	0.630	0.763	0.851	0.373	0.630	0.767	0.209	0.431	0.635
T4- POP (KAU)	0.774	0.843	1.048	0.468	0.756	0.864	0.326	0.566	0.706
T5- Absolute control	0.440	0.525	0.705	0.249	0.521	0.567	0.110	0.354	0.517
CD (0.05)	0.205	0.241	0.212	0.172	0.136	0.103	0.091	0.074	0.090

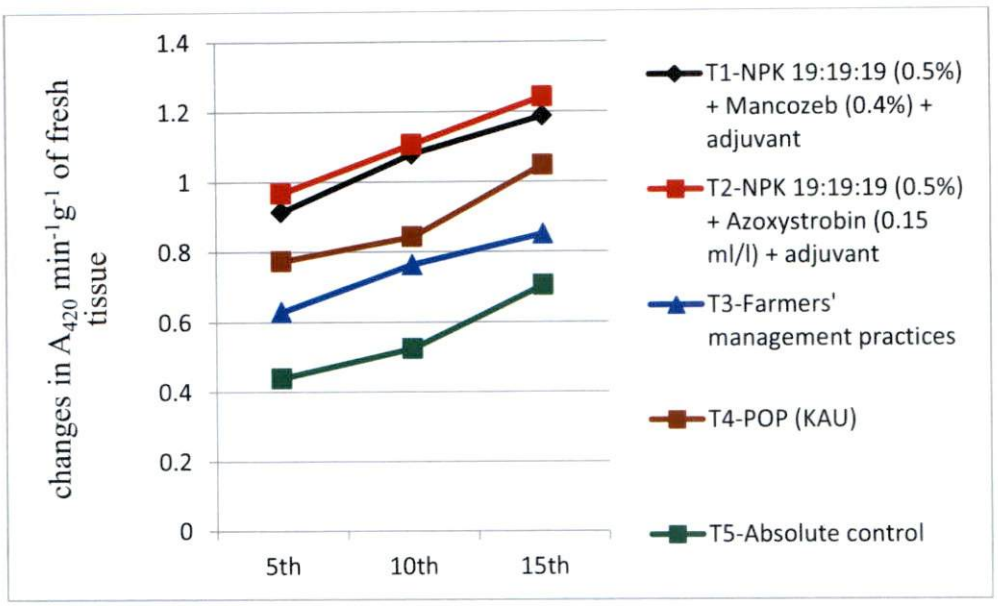


Fig. 12a. Activity of peroxidase activity in culinary melon plants –Venganoor

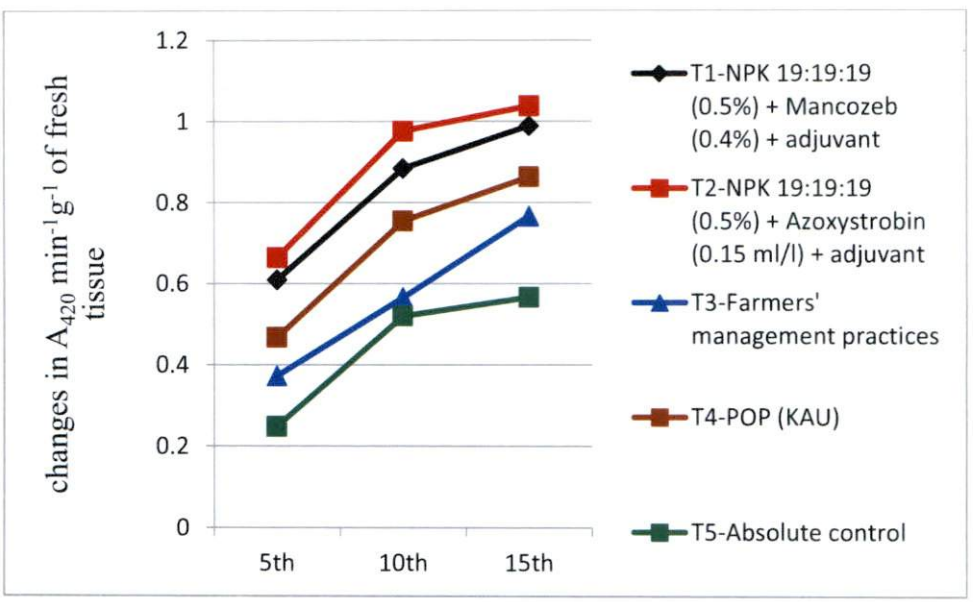


Fig. 12b. Activity of peroxidase activity in culinary melon plants – Vavamoola

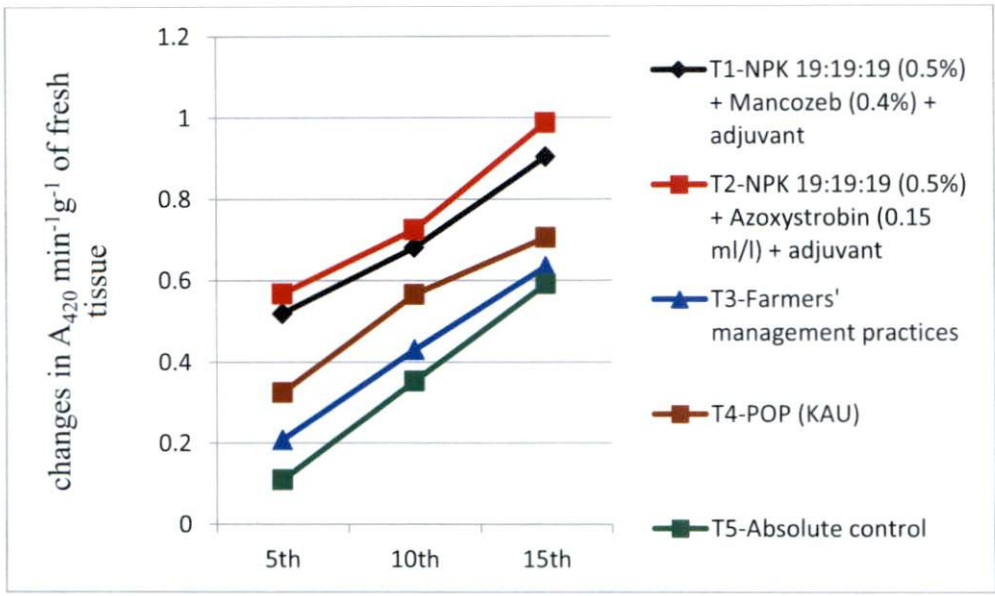


Fig. 12c. Activity of peroxidase activity in culinary melon plants – Venjaramoodu

Table 20. Activity of polyphenol oxidase in culinary melon plants of farmers' field trials – Venganoor, Vavamoola and Venjaramoodu (changes in A_{490} min^{-1} g^{-1} of fresh tissue)

Treatments	Venganoor			Vavamoola			Venjaramoodu		
	5th	10th	15th	5th	10th	15th	5th	10th	15th
T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	0.819	0.887	1.092	1.186	1.308	1.585	0.981	1.110	1.185
T2- NPK 19:19:19 (0.5%) +Azoxystrobin (0.15 ml/l) +adjuvant	0.877	0.992	1.114	1.263	1.395	1.661	1.006	1.124	1.262
T3- Farmers' management practices	0.515	0.622	0.838	0.959	1.117	1.344	0.673	0.842	0.995
T4- POP (KAU)	0.603	0.747	0.946	0.777	1.145	1.466	0.806	0.966	1.064
T5- Absolute control	0.435	0.515	0.745	0.740	0.935	1.237	0.432	0.685	0.772
CD (0.05)	0.078	0.074	0.072	0.235	0.202	0.229	0.083	0.069	0.071

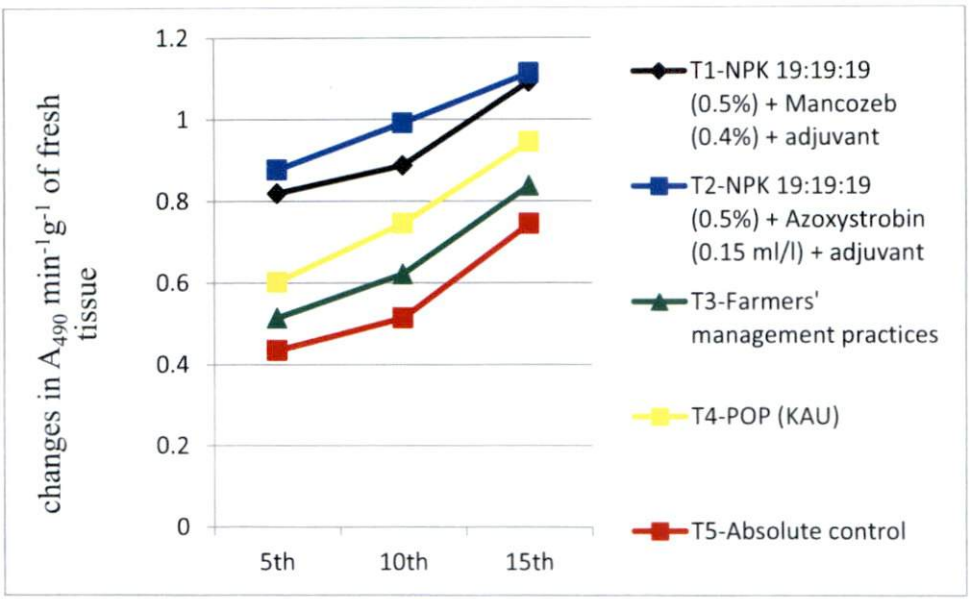


Fig. 13a. Activity of polyphenol oxidase activity in culinary melon plants – Venganoor

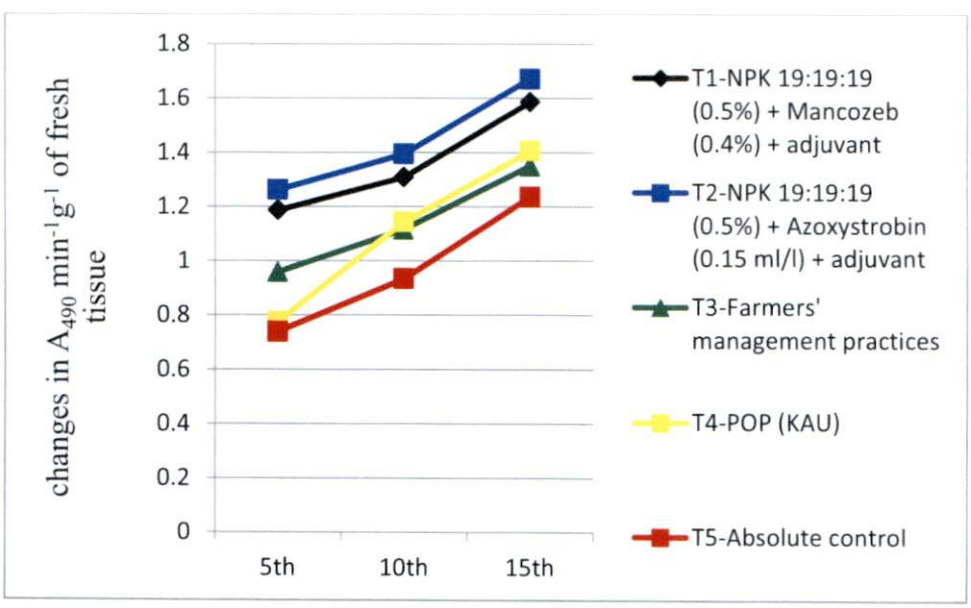
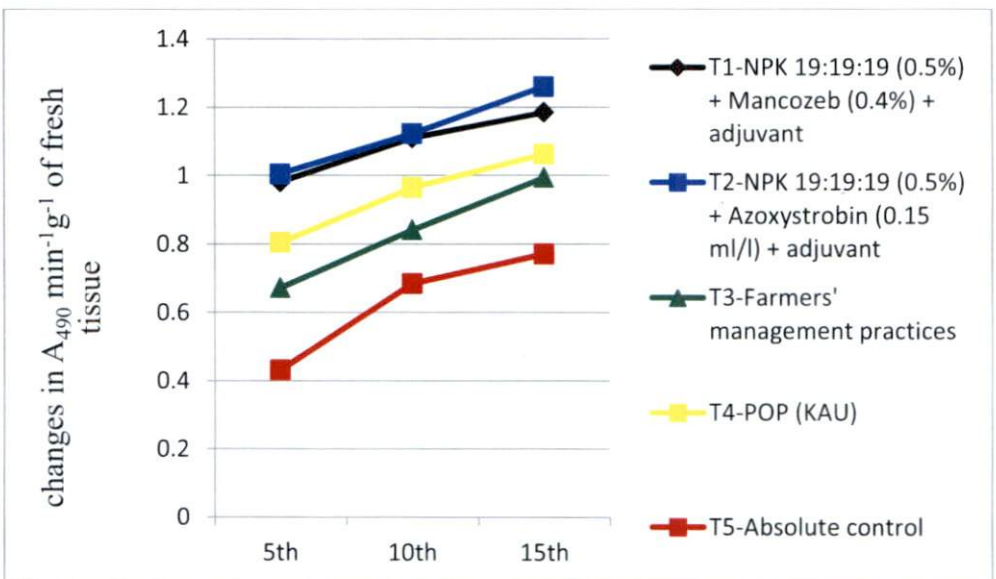


Fig. 13b. Activity of polyphenol oxidase activity in culinary melon plants – Vavamoola



Days after treatment application

Fig. 13c. Activity of polyphenol oxidase activity in culinary melon plants – Venjaramoodu

declined on 15th day. Plants treated with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant recorded maximum polyphenol oxidase (PPO) activity (1.262 changes in A_{490} $min^{-1}g^{-1}$ of fresh tissue) followed by foliar spray of NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.185 changes in A_{490} $min^{-1}g^{-1}$ of fresh tissue) on 15th day after treatment of the plants in three location respectively. Lower polyphenol oxidase (PPO) activity was recorded in the plants of control plot (0.745, 1.237, 0.772 changes in A_{490} $min^{-1}g^{-1}$ of fresh tissue) on 5th, 10th and 15th days after treatment of the plants (Table 20) (Fig. 13a, 13b, 13c).

4.8.4. Changes in the β-1, 3 glucanase activity in culinary melon plants treated with nutrients and fungicides

In all three locations namely (Venganoor, Vavamoola and Venjaramoodu), β-1, 3 glucanase activity gradually increased in culinary melon leaves following treatments with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (1.209, 1.297 µg of glucose $min^{-1}g^{-1}$ of fresh tissue) and foliar spray of NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.183, 1.204 µg of glucose $min^{-1}g^{-1}$ of fresh tissue) which reached maximum level at 10th day after treatment when compared to control. The enzyme activity subsequently declined on 15th day. Plants treated with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant recorded maximum β-1, 3 glucanase activity (1.024 µg of glucose $min^{-1}g^{-1}$ of fresh tissue) followed by foliar spray of NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (0.991 µg of glucose $min^{-1}g^{-1}$ of fresh tissue) on 15th day after treatment of the plants in three location respectively. Lower β-1, 3 glucanase activity was recorded in the plants of control plot (0.845, 0.804, 0.620 µg of glucose $min^{-1}g^{-1}$ of fresh tissue) on 5th, 10th and 15th days after treatment of the plants (Table 21) (Fig. 14a, 14b, 14c).

Table 21. Activity of β – 1,3glucanase in culinary melon plants of farmers' field trials – Venganoor, Vavamoola and Venjaramoodu (μg of glucose released min^{-1} g^{-1} of fresh tissue)

Treatments	Venganoor			Vavamoola			Venjaramoodu		
	5th	10th	15th	5th	10th	15th	5th	10th	15th
T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	0.886	1.091	1.183	1.000	1.109	1.204	0.794	0.915	0.991
T2- NPK 19:19:19 (0.5%) +Azoxystrobin (0.15 ml/l) +adjuvant	0.965	1.133	1.209	1.026	1.171	1.297	0.874	0.974	1.024
T3- Farmers' management practices	0.625	0.844	1.045	0.747	0.875	0.994	0.502	0.634	0.765
T4- POP (KAU)	0.728	0.936	0.984	0.842	0.945	1.072	0.620	0.704	0.842
T5- Absolute control	0.513	0.728	0.845	0.650	0.751	0.804	0.414	0.453	0.620
CD (0.05)	0.071	0.069	0.070	0.196	0.202	0.064	0.076	0.064	0.064

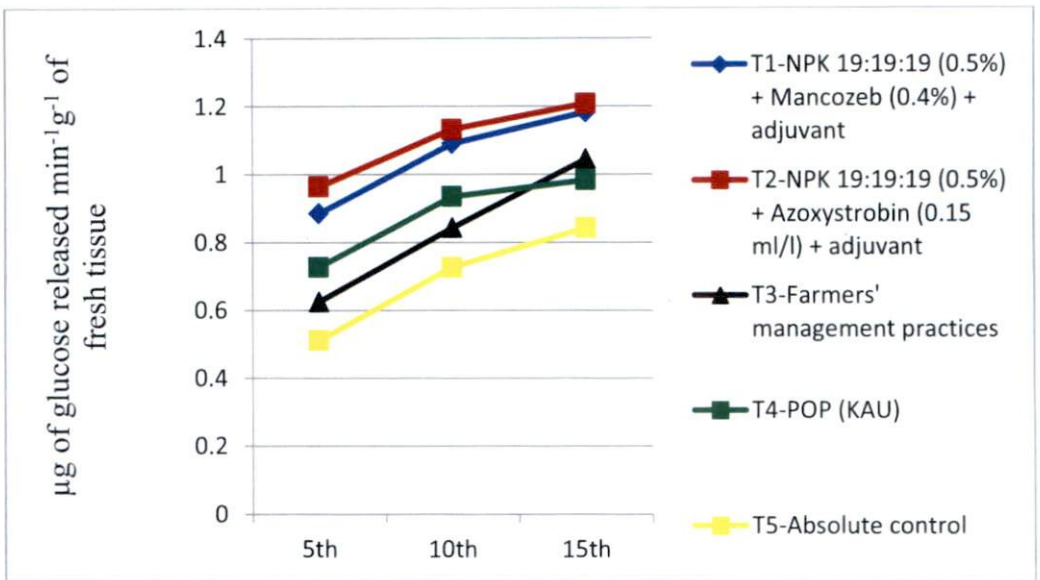


Fig. 14a. Activity of β - 1, 3 glucanase activityin culinary melon plants – Venganoor

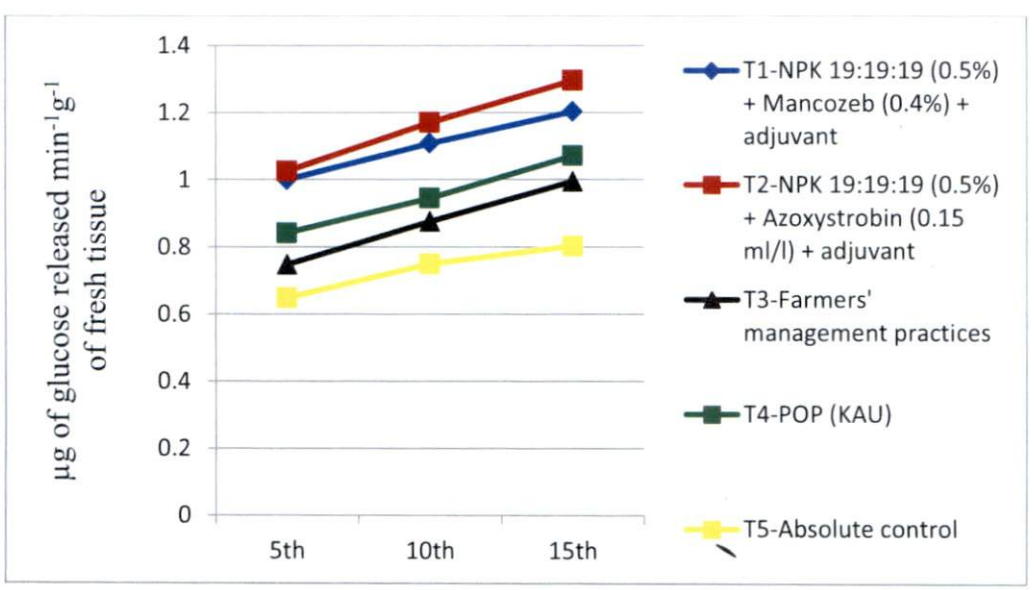


Fig. 14b. Activity of β - 1, 3 glucanase activity in culinary melon plants – Vavamoola

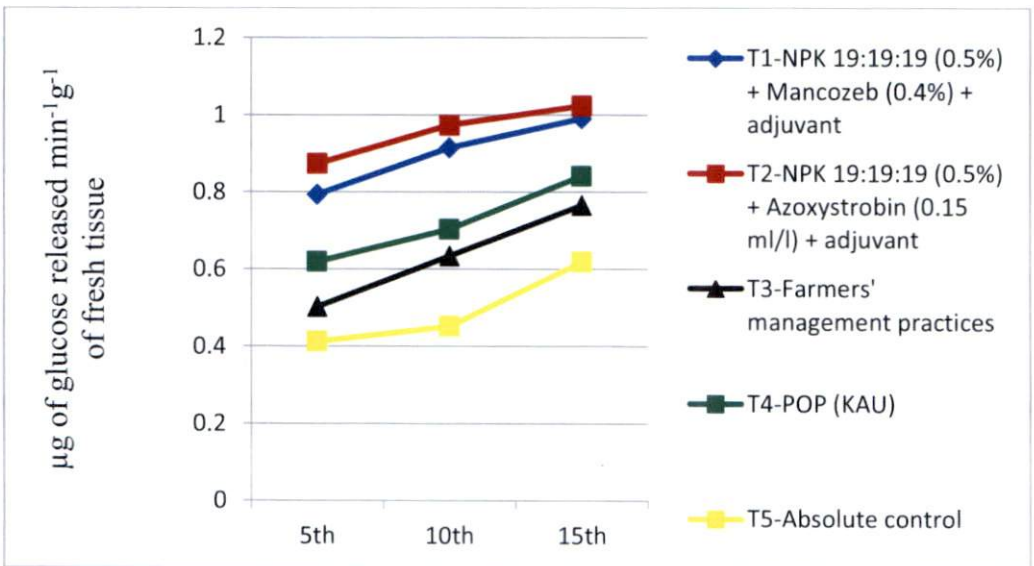


Fig. 14c. Activity of β - 1, 3 glucanase activity in culinary melon plants – Venjaramoodu

4.8.5. Changes in the super oxide dismutase (SOD) activity in culinary melon plants treated with nutrients and fungicides

In all three locations namely (Venganoor, Vavamoola and Venjaramoodu), super oxide dismutase (SOD) activity gradually increased in culinary melon leaves following treatments with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (0.984, 1.146 unit $min^{-1}g^{-1}$ of fresh tissue) and foliar spray of NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (0.921, 1.111 unit $min^{-1}g^{-1}$ of fresh tissue) which reached maximum level on 10^{th} day after treatment when compared to control. The enzyme activity subsequently declined on 15^{th} day. Plants treated with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant recorded maximum super oxide dismutase (SOD) activity (0.825 unit $min^{-1}g^{-1}$ of fresh tissue) followed by foliar spray of NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (0.778 unit $min^{-1}g^{-1}$ of fresh tissue) on 15^{th} day after treatment of the plants in three location respectively. Lowest super oxide dismutase (SOD) activity was recorded in the plants of control plot (0.535, 0.672, 0.414 unit $min^{-1}g^{-1}$ of fresh tissue) on 5^{th}, 10^{th} and 15^{th} days after treatment of the plants (Table 22) (Fig. 15a, 15b, 15c).

4.8.6. Changes in the phenolic content in culinary melon plants treated with nutrients and fungicides

In all three locations namely (Venganoor, Vavamoola and Venjaramoodu), phenolic content gradually increased in culinary melon leaves following treatments with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (0.15ml/l) + adjuvant (1.342, 0.963 µg of catechol g^{-1} of fresh tissue) and foliar spray of NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (1.289, 0.912 µg of catechol g^{-1} of fresh tissue) which reached maximum level at 10^{th} day after treatment when compared to control. The enzyme activity subsequently

Table 22. Activity of super oxide dismutase (SOD) in culinary melon plants of farmers' field trials – Venganoor, Vavamoola and Venjaramoodu (unit $min^{-1} g^{-1}$ of fresh tissue)

Treatments	Venganoor			Vavamoola			Venjaramoodu		
	5th	10th	15th	5th	10th	15th	5th	10th	15th
T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	0.569	0.686	0.921	0.527	0.813	1.111	0.530	0.622	0.778
T2- NPK 19:19:19 (0.5%) +Azoxystrobin (0.15 ml/l) +adjuvant	0.608	0.796	0.984	0.546	0.875	1.146	0.595	0.669	0.825
T3- Farmers' management practices	0.340	0.406	0.619	0.313	0.476	0.724	0.210	0.387	0.555
T4- POP (KAU)	0.414	0.536	0.713	0.479	0.692	0.946	0.321	0.444	0.631
T5- Absolute control	0.214	0.311	0.535	0.286	0.351	0.672	0.105	0.296	0.414
CD (0.05)	0.106	0.074	0.078	0.068	0.073	0.069	0.062	0.062	0.083

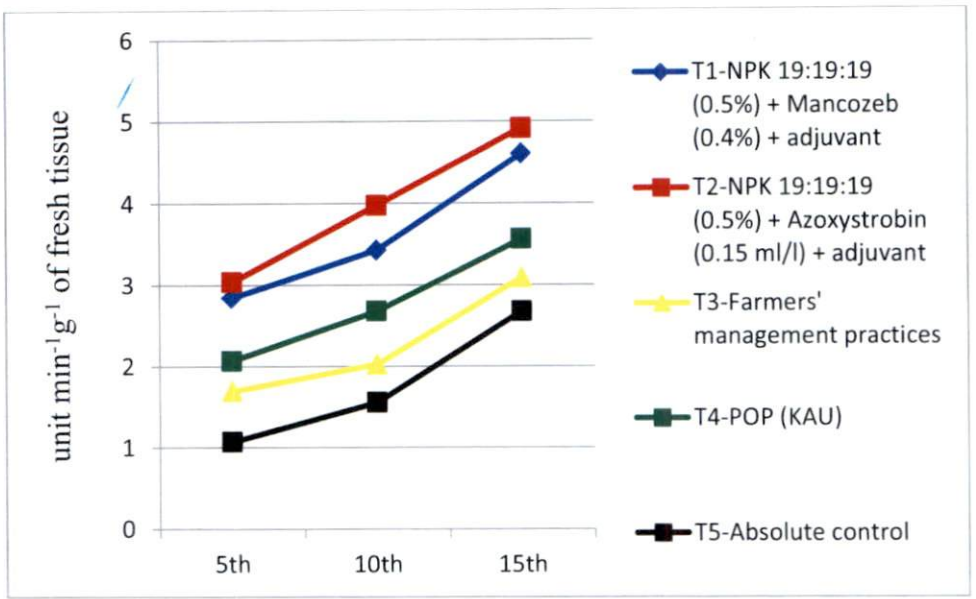


Fig. 15a. Activity of super oxide dismutase (SOD) activity in culinary melon plants – Venganoor

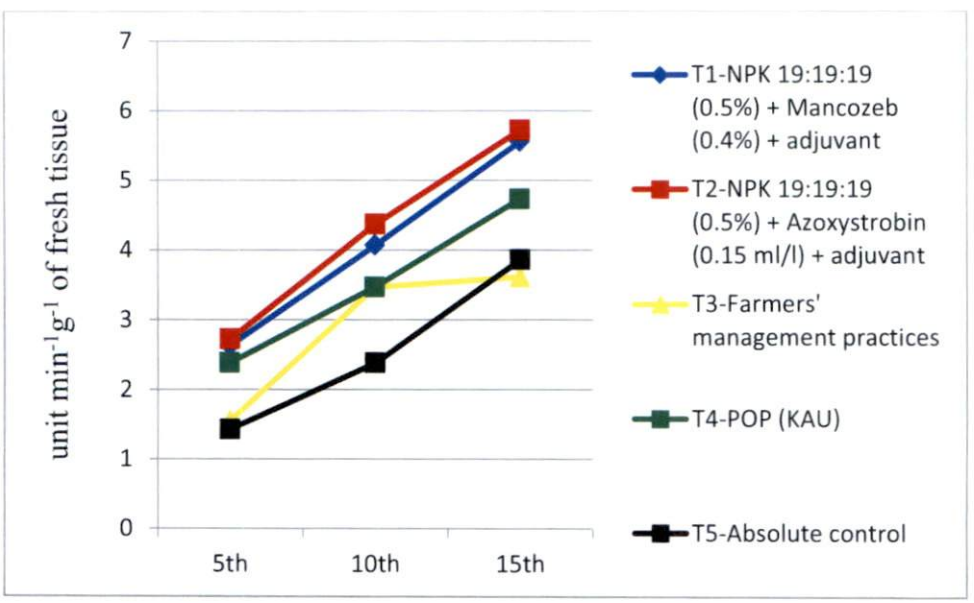
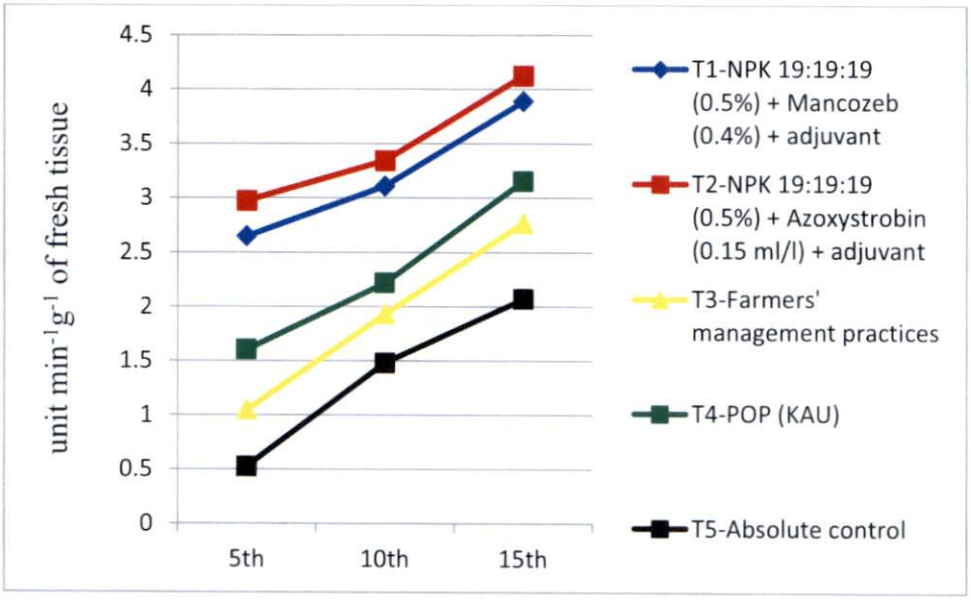


Fig. 15b. Activity of super oxide dismutase (SOD) activity in culinary melon plants – Vavamoola



Days after treatment application

Fig. 15c. Activity of super oxide dismutase (SOD) activity in culinary melon plants – Venjaramoodu

declined on 15th day. Plants treated with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant recorded maximum phenolic content (1.384 µg of catechol g^{-1} of fresh tissue) followed by foliar spray of (0.15ml/l) + mancozeb (0.4 per cent) + adjuvant (1.309 µg of catechol g^{-1} of fresh tissue) on 15th day after treatment of the plants in three location respectively. Lowest phenolic content was recorded in the plants of control plot (0.954, 0.352, 1.027 µg of catechol g^{-1} of fresh tissue) on 5th, 10th and 15th days after treatment of the plants (Table 23) (Fig. 16a, 16b, 16c).

4.9. Assessment of physiological parameters

4.9.1. Nutrient content (NPK %)

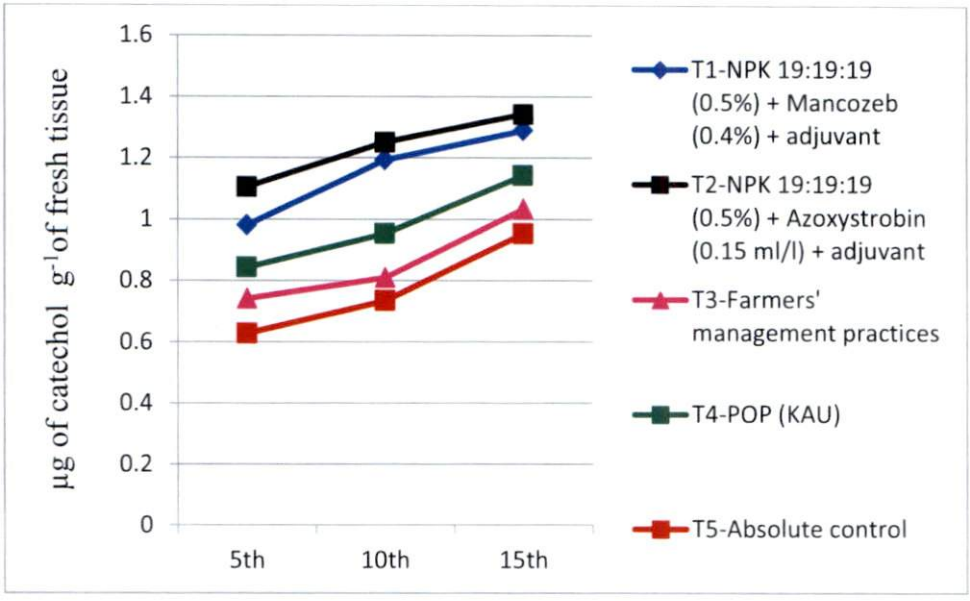
4.9.1.1. Venganoor

Nitrogen content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (2.50%) which was on par with that of plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2.31%). N content of all other treatment samples were significantly higher than that of plants in control plot (1.29%). Lowest N content was recorded in leaf samples obtained from control plant (1.29%) (Table 24a) (Fig. 17a).

Phosphorus content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (0.62%) which was on par with that of plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (0.57%). P content of all other treatment samples were significantly higher than that of control (0.31%). Lowest P content was recorded in leaf samples obtained from that of plants in control plot (0.31%) (Table 24a) (Fig. 17b).

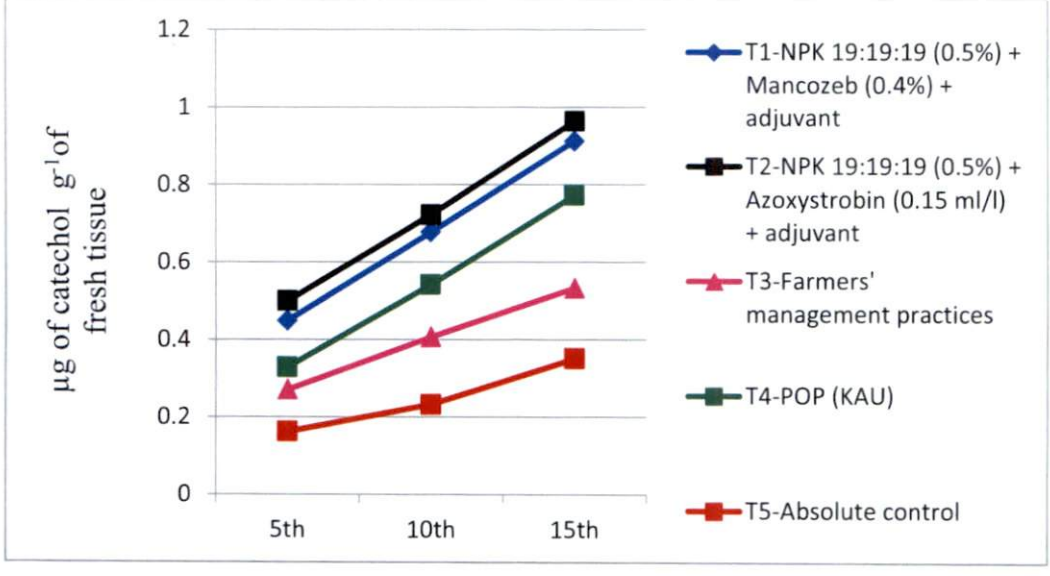
Table 23. Activity of phenol in culinary melon plants of farmers' field trials – Venganoor, Vavamoola and Venjaramoodu

Treatments	Venganoor			Vavamoola			Venjaramoodu		
	5th	10th	15th	5th	10th	15th	5th	10th	15th
T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	0.981	1.193	1.289	0.449	0.678	0.912	1.125	1.211	1.309
T2- NPK 19:19:19 (0.5%) +Azoxystrobin (0.15 ml/l) +adjuvant	1.105	1.250	1.342	0.497	0.723	0.963	1.186	1.294	1.384
T3- Farmers' management practices	0.742	0.810	1.034	0.272	0.408	0.533	0.809	0.938	1.108
T4- POP (KAU)	0.844	0.954	1.143	0.330	0.542	0.773	0.974	1.007	1.145
T5- Absolute control	0.629	0.735	0.954	0.163	0.233	0.352	0.724	0.866	1.027
CD (0.05)	0.140	0.066	0.069	0.069	0.097	0.069	0.071	0.076	0.077



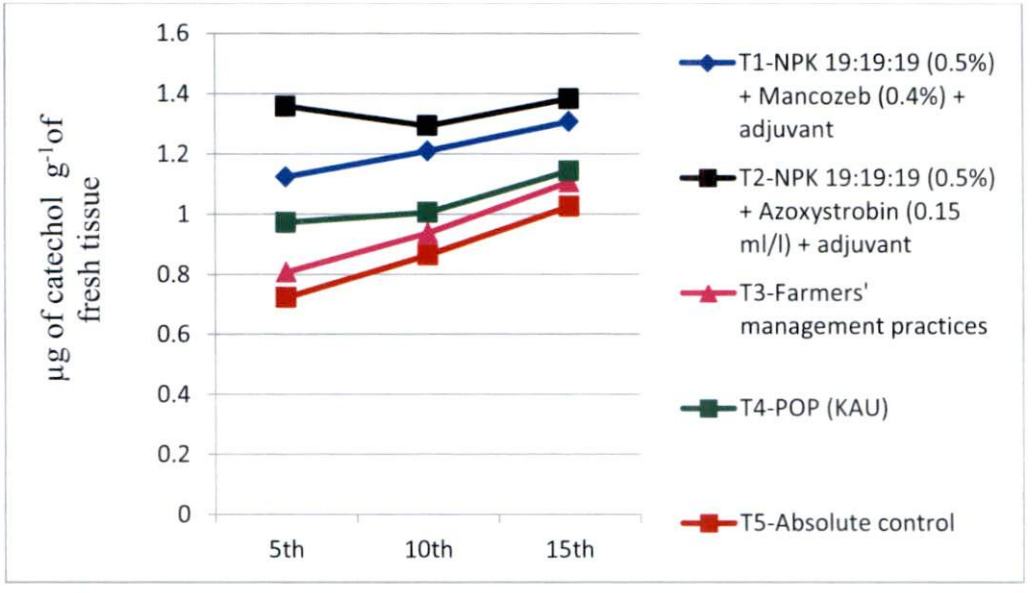
Days after treatment application

Fig. 16a. Activity of phenolic content in culinary melon plants – Venganoor



Days after treatment application

Fig. 16b. Activity of phenolic content in culinary melon plants – Vavamoola



Days after treatment application

Fig. 16c. Activity of phenolic content in culinary melon plants – Venjaramoodu

Potassium content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (3.71%) which was on par with that of plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (3.64%). K content of all other treatment samples were significantly higher than that of control (1.21%). Lowest K content was recorded in leaf samples obtained from that of plants in control plot (1.21%) (Table 24a) (Fig. 17c).

4.9.1.2. Vavamoola

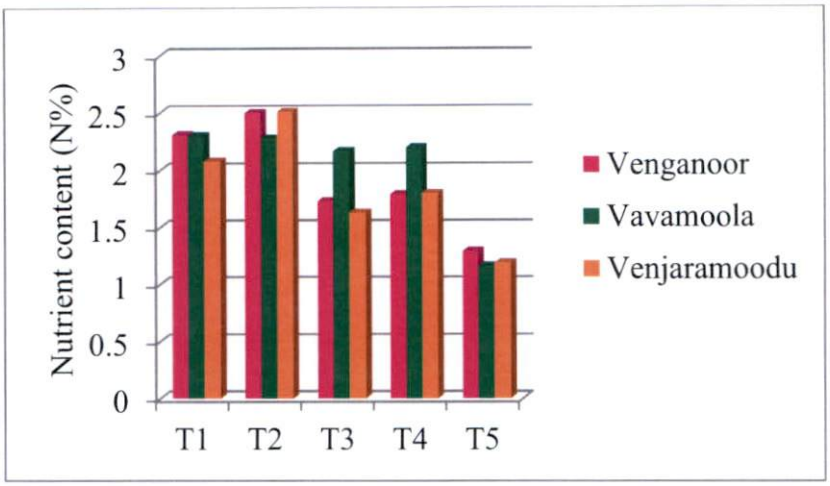
Nitrogen content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2.30%) which was on par with of plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (2.28%). N content all other treatments were significantly higher than that of plants in control plot (1.16%). Lowest N content was recorded in leaf samples obtained from plants of control plot (1.16%) (Table 24a) (Fig. 17a).

Phosphorus content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant and NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (0.63%) which were equal and on par with that of plants raised according to (POP) KAU (0.46%) and farmers' management practices (0.45%). P content all other treatment samples were significantly higher than that of plants in control plot (0.33%). Lowest P content was recorded in leaf samples obtained from plants of control plot (0.33%) (Table 24a) (Fig. 17b).

Potassium content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (3.64%) which was on par with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) +

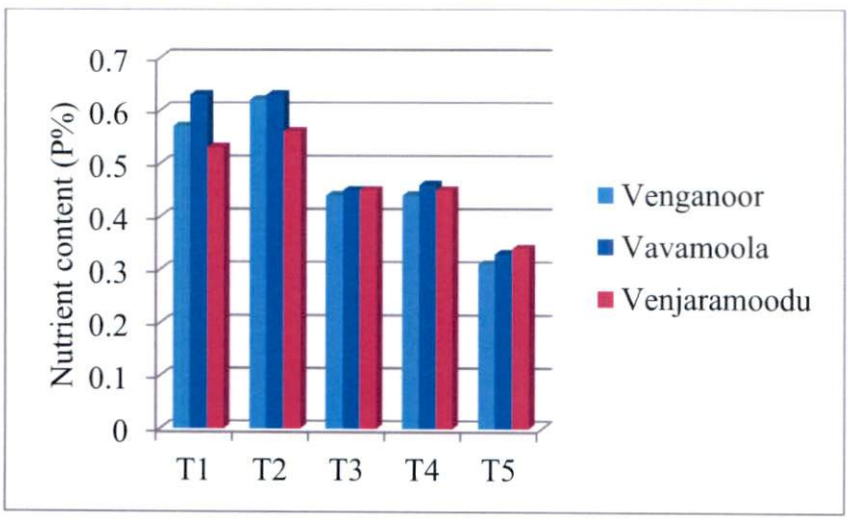
Table 24a. Effect of different treatments on nutrient content (%) of culinary melon plants in confirmation trials

Sl.no	Treatments	Venganoor			Vavamoola			Venjaramoodu		
		N	P	K	N	P	K	N	P	K
1	T1- NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant	2.31	0.57	3.64	2.30	0.63	3.63	2.08	0.53	3.70
2	T2- NPK 19:19:19 (0.5%) + Azoxystrobin(0.15 ml/l) + adjuvant	2.50	0.62	3.71	2.28	0.63	3.64	2.51	0.56	3.79
3	T3- Farmers' management practices	1.73	0.44	2.85	2.17	0.45	3.39	1.63	0.45	3.24
4	T4- POP (KAU)	1.79	0.44	3.28	2.20	0.46	3.40	1.80	0.45	3.35
5	T5- Absolute control	1.29	0.31	1.21	1.16	0.33	1.24	1.19	0.34	1.16
	CD (0.05)	0.324	0.266	0.447	0.290	0.204	0.271	0.577	0.192	0.286



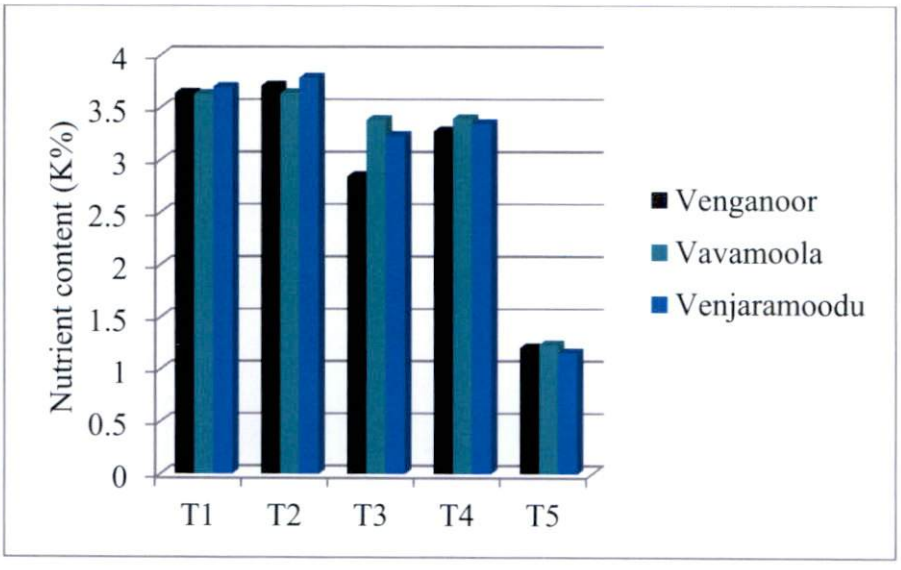
T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 17a. Nutrient content (N %) of culinary melon plants in confirmation trials



T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 17b. Nutrient content (P %) of culinary melon plants in confirmation trials



T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 17c. Nutrient content (K %) of culinary melon plants in confirmation trials

adjuvant (3.63%), plants raised according to (POP) KAU (3.40%) and farmers' management practices (3.39%). K content all other treatment samples were significantly higher than that of control (1.24%). Lowest K content was recorded in leaf samples obtained from plants of control plot (1.24%) (Table 24a) (Fig. 17c).

4.9.1.3. Venjaramoodu

Nitrogen content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (2.51%) which was on par with that of plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2.08%) and plants raised according to (POP) KAU (1.80%). N content all other treatment samples were significantly higher than that of plants in control plot (1.19%). Lowest N content was recorded in leaf samples obtained from plants in control plot (1.19%) (Table 24a) (Fig. 17a).

Phosphorus content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (0.56%) which was on par with that of plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (0.53%). P content all other treatment samples were significantly higher than that of plants in control plot (0.34%). Lowest P content was recorded in leaf samples obtained from plants in control plot (0.34%) (Table 24a) (Fig. 17b).

Potassium content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (3.79%) which was on par with that of plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (3.70%), plants raised according to (POP) KAU (3.35%) and farmers' management practices (3.24%). K content all other treatment samples were significantly higher than that of plants in control plot (1.16%). Lowest

K content was recorded in leaf samples obtained from plants in control plot (1.16%) (Table 24a) (Fig. 17c).

4.9.2. Nutrient use efficiency

4.9.2.1. Venganoor

Nutrient use efficiency (NPK) was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (1.89%) and was significantly higher than that of other treatments. Nutrient use efficiency was not recorded in the plants of control plot (Table 24b) (Fig. 18a).

4.9.2.2. Vavamoola

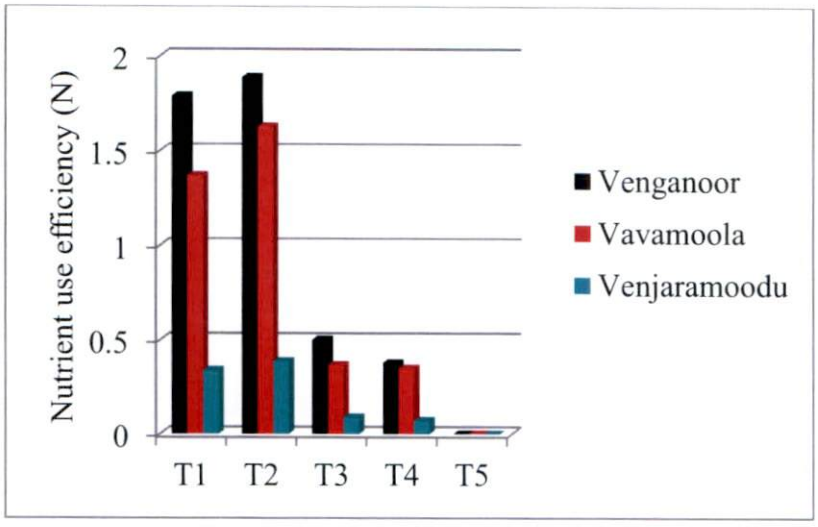
Nutrient use efficiency (NPK) was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (1.63%) and was significantly higher than that of other treatments. Nutrient use efficiency was not recorded in the plants of control plot (Table 24b) (Fig. 18b).

4.9.2.3. Venjaramoodu

Nutrient use efficiency (NPK) was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (0.39%) and was significantly higher than that of other treatments. Nutrient use efficiency was not recorded in the plants of control plot (Table 24b) (Fig. 18c).

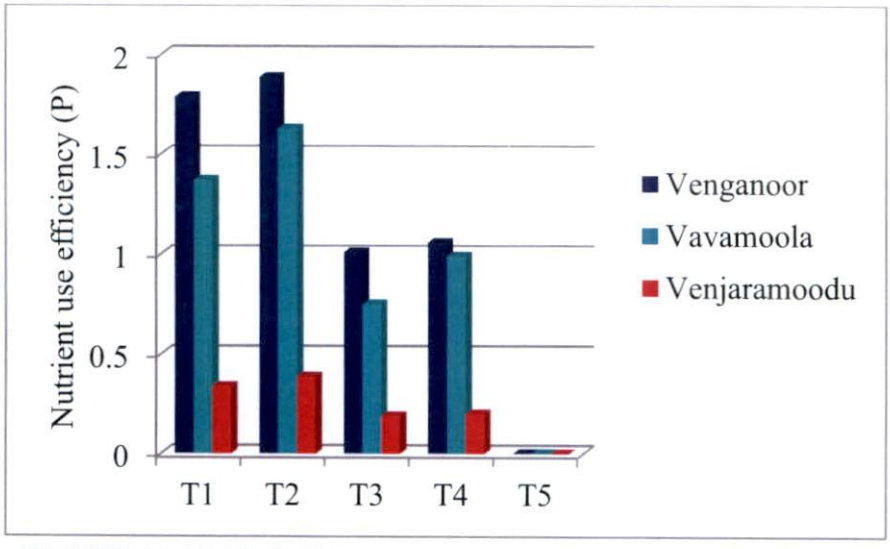
Table 24b. Effect of different treatments on nutrient use efficiency of culinary melon plants in confirmation trials

Sl.no	Treatments	Venganoor			Vavamoola			Venjaramoodu		
		N	P	K	N	P	K	N	P	K
1	T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	1.79	1.79	1.79	1.37	1.37	1.37	0.34	0.34	0.34
2	T2- NPK 19:19:19 (0.5%) +Azoxystrobin(0.15 ml/l) +adjuvant	1.89	1.89	1.89	1.63	1.63	1.63	0.39	0.39	0.39
3	T3- Farmers' management practices	0.503	1.01	1.01	0.37	0.75	0.75	0.09	0.19	0.19
4	T4- POP (KAU)	0.38	1.06	1.06	0.35	0.99	0.99	0.07	0.2	0.2
5	T5- Absolute control	0	0	0	0	0	0	0	0	0



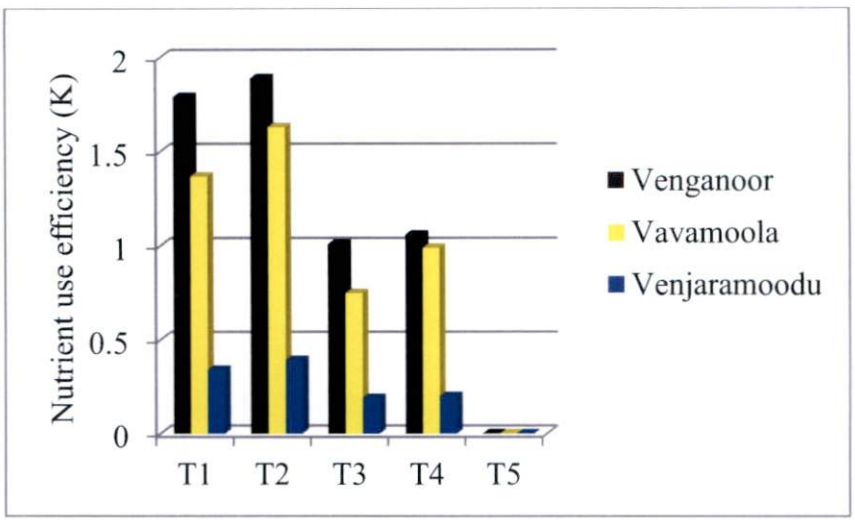
T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 18a. Nutrient use efficiency (N) of culinary melon plants in confirmation trials



T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) +Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 18b. Nutrient use efficiency (P)of culinary melon plants in confirmation trials



T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 18c. Nutrient use efficiency (K) of culinary melon plants in confirmation trials

4.9.3. Pigments status of the leaves - chlorophyll a, b and total chlorophyll content

4.9.3.1. Venganoor

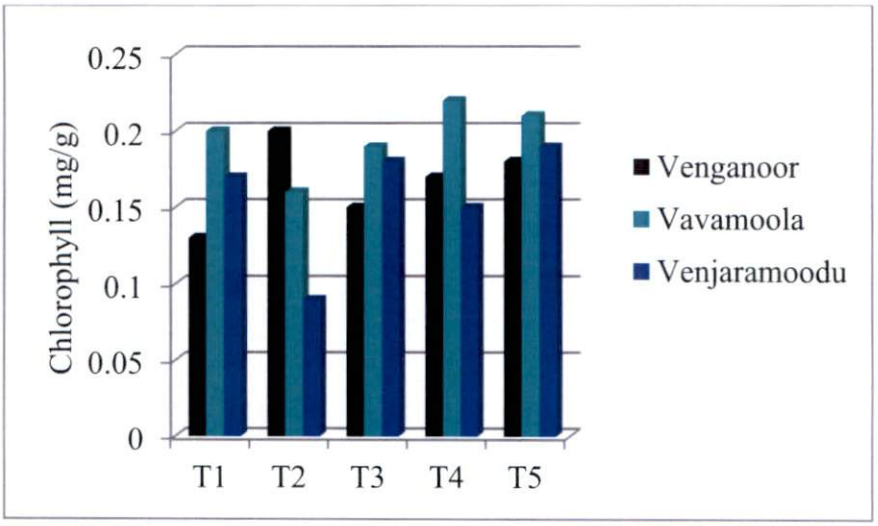
Chlorophyll a content in the leaf samples of plants applied with different treatments in the trial at Venganoor ranged from 0.13 mg/g to 0.20 mg/g. Chlorophyll a content was highest in leaf samples of plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (0.20 mg/g). Chlorophyll b content in the leaf samples of the different treatments ranged from 0.03 mg/g to 0.09 mg/g and highest chlorophyll b content was recorded in the leaf samples of plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (0.09 mg/g), and from plants raised according to farmers' management practices (0.09 mg/g) which were equal. Total chlorophyll content in the leaf samples of different treatments ranged from 0.21 mg/g to 0.32 mg/g. Total chlorophyll content was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (0.32 mg/g) (Table 25) (Fig. 19a).

4.9.3.2. Vavamoola

Chlorophyll a content in the leaf samples of plants applied with different treatments in the trial at Vavamoola ranged from 0.16 mg/g to 0.22 mg/g. Chlorophyll a content in the leaf samples obtained from plants raised according to package of practices recommendations (POP) (KAU) (0.22 mg/g) was the highest and was on par with those from control plants (0.21 mg/g). Chlorophyll b content in the leaf samples of plants applied with different treatments ranged from 0.09 mg/g to 0.15 mg/g. Chlorophyll b content was highest in the leaf samples obtained from plants raised according to package of practices recommendations (POP) (KAU) and those of plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) +

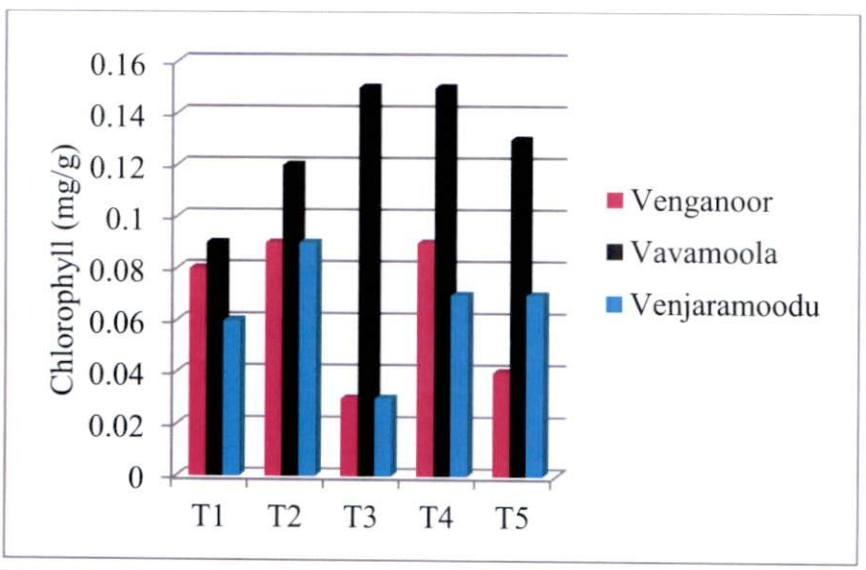
Table 25. Pigments status (chlorophyll a,b) of leaves of culinary melon plants in confirmation trials

Sl.no	Treatments	Venganoor			Vavamoola			Venjaramoodu		
		Chlorophyll (mg/g)			Chlorophyll (mg/g)			Chlorophyll (mg/g)		
		a	b	Total a, b	a	b	Total a, b	a	b	Total a, b
1	T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	0.13	0.09	0.28	0.20	0.15	0.36	0.17	0.07	0.26
2	T2- NPK 19:19:19 (0.5%) +Azoxystrobin(0.15 ml/l) +adjuvant	0.20	0.08	0.32	0.16	0.09	0.26	0.09	0.06	0.15
3	T3- Farmers' management practices	0.15	0.09	0.25	0.19	0.12	0.32	0.18	0.09	0.28
4	T4- POP (KAU)	0.17	0.03	0.21	0.22	0.15	0.37	0.15	0.03	0.19
5	T5- Absolute control	0.18	0.04	0.22	0.21	0.13	0.34	0.19	0.07	0.24
	CD (0.05)	0.300	0.157	0.293	0.145	0.171	0.122	0.102	0.135	0.090



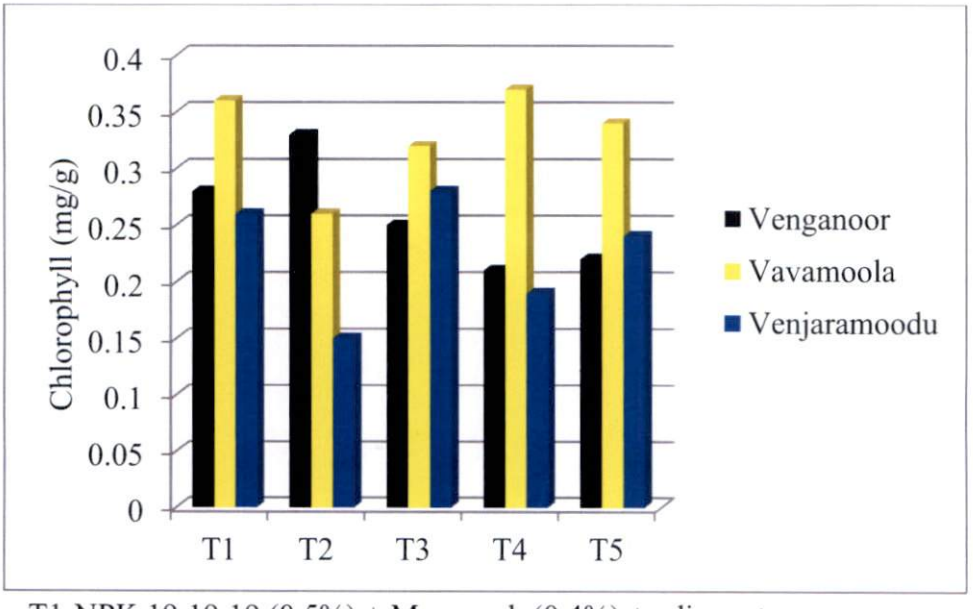
T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 19a. Pigments status of leaves of culinary melon plants - chlorophyll a



T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 19b. Pigments status of leaves of culinary melon plants - chlorophyll b



T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) +Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 19c. Pigments status of leaves of culinary melon plants – Total chlorophyll

adjuvant (0.15 mg/g) which were equal. Total chlorophyll content in the leaf samples of the different treatments ranged from 0.26 mg/g to 0.37 mg/g. Total chlorophyll content recorded in leaf samples collected from plants raised according to package of practices recommendations (POP) (KAU) was highest (0.37 mg/g) and was on par with those from plants sprayed according to NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (0.36 mg/g) (Table 25) (Fig. 19b).

4.9.3.3. Venjaramoodu

Chlorophyll a content in the leaf samples of plants applied with different treatments in the trial at Venjaramoodu ranged from 0.09 mg/g to 0.19 mg/g. Chlorophyll a content in the leaf samples obtained from plants of control plot (0.19 mg/g) was the highest and was on par with those of plants raised according to farmers' management practices (0.18 mg/g). Chlorophyll b content in the leaf samples of the different treatment ranged from 0.03 mg/g to 0.09 mg/g. Chlorophyll b content was highest in the leaf samples obtained from plants raised according to farmers' management practices (0.09 mg/g) which was significantly different from plant samples applied with other treatments. Total chlorophyll content in the leaf samples of different treatments ranged from 0.15 mg/g to 0.28 mg/g. Total chlorophyll content in the leaf samples collected from plants raised according to farmers' management practices was the highest (0.28 mg/g) and was significantly different from all other treatments (Table 25) (Fig. 19c).

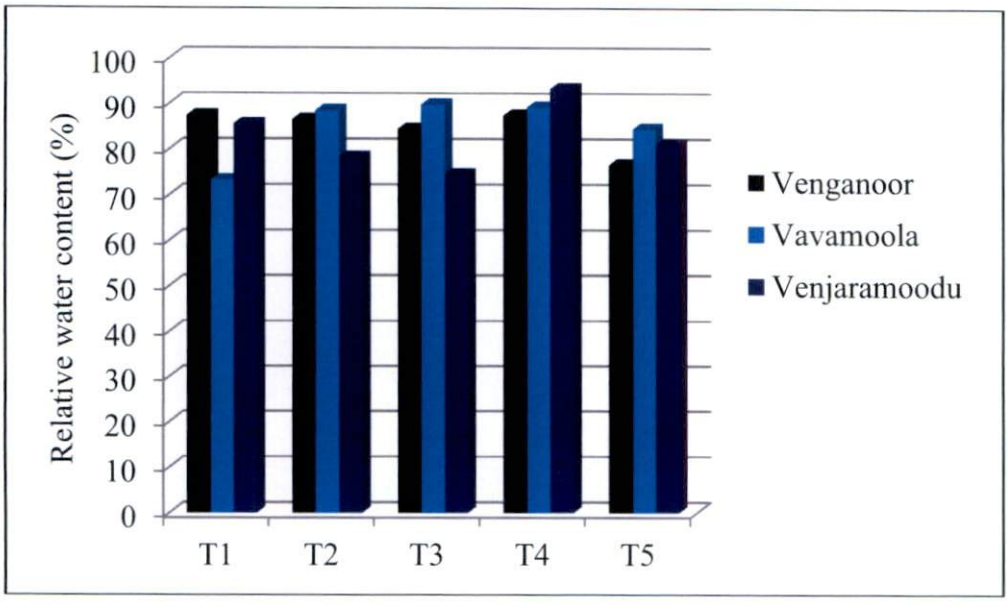
4.9.4. Relative water content (RWC %)

4.9.4.1. Venganoor

The relative water content of plants varied with different treatments and ranged from 76.71 to 87.50. A higher RWC was observed in plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (87.50%) and was

Table 26. Relative water content (%) of culinary melon plants in confirmation trials

Sl.no	Treatments	Venganoor	Vavamoola	Venjaramoodu
1	T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	87.50	73.41	85.66
2	T2- NPK 19:19:19 (0.5%) +Azoxystrobin(0.15 ml/l) +adjuvant	86.62	88.58	78.40
3	T3- Farmers' management practices	84.49	89.89	74.67
4	T4- POP (KAU)	87.08	89.28	93.42
5	T5- Absolute control	76.71	84.51	81.14
	CD (0.05)	1.519	5.238	1.591



Treatments
T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 20. Relative water content (%)

on par with those of plants raised according to package of practices recommendations (POP) (KAU) (87.08%) which were almost equal. Lowest RWC was observed in control plants (76.71%) (Table 26) (Fig. 20).

4.9.4.2. Vavamoola

The relative water content of plants varied with different treatments and ranged from 73.41 to 89.89. A higher RWC was observed in plants raised according to farmers' management practices (89.89%) which was on par with that of plants raised according to package of practices recommendations (POP) (KAU) (89.28%). Lowest RWC was observed in plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (73.41%) (Table 26) (Fig. 20).

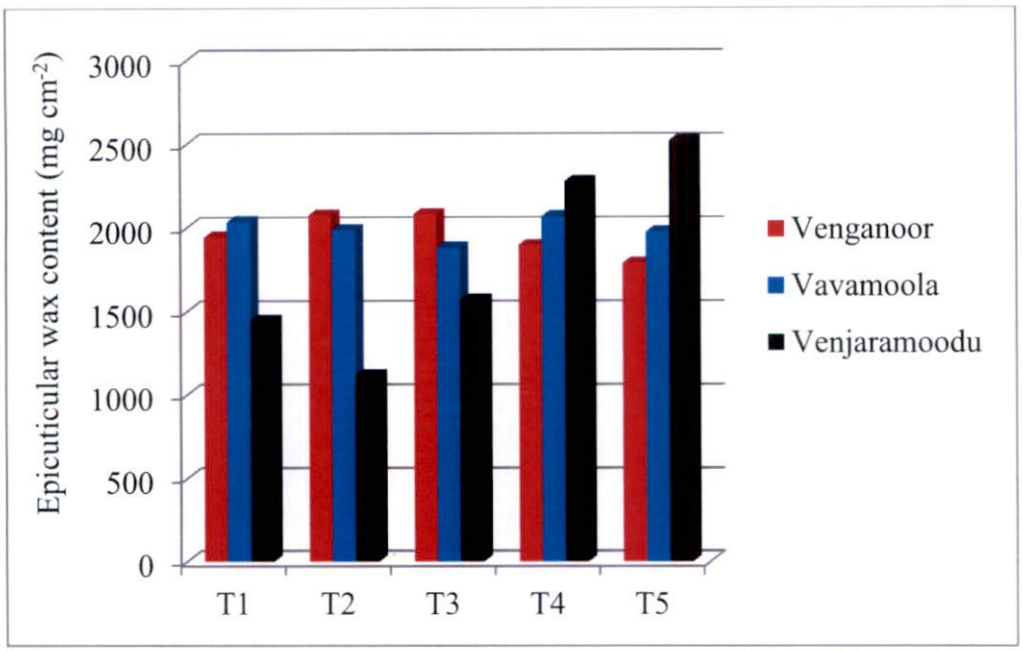
4.9.4.3. Venjaramoodu

The relative water content of plants varied with different treatments and ranged from 74.67 to 93.42. A higher RWC was observed in plants raised according to package of practices recommendations (POP) (KAU) (93.42%). This was followed by plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (85.66%). Lowest RWC was observed in plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (78.40%) (Table 26) (Fig. 20).

4.9.5. Epicuticular wax content

4.9.5.1. Venganoor

Epicuticular wax content was highest in leaf samples of plants raised according to farmers' management practices (2082.00 mg cm^{-2}) which was on par with that of leaf samples collected from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (2076.67 mg cm^{-2}). Lowest content of



Treatments
T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 21. Epicuticular wax content

epicuticular wax was observed in leaf samples of plants of control plot (1787.33 mg cm^{-2}) (Table 27) (Fig. 21).

4.9.5.2. Vavamoola

Epicuticular wax content was highest in leaf samples collected from plants raised according to package of practices recommendations (POP) (KAU) (2066.00 mg cm^{-2}) and was on par with that of plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2036.33 mg cm^{-2}). Lowest content of epicuticular wax was observed in leaf samples collected from plants raised according to farmers' management practices (1880.00 mg cm^{-2}) (Table 27) (Fig. 21).

4.9.5.3. Venjaramoodu

Epicuticular wax content was highest in leaf samples obtained from plants of control plot (2519.67 mg cm^{-2}) and was on par with that of leaf samples obtained from plants raised according to package of practices recommendations (POP) (KAU) (2277.33 mg cm^{-2}). Lowest content of epicuticular wax was observed in leaf samples of plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (1118.67 mg cm^{-2}) (Table 27) (Fig. 21).

4.9.6. Stomatal frequency

4.9.6.1. Venganoor

Stomatal frequency on upper and lower surfaces of leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant was highest (2718.80 no cm^{-2}, 2867.78 no cm^{-2}). This was followed by stomatal frequency on upper surface of leaf samples of plants in control plot (2607.07 no cm^{-2}) and that of lower surface of leaf samples collected from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant as

Table 27. Epicuticular wax content in leaves of culinary melon plants in confirmation trials

Sl.no	Treatments	Venganoor (mg cm^{-2})	Vavamoola (mg cm^{-2})	Venjaramoodu (mg cm^{-2})
1	T1- NPK 19:19:19 (0.5%) +Mancozeb (0.4%) + adjuvant	1943.33	2036.33	1447.00
2	T2- NPK 19:19:19 (0.5%) +Azoxystrobin(0.15 ml/l) +adjuvant	2076.67	1986.67	1118.67
3	T3- Farmers' management practices	2082.00	1880.00	1568.33
4	T4- POP (KAU)	1894.67	2066.00	2277.33
5	T5- Absolute control	1787.33	1974.33	2519.67
	CD (0.05)	19.047	4.645	8.728

well as those from plants of control plot (2756.05 no cm^{-2}) which were equal. Lowest stomatal frequency on upper and lower surface of leaf samples was recorded from plants raised according to farmers' management practices (2197.40 no cm^{-2}, 2569.83 no cm^{-2}) respectively (Table 28) (Fig. 22a, 22b).

4.9.6.2. Vavamoola

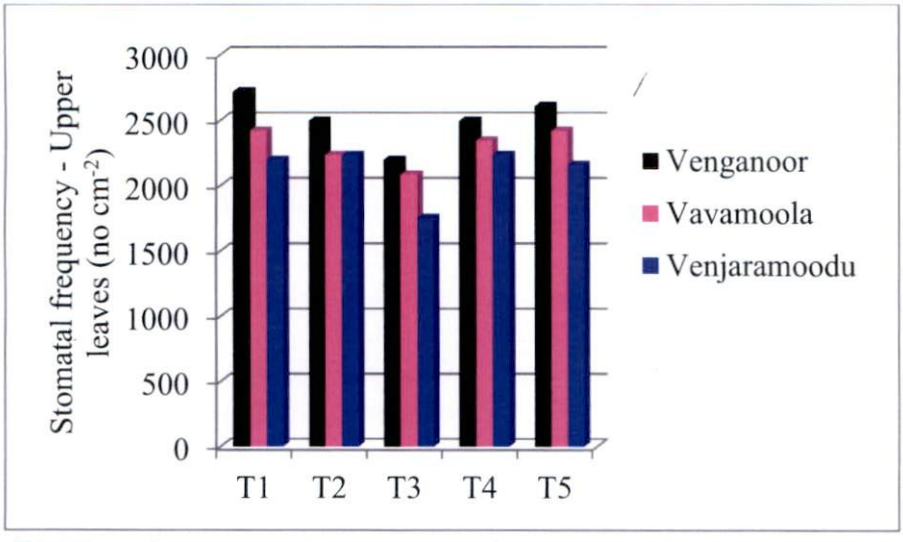
Stomatal frequency on upper surfaces of leaf samples collected from plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2420.92 no cm^{-2}) and those of plants in control plot (2420.85 no cm^{-2}) were highest and were on par with each other. Lowest stomatal frequency of upper surface of leaf samples was recorded from plants raised according to farmers' management practices (2085.66 no cm^{-2}). Stomatal frequency on lower surfaces of leaf samples was highest in plants of control plot (2681.56 no cm^{-2}). This was followed by stomatal frequency on lower surface of leaf samples of plants raised according to package of practices recommendations (POP) (KAU) (2644.32 no cm^{-2}) and plants sprayed with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2644.31 no cm^{-2}) which were almost equal. Lowest stomatal frequency on lower surface of leaf samples was recorded in leaves of plants raised according to farmers' management practices (2420.86 no cm^{-2}) (Table 28) (Fig. 22a, 22b).

4.9.6.3. Venjaramoodu

Stomatal frequency on upper surfaces of leaf samples of plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant and that of plants raised according to package of practices recommendations (POP) (KAU) were highest (2234.64 no cm^{-2}) and were equal. This was followed by NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2197.39 no cm^{-2}). Stomatal frequency on lower surface of leaf samples of plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (2577.65 no cm^{-2})

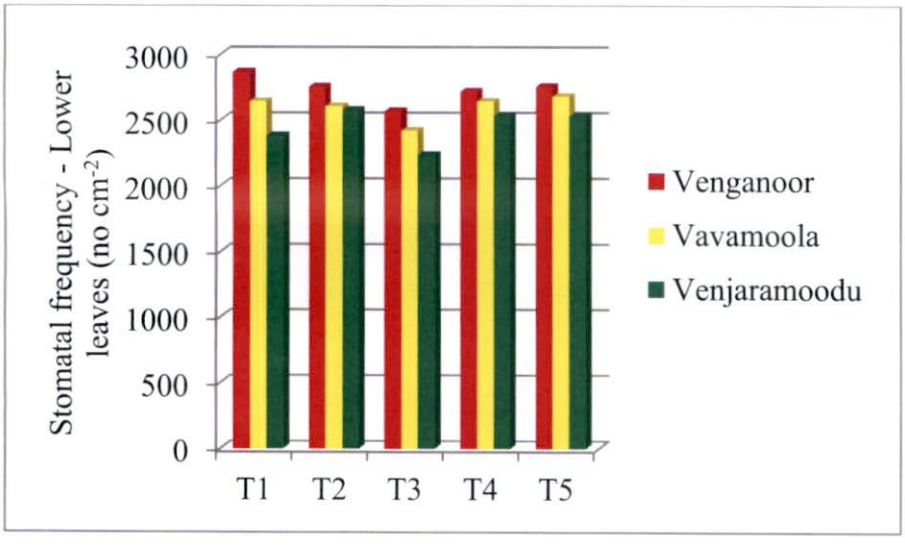
Table 28. Stomatal frequency in leaves of culinary melon plants of confirmation trials

Sl.no	Treatments	Venganoor (no cm^{-2})		Vavamoola (no cm^{-2})		Venjaramoodu (no cm^{-2})	
		Upper surface	Lower surface	Upper surface	Lower surface	Upper surface	Lower surface
1	T1- NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant	2718.80	2867.78	2420.92	2644.31	2197.39	2383.61
2	T2- NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) +adjuvant	2495.34	2756.05	2234.64	2607.07	2234.64	2577.65
3	T3- Farmers' management practices	2197.40	2569.83	2085.66	2420.86	1750.47	2234.64
4	T4- POP (KAU)	2495.34	2718.81	2346.37	2644.32	2234.64	2532.59
5	T5- Absolute control	2607.07	2756.05	2420.85	2681.56	2160.15	2532.59
	CD (0.05)	15.292	15.098	16.482	13.459	16.006	17.928



T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 22a. Stomatal frequency - Upper leaves



T1-NPK 19:19:19 (0.5%) + Mancozeb (0.4%) + adjuvant
T2-NPK 19:19:19 (0.5%) + Azoxystrobin (0.15 ml/l) + adjuvant
T3-Farmers' management practices
T4-POP (KAU)
T5-Absolute control

Fig. 22b. Stomatal frequency - Lower leaves

was highest. This was followed by stomatal frequency on lower surface of leaf samples obtained from plants raised according to package of practices recommendations (POP) (KAU) and NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (2532.59 no cm^{-2}) which were equal. Lowest stomatal frequency on upper and lower surface of leaf samples was recorded from plants raised according to farmers' management practices (1750.47 no cm^{-2}, 2234.64 no cm^{-2}) (Table 28) (Fig. 22a, 22b).

Discussion

5. DISCUSSION

Anthracnose leaf spot and downy mildew were the major foliar diseases diagnosed during the survey conducted in culinary melon fields located at Instructional Farm, College of Agriculture (CoA), Vellayani as well as in the villages of Kalliyoor and Venganoor panchayats of Thiruvananthapuram district. During the survey, severe incidence of a few diseases such as Colletotrichum leaf spot and downy mildew were observed. Jenkins and Wehner (1983) observed that diseases of cucumber were mostly caused by fungi some of which were anthracnose (*Colletotrichum lagenarium*), downy mildew (*Pseudoperonospora cubensis*), powdery mildew (*Erysiphe cichoracearum*), scab (*Cladosporium cucumerinum*), target spot (*Corynespora cassicola*) and Alternaria leaf spot (*Alternaria cucumerinum*). These fungal pathogens caused injury to all above-ground parts including the leaves, stems, petioles, peduncles and fruits.

Typical symptoms of anthracnose observed in the surveyed fields appeared as small yellowish and water soaked area that later turned circular, brown and was surrounded by a yellow halo. Diseased tissues finally dried up and at times the necrotic areas disintegrated at the centre of the lesion to form shot-hole. The disease was detected in all the ten surveyed locations of Kalliyoor and Venganoor panchayats. Averre (1991) observed that the symptoms of anthracnose vary somewhat on different hosts. On cucumber leaves the spots started as water soaked area and expanded into brown spot which was roughly circular, reaching about 1/4 to 1/2 inch in diameter. Small, growing leaves may be distorted and severe spotting may cause entire leaves to blight. The cucumber plants both in greenhouse and field produced roughly circular to brown lesions that were usually large and more than 10 mm diameter on all the above-ground tissues including leaves, stems, petioles and fruits after infection by *Colletotrichum orbiculare* (Lanston *et al.*, 1999, Goldberg, 2004 and Akem and Jovicich, 2011). Cucumber anthracnose, caused by

Colletotrichum orbiculare (Berk & Mont.) Arx (*C. lagenarium* (Pass.) Ellis & Halst.) is one of the most destructive diseases of cucurbits and occurs all over the world where cucumber is grown. The symptoms appeared on leaves, stems, fruits and the defoliation caused by foliar infections resulted in reductions in bulk yield (Amin and Ullasa, 1981). Ferrin, (2008) reported that lesions were brown to black with irregular margins often restricted by leaf veins. Anthracnose disease caused by *Colletotrichum orbiculare* had recently been considered to be particularly important wherever cucurbits were cultivated under highly controlled conditions. Severe infections caused formation of numerous leaf lesions and vine defoliation resulting in poor quality fruit, and yield loss (Egel, 2014). *C. gloeosporioides* infects about 470 different host genera some among which are economically important crops such as: avocado, mango, beans, cashews, cassava, citrus plant, cotton, cow-pea, cucumber, eggplant, green gram, mango, onion, pepper, pumpkin, papaya, sorghum, soybean, tomato, watermelon, wheat, yam, zucchini/courgette, cereals, legumes and spinach (Sharma and Kulshrestha, 2015).

Cylindrical and hyaline, conidia typical of those produced by *Colletotrichum gloeosporioides*, were observed on microscopic examination of the leaf samples that exhibited symptoms of anthracnose leaf spot. The fungus *Colletotrichum lagenarium* (syn. *C. orbiculare*) was reported as the causal agent of anthracnose disease on infected leaves of melon (Prakash *et al.* (1974); Timchenko (1977); Pegerine *et al.* (1984); Wei *et al.* (1991) and Kehinde, 2011). The conidia were oval, or pill-shaped, clear, and had no cross walls (Zitter *et al.,* 1998). According to Perez-Brito (2010) colonies of the pathogen isolated from infected leaf tissue produced white to greyish dense aerial mycelium and dark brown to black conidial masses on PDA. Conidia were hyaline, straight, and rounded at both ends and the fungal isolate was identified as *C. gloeosporioides*. Rampersad (2010) reported for the first time *C. gloeosporioides* as causing widespread anthracnose infection in pumpkin in Trinidad. He indicated that although anthracnose was a serious threat to cucurbit

production, infection was not common in pumpkin and squash. Foliar chlorosis and necrosis symptoms were observed in 15 commercial pumpkin fields and the plants severely infected were unable to support fruit maturation, which resulted in yield loss. The pathogen *C. gloeosporioides* isolated from surface-sterilized tissues of symptomatic plants, produced on potato dextrose agar (PDA) white to cream coloured colonies with gray spore masses in the center. Conidia were hyaline, cylindrical with rounded ends and aseptate.

Symptoms of downy mildew disease were observed in a few locations viz., Palapoor, Papanchani, Punjakari and Kalliyoor during the period of survey. The initial symptoms appeared on upper surface of the leaves as pale green to yellow angular patches resembling mosaic mottling which later developed to a deep brown colour as they grew older. On the underside of leaves, opposite to these patches, purplish downy growth of the pathogen was evident especially under moist conditions. The infected leaves withered quickly and as a result, the entire plant wilted.

Downy mildew is a destructive disease of cucumber, muskmelon, and watermelon. Occasionally it causes damage to gourd, pumpkin, and squash and is favored by warm, moist weather. It is most prevalent in regions where rain falls during the growing season. The disease is incited by the fungus *Pseudoperonospora cubensis*. Seebold (2010) observed that symptoms of downy mildew first appeared as pale-to-bright yellow spots on the upper surface of leaves in the crown area of the plant. Leaf spots were irregular or "blocky" in appearance and were limited by leaf veins. As lesions expanded and the number of lesions increased, leaves became necrotic and plants appeared scorched. On the underside of leaves, lesions were water-soaked and slightly sunken and profuse sporulation light to dark gray or purple in color was evident as a fuzzy or "downy" growth on lower leaf surfaces when humidity was high. Downy mildew is one of the most important melon diseases in

Northeast Brazil which caused up to 60 per cent reduction in fruit production (Cardoso *et al.*, 2002a) and 49 per cent in the content of soluble solids (Cardoso *et al.*, 2002b). Downy mildew symptoms started showing up in older leaves, as angular light-yellow spots, limited by the veins, following which lesions coalesced and caused rotting of the tissue which assumed a bronze to brown hue. Severe infection resulted in early leaf dropping, producing malformed and under developed fruits. Kehinde (2011) reported that at the early stage of downy mildew infection, symptoms appeared on the upper surface of the oldest leaves near the crown, as small and pale-green to yellow angular spots. The underside of the leaves opposite the yellow spots became covered with layers of grey mycelial growth. The leaf veins confined the spots and at advanced stage, severely infected leaves became chlorotic, turned light brown and shriveled

When the leaf samples were examined under the microscope, coenocytic mycelium of the pathogen associated with the disease, was observed. The mycelium developed sporangiophores that appeared singly or in groups of two to five and were branched dichotomously at acute angles and tapered at the tips, on which the sporangia were borne. The sporangia were greyish to olivaceous, ovoid, thin-walled and had a papilla at the distal end. Rego and Carrijo, (2000) and Tavares, (2002) observed olive-green to purple frutifications, sporangiophores and spores of the downy mildew pathogen in the adaxial leaf surface.

The assessment of diseases affecting culinary melon indicated that anthracnose leaf spot were prevalent during the entire period of survey (September 2013-December 2013) in all the ten locations. Average incidence of anthracnose leaf spot for the period of survey ranged between (41.42 to 16.28) and disease index ranged between (30.16 to 11.42). The highest average incidence as well as severity were recorded from the same locations i.e., culinary melon fields of Chavadinada. Similarly the minimum average incidence and severity were recorded

from the same location viz., IF, Vellayani. Average incidence and severity of anthracnose leaf spot was highest during month of November 2013, which recorded the highest total rainfall during the period of survey. This implied that the crop is more susceptible to the disease during spells of rains which favoured the dispersal of spores, infection as well as disease development according to Averre, (1991) and Kehinde, (2011). The highest average index and severity of anthracnose leaf spot observed at Chavadinada may be due to the continuous cropping of the same field with culinary melon in accordance to the response to questionnaire provided to farmers during the survey. Kehinde (2011) reported that the pathogen (*Colletotrichum*) had the ability to survive in fields on plant debris from previously infected plants. In general, as observed from the responses in the questionnaire, there was no adequate application of fertilizers which was evident from the pale colour and poor growth stature of the crop in the farmers' fields that were surveyed. Environmental conditions have a significant influence on the disease progression of anthracnose on cucumber. Anthracnose is less likely to infect cucumber when temperatures get above 86°F (30°C), even if rainfall occurs (Thompson and Jenkins, 1985). Minimum incidence of anthracnose leaf spot in the culinary melon plants grown at IF, Vellayani can be attributed to the proper management practices adopted according to the package of practices recommendations of (POP) KAU (2011).

At the same time, incidence of another serious fungal disease, *viz.*, downy mildew was recorded only in four out of the ten locations surveyed. The temperature and relative humidity conditions that were prevalent during the period seems to have hindered the progressive spread of the disease. According to Seebold (2010) and Kehinde (2011) although downy mildew pathogen is easily dispersed by rain splash, its spore development and further multiplication is favoured by moderately warm and dry weather.

The pathogen associated with anthracnose leaf spot disease of culinary melon plant was isolated from leaf samples exhibiting typical symptoms of the disease. Four similar fungal isolates resembling *Colletotrichum* sp. in external cultural characters were obtained from leaf samples of various surveyed locations. All the isolates produced grey mycelial growth on PDA and microscopic examination of the culture revealed that the mycelium was composed of septate, hyaline hyphae. In the older regions of the culture, masses of cylindrical and hyaline conidia, typical of those produced by *Colletotrichum gloeosporioides*, were observed. The isolates of the pathogen associated with anthracnose leaf spot of culinary melon was thus tentatively identified as, *C. gloeosporioides* based on their morphological and cultural characters. In subsequent pathogenicity studies, the four fungal isolates produced typical symptoms of anthracnose leaf spot disease in thirty-days old culinary melon plants, three to five days after artificial inoculation. Plant sprayed with distilled water that was maintained as control, remained healthy. The fungal isolates that were re-isolated from the respective artificially infected leaf tissues shared the same morphological characters of the original isolates. Earlier findings revealed *Colletotrichum lagenarium* is (syn. *C. orbiculare*) as the causal pathogen of leaf, stem and fruit anthracnose of melon (Prakash *et al.* 1974; Timchenko 1977; Peregrine *et al.*, 1984 and Wei *et al.* 1991). *C. orbiculare* is recognized world-wide as the anthracnose pathogen of cucurbits. It is a widespread pathogen of cucurbits and causes anthracnose of cucumber, watermelon, muskmelon, squash and pumpkin.

Rampersad (2010) isolated *C. gloeosporioides* from surface-sterilized tissues of symptomatic pumpkin plants in Trinidad and obtained on potato dextrose agar (PDA), white to cream colonies of the pathogen with gray spore masses in the center. Among the very few reports from India, pertaining to anthracnose of cucurbits, Sharma and Kulshrestha (2015) included cucumber and pumpkin in the 470 different host genera of the pathogen. Shamsi and Naher (2015) observed highest per cent frequency of association with *C. gloeosporioides* in *C. maxima.*

Shoji *et al.* (2013) observed that when fungal disks (5mm diameter) of the isolate SknCSY1 grown on PDA was inoculated on leaves and stems, black spots appeared both on inoculated leaves and stems and the fungal isolate was re-isolated from the diseased leaves and stems. Pathogenicity studies of the four fungal isolates produced typical symptoms of anthracnose leaf spot disease in thirty-days old culinary melon plants, three to five days after artificial inoculation. Plant sprayed with distilled water that was maintained as control, remained healthy.

Among the four pathogenic isolates of *Colletotrichum gloeosporioides*, the most virulent one was screened by estimating the incubation period (IP) and disease development time (DDT) for each isolate, in a separate experiment. IP of 3-5 days were recorded for the four isolates and the shortest IP of 3 days was observed in the IF, Vellayani isolate. DDT ranged from 7 to 10 days for the different isolates and the shortest period of 7 days was recorded for the IF, Vellayani isolate. The IF, Vellayani isolate that had the shortest IP and DDT was screened as the most virulent isolate and was used in subsequent studies. According to Zitter *et al.,* (1998) plant disease symptoms appeared about 4 days after infection. Palenchar *et al.,* (2009) reported that initial symptoms of anthracnose appeared on cucumber leaves 6 days after artificial inoculation with *C. orbiculare*. In general, the first anthracnose lesion appeared on the inoculated leaf 3 days after inoculation cucumber leaves. The brown lesions enlarged with time, up to 5 days after the appearance of initial symptom and coalesced with each other to form large diseased area on the leaves (Negishi *et al.,* 2011). In pathogenicity tests of five isolates of anthracnose pathogen (*C. gloeosporioides*) infecting pumpkin, the plants (cv. Jamaican squash) showed symptoms of infection, 7 days post-inoculation while there was no symptom on control plants (Rampersad, 2013). Therefore the period of development of symptoms observed in the pathogenicity tests of the isolates of anthracnose pathogen were comparable to the infection period reported in several other studies pertaining to anthracnose of cucurbitaceous plants.

The fungal isolates that were re-isolated from the respective artificially infected leaf tissues shared the same morphological characters of the original isolates. Earlier findings revealed *Colletotrichum lagenarium* as the causal pathogen of leaf, stem and fruit anthracnose of egusi melon. The fungus was reported by Prakash *et al.* (1974), Timchenko (1977); Peregrine *et al.*, (1984) and Wei *et al.* (1991) as the causal agent of anthracnose diseases on infected leaves of melon. Dark brown lesions were observed which were more or less circular. Ferrin (2008) reported that lesions were brown to black with irregular margins often restricted by leaf veins and stated that the differences in disease symptom exhibited may be due to the differences in cultivars assessed. In pathogenicity tests, six plants (cv. Jamaican squash) for each of five isolates were spray inoculated to runoff with a conidial suspension
(1.0×10^6 conidia/ml). Negative controls were sprayed with sterile distilled water. In repeated tests, plants were symptomatic of infection 7 days post-inoculation. There were no symptoms on control plants (Rampersad, 2010). Koch's postulates were fulfilled with the re-isolation of the pathogen from symptomatic leaf tissues. Shoji *et al.* (2013) observed when that fungal disks (5mm diameter) of a representative isolate SknCSY1 grown on PDA were inoculated on leaves and stems of sweet pepper, black spots appeared on both inoculated leaves and stems. The isolate was also re-isolated from the diseased leaves and stems.

In the present study, the morphological and cultural characters of the most virulent isolate, *viz.*, IF, Vellayani were studied. Colonies on PDA were initially floccose, white and later became grey, while it was olivaceous grey on the reverse. Hyphae was hyaline, pigmented, smooth walled, septate and measured 1.72 – 8.72µm in width. Setae were absent; conidia were hyaline, cylindrical with obtuse ends, aseptate, smooth thin-walled and size ranged from 31.34µm x 4.1µm. Based on morphological characters the fungal isolate was tentatively identified as *Colletotrichum gleosporioides* at the Department of Plant Pathology, College of

Agriculture, Vellayani. The identity of this isolate was further confirmed by morphological characterization conducted at the Institute of National Fungal Culture Collections of India (NFCCI), Pune, where molecular characterization was also performed by partial sequencing of ITSr of rDNA. The sequence analysis of ITS_4 and ITS_5 revealed that the IF,Vellayani isolate was included in the same cluster with *Colletotrichum fructicola* and was related to *Colletotrichum fructicola* with 100 per cent identity. Therefore the virulent IF,Vellayani isolate of the anthracnose pathogen isolated from culinary melon plant was identified as *Colletotrichum fructicola* which belonged to the same clade of *Colletotrichum gleosporioides.* The isolate of *C. fructicola* was deposited at the Institute of National Fungal Culture Collections of India (NFCCI), Pune, with (Accession No.3808).

The morphological characters of IF,Vellayani isolate observed in the present study matched closely with the descriptions of *C. gloeosporioides* and *C. fructicola* (Prihastuti *et al.* 2009). Similar findings were also documented by Adhikary *et al.,* (2013) stated that conidia of *Colletotrichum gleosporioides* measured 8-20 ×4-7µ. and were hyaline, single celled and smooth walled. *Colletotrichum fructicola* was first isolated from the berries of *Coffea arabica* in the Chiang Mai region of Thailand (Prihastuti *et al.,* 2009) and it has been found in tropical fruits such as chili, papaya, and longan (Phoulivong *et al.,* 2010). Shoji *et al.* (2013) stated that colony of *Colletotrichum fructicola* on PDA were gray, cottony, pale gray to pale orange, sometimes with dark flecking on the reverse. Conidia were colorless, sub cylindrical, attenuated and had blunt ends when produced on synthetic nutrient-poor agar medium (SNA). The size was 14.5-20.4 × 3.6-5.7 µm. *Colletotrichum fructicola* was originally described on coffee berries (Prihastuti *et al.* 2009) and cacao (Rojas *et al.* 2010). Traditionally the identification and characterization of *Colletotrichum* spp. with based on differences in morphology features such as colony color, size, and shape of conidia and appressorium, optimal temperature for growth, growth rate, presence or absence of setae (Arx, 1957; Smith and Black, 1990; Gunnel and Gubler,

1992; Sutton, 1992). Later molecular techniques provided alternative methods for taxonomic studies and have become important tools in solving the problem of species delimitation (MacLean *et al.,* 1993). Sharma and Shenoy (2014) investigated the phylogenetic relationships of 52 fungal isolates associated with chilli anthracnose in southern India. All the 52 isolates which were sequenced for partial ITS/5.8S rRNA and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) genes showed affinities with *Colletotrichum siamense* and *C. fructicola* within *Colletotrichum. gloeosporioides* species complex and this was the first report that indicated association of *C. fructicola* and *C. siamense* in causing chilli anthracnose in India. Many recent reports from China indicated *C. fructicola* as the incitant of anthracnose disease in the fruit plant *Pyrus bretschneideri* and an important landscape bush *Aucuba japonica* (Li, *et al.*, 2013 and Li, *et al.*, 2016). The present investigation has thus revealed the occurrence of *C. fructicola* as the incitant of anthracnose pathogen of culinary melon in Kerala as well as its phylogenetic relationship with *C. gloeosporioides* which shares almost similar morphological identity with *C. fructicola*. The pathogen that has been isolated from anthracnose infected culinary melon leaves and identified as *C. fructicola* in this study, presents the first report of the fungus causing anthracnose leaf spot of culinary melon in India.

Following the survey and isolates of the anthracnose Colletotrichum leaf spot pathogen the subsequent studies were focused on the major objective of the thesis programme for undertaking the integrated management of foliar fungal disease of Culinary melon. Among the 8 treatments tested, in the preliminary *in vitro* study, maximum inhibition (100 per cent) of mycelia growth of *C. fructicola* was observed in PDA amended with combination of NPK 19:19:19 (0.5 per cent) and mancozeb (0.4 per cent), compared to all other treatments. Hussain *et al.* (2008) indicated that mancozeb which is a derivative of dithiocarbamic acid is toxic to fungi because they are metabolized to isothiocyanate radicals inside the pathogen cells, which inactivates the –SH group of aminoacids and enzymes. Fitsum *et al.* (2014) reported that when

three synthetic fungicides at different concentrations were evaluated by the poisoned food technique against the bean anthracnose pathogen *C. lindemuthianum,* there was least mycelia growth of the pathogen in medium amended with mancozeb at 250 ppm and there was no growth at all, of the mycelium in media amended with mancozeb at 500ppm, when compared to the other two fungicides *viz.,* mancolaxyl and folpan. The present study therefore denotes the inhibitory effect of the fungicide mancozeb even when mixed with the foliar fertilizer.

The *in vitro* study also revealed the remarkable antagonistic activity of talc based formulation of bio-control agents *Pseudomonas fluorescens* at two per cent concentration that inhibited the mycelia growth of *C. fructicola* by (87.14 per cent) followed by (86.33 per cent) growth inhibition of the pathogen caused by the talc based formulation of fungal antagonist *Trichoderma viride* at two per cent Jeyalakshmi and Seetharaman (1999) reported that *T. viride* reduced the mycelial growth of *Colletotrichum* spp by over growing the pathogen, causing hyphal coiling, hyphal abnormalities, lysis of hyphae and sclerotia. Padder *et al.* (2010) reported mycelia growth inhibition of (69.21 per cent) and (64.20 per cent) by *T. viride* and *T. harzianum,* respectively, against a local strain of *C. lindemuthianum.* When Ngullie *et al.* (2010) tested seven antagonists against *C. gloeosporioides, Pseudomonas fluorescens* exerted the maximum inhibition (67.42 per cent) of mycelial growth of *C. gloeosporioides* followed by *T. viride* and *Bacillus subtilis* that inhibited mycelial growth of *C. gloeosporioides* by (63.34 per cent) and (56.86 per cent) respectively compared to the control. Out of nineteen isolates of antagonistic bacteria and twelve isolates of yeast evaluated for bio-control efficiency both under *in vitro* and *in vivo* conditions, *Pseudomonas fluorescens* (FP7) recorded the maximum inhibition of mycelial growth of *C. musae* (Faisal *et al.,* 2013). Fitsum *et al.,* (2014) stated that antagonistic effects of the three bio-agents tested showed high percentage inhibition of the mycelial growth (PIMG) of *C. lindemuthianum.* The

highest PIMG resulted from *T. viride* (80.39 per cent) followed by *T. harzianum* (75.49 per cent) and *P. fluorescens* (40.20 per cent).

The percentage inhibition of mycelial growth of *C. fructicola* was lower (62.59 per cent) due to amendment of the medium with combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and azoxystrobin (0.15 ml/l), compared to amendment with combination of foliar fertilizer and the fungicide mancozeb (0.4 per cent) or the amendment of media with bio-control agents. Inhibitory activity of azoxystrobin (0.15 ml/l) has been reported in several studies. Earlier findings revealed that the active ingredient of the fungicide azoxystrobin is characterized by its ability to limit the growth of a wide spectrum of pathogens and that it is recommended for onion, bean, pumpkin plants and tomato for control of anthracnose Warzywa (2008). Sundravadana *et al.*, (2007) observed (100 per cent) inhibition of mycelial growth of *C. gloeosporioides*, the causal agent of mango anthracnose, by azoxystrobin. Mycelial growth and sporulation of *Alternaria alternate* (Reuveni and Sheglov, 2002), *Botryosphaeria parva* and *Phomopsis* sp. (Everett *et al.*, 2005) were also inhibited by azoxystrobin. However Filoda (2008) indicated that the fungicide azoxystrobin was not satisfactory in inhibitory mycelial growth of *C. gloeosporioides* (68.00 per cent) compared to benzimidazole at the same concentration which resulted in (81.00 per cent) inhibition of the pathogen whereas a high percentage of growth inhibition was observed when azoxystrobin was used at higher concentrations of 0.1% and 0.2% (80.00 per cent and 82.00 per cent respectively). In the present experiment, only a lower concentration of the fungicide (0.15m/l) was tested and therefore further studies on the evaluation of the fungicide at higher concentrations, for the inhibition of mycelia growth of anthracnose leaf spot pathogen, is recommended.

When the medium was incorporated only with the nutrient NPK 19:19:19, the mean colony diameter of the pathogen recorded was 6.33 cm resulting in

comparatively low (29.63 per cent) inhibition of mycelial growth when compared to its combination with fungicides. From the results of this study it is obvious that combining fungicides like mancozeb (100 per cent) or azoxystrobin (62.59 per cent) with the foliar nutrient NPK 19:19:19 enhances its inhibition of the mycelial growth of the anthracnose leaf spot pathogen of culinary melon, *C. fructicola*. Earlier, Reuveni *et al.* (1993) had exemplified the benefits of integrating foliar fertilizers with fungicides for control of several crop diseases.

It was observed in the study that the per cent inhibition of growth of *C. fructicola* was further lowered with corresponding increase in mycelial growth when the nutrient calcium nitrate (5g/l) was amended in PDA in which the pathogen was grown. Maximum mean colony diameter (7.37cm) and least per cent inhibition of the mycelia growth of the pathogen (18.17 per cent) as compared to control, were recorded in PDA amended with a combination of foliar fertilizer (0.5 per cent) mixed with the nutrient calcium nitrate (5g/l) indicating that the addition of the calcium nitrate to the foliar fertilizer had lowered the percentage inhibition of the fertilizer from (29.63 per cent) to (18.17 per cent) and therefore any stimulatory effect of calcium nitrate on the growth of the anthracnose pathogen has to be ascertained. The addition of calcium has usually resulted in reduced susceptibility to rotting in most temperate and tropical fruit, however in some experiments on strawberry (*B. cinerea*) and mango (*Dothiorella dominicana*) no reduction in rotting was recorded (Ellis *et al.*, 1996, Johnson *et al.*, 1990) while in guava addition of low doses of calcium post-harvest, stimulated growth of the fungus *Colletotrichum gloeosporioides* up to a certain concentration, above which fungal growth was inhibited.

As observed in the study on inhibition of mycelia growth of *C. fructicola*, maximum inhibition of conidial germination (100 per cent) of the pathogen *C. fructicola* was observed in combination of NPK 19:19:19 (0.5 per cent) and

mancozeb (0.4 per cent). Singh *et al.* (1990) stated that Dithane M-45 and Redomil were the most effective fungicides against *C. gloeosporioides*. Imtiaj *et al.*, (2005) stated that Dithane M-45 and Redomil completely inhibited conidial germination of *C. gloeosporioides* when the fungus was immersed in the chemicals for 10-20 minutes at 500-1000 ppm concentrations. In case of bio-control agents treatment of the spore suspension with talc based formulation of bacterial antagonist *viz.*, *Pseudomonas fluorescens* (two percent) resulted in (95 per cent) inhibition of germination of conidia of *C. fructicola*. Sporulation was stimulated in *Alternaria solani, Curvularia pallescens, Helminthosporium speciferum, Leptoxyphium axillatum* and *Mucor* spp., at one per cent and two per cent concentrations of *Pseudomonas fluorescens*. However, at higher concentrations (three per cent, four per cent and five per cent), sporulation was reduced (Maurya *et al.*, 2006). There was (93.67 per cent) inhibition of conidial germination due to the fungal antagonist *Trichoderma viride.* Rahman *et al.* (2011) stated that hundred per cent inhibition of conidial germination and shortest germ tube formation was exhibited when treated with 2000 mg/l concentration of 30-day-old metabolites of *T. harzianum* IMI-392433 and the lowest inhibition of conidial germination and longest germ tube formation was recorded at 1000 mg/l concentrations of 10-day-old metabolites of *T. pseudo koningii* IMI-392431after 4 to 24 hours of incubation. Treating spore suspension with combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and azoxystrobin (0.15ml/l) resulted in (91.33 per cent) inhibition of conidial germination. Sporulation of *Alternaria alternate* (Reuveni and Sheglov, 2002), *Botryosphaeria parva* and *Phomopsis* sp. (Everett *et al.*, 2005) were also inhibited by azoxystrobin.

Inhibition of conidial germination of *C. fructicola* due to amendment of the spore suspension with NPK 19:19:19 (0.5 per cent) was higher than the inhibition observed on treatment with calcium nitrate (84.00 per cent). Maximum spore germination (19.33 per cent) and correspondingly lowest per cent inhibition (80.67 per cent) of spore germination of the pathogen as compared to control was

recorded when the spore suspension was treated with combination of foliar fertilizer NPK 19:19:19 and calcium nitrate (5g/l).

Results of the studies on inhibition of mycelia growth and spore germination were comparable, as the efficacies of the treatments tested were almost similar in both experiments. However the per cent inhibition of conidial germination ranged from (80.67 per cent to 100 per cent) while there was a wide range (18.17 per cent to 100 per cent) in the inhibition of mycelia growth of the pathogen indicating that most of the treatments were more effective in inhibiting the sporulation and perpertuation of the fungus. This was more evident in the fungicide azoxystrobin (0.15 ml/l) which induced only (62.59 per cent) inhibition of mycelia growth of the *C. fructicola* whereas the conidial germination of pathogen was inhibited by (91.33 per cent).

As in the inhibition studies of mycelia growth of *C. fructicola*, inhibitory effect of calcium nitrate on spore germination was not as high as in other treatments. Spore germination was further increased when the foliar fertilizer NPK 19:19:19 was mixed with calcium nitrate which was similar to the effect of the treatment on mycelia growth of the pathogen.

Two greenhouse experiment conducted by during March - June 2014 and August - October 2014 to validate the efficacies of the treatments tested in the *in vitro* experiment, indicated that minimum disease incidence (DI) and per cent disease index (PDI) were recorded in plants that were treated with a combination of NPK 19:19:19 (0.5 per cent) and mancozeb (0.4 per cent) along with adjuvant. The foliar fertilizers were applied at 10 days interval starting from 10 days after sowing and the fungicide was incorporated in the foliar fertilizer with the appearance of initial symptoms. This was followed by the reduction in DI and PDI of plants that were sprayed with a combination of NPK 19:19:19 (0.5 per cent) and the newer fungicide azoxystrobin (0.15 ml/l) and adjuvant. Mixing the nutrient calcium nitrate

(0.5 per cent) along with the foliar fertilizer NPK 19:19:19 (0.5 per cent) and adjuvant was also effective in reducing the disease incidence (DI) and percentage disease index (PDI) of anthracnose leaf spot though not as effective as the amendment with fungicides. Mere spraying of foliar fertilizer without any fungicide or nutrient amendment on cucurbit plants also gave appreciable control of the disease which was comparable with the combination of foliar fertilizer and calcium nitrate. The fungicide treatments given separately following the application of foliar fertilizers, were not as effective as the combination of the two chemicals. Meanwhile the efficacy of the two bio-control agents *viz.*, *Pseudomonas fluorescens* and *Trichoderma viride* were not translated to the greenhouse experiment as they were not as effective as the previous treatments in suppressing anthracnose leaf spot of culinary melon. The extent of control of anthracnose leaf spot was similar in case of plants that received plant protection measures that were adopted by farmers as well as those adopted according to package of practices recommendations of (POP) KAU (2011). Disease incidence and severity were comparatively higher in plants maintained as absolute control which did not receive any plant management or plant protection measures. Therefore this study indicated that application of foliar fertilizer had moderate effect in suppressing anthracnose leaf spot and that the efficacy could be further enhanced by mixing with any of the fungicides like mancozeb or azoxystrobin or even the nutrient calcium nitrate. Concomitant to the control of anthracnose leaf spot, plant biometric characters *viz.*, plant height, leaf length, leaf breadth, number of branches and number of fruits produced were all highest in plants that received combination of NPK 19:19:19 (0.5 per cent) and mancozeb (0.4 per cent) along with adjuvant followed by those that were sprayed with a combination of NPK 19:19:19 (0.5 per cent) and the newer fungicide azoxystrobin (0.15 ml/l) along with adjuvant. For all other treatments the same trend as in the control of anthracnose leaf spot was observed with respect to the biometric characters that were recorded. Therefore from this study it is evident that the foliar fertilizer application has a moderate effect in suppressing anthracnose leaf spot and thereby improving the plant

growth and yield, which in turn is enhanced by mixing it with the fungicides like mancozeb (0.4 per cent), azoxystrobin (0.15 ml/l) and nutrient calcium nitrate (0.5 per cent). In Malaysia the use of combined spray applications of mancozeb, the insecticide dicrotophos and foliar fertilizer gave excellent control of mango anthracnose and boosted fruit yields when used at intervals of 7-10 days from the beginning of flower bud formation (Kwee and Chong, 1985). Foliar application may also overcome the block of nutrient uptake and enrich the target organs (*viz.*, the foliage) directly with the appropriate amount of nutrients. There are previous lines of evidence that suggested that foliar application of nutrients were effective in reducing Alternaria leaf spot severity in tomato (Stevenson and Stewert, 1988 and Zitter and Wolfe, 1989). Reuveni *et al.,* (1993) had elaborated on the benefits of integrating foliar fertilizers with fungicides for control of several crop diseases including powdery mildew of cucumber. Field experiments conducted by Maczynska and Glazek (2005) indicated that foliar fertilization of all experimental plots improved leaf condition and therefore halted the development of wheat leaf diseases. They also observed increases of 1000 grain mass and yield was high for each plot where a fertilizer and a full or half dose of a fungicide was applied. Cushman *et al.,* (2007) reported that among spray treatments consisting of azoxystrobin/chlorothalonil, alone or in combination with potassium bicarbonate, foliar phosphite (0N–12.2P–21.6K), or foliar nitrogen (25N–0P–0K), all fungicide treatments reduced foliar diseases of pumpkin and significantly increased the yield from the crop.

Calcium is a vital nutrient elemental nutrient for normal plant development by taking part in copious physiological and biosynthesis processes (Barker and Pilbeam, 2007). With respect to calcium nitrate there are controversial reports on the impact of the element in suppressing plant diseases. Despite successful control of tip burn disease of lettuce in the laboratory, foliar application of calcium salts have not been effective in controlling the disease in the fields (Corgan and Cotter, 1971). However Walter *et al.* (2008) indicated that calcium nitrate when used as a source of

nitrogen in strawberry reduced the disease caused by *Botrytis cinerea*. Similarly Singh *et al.*, (2009) observed that application of calcium carbonate or calcium nitrate reduced *Phytophthora* stem rot of soyabean. Eryani-Raqeeb *et al.* (2009) indicated that calcium could be used as an alternative firming agent for fruit and that its usage decreased anthracnose disease of papaya fruits after harvesting.

There are many reports on the effects of bio-control agents in reducing severity of anthracnose leaf spot. Several biotic and abiotic factors, including chemical and physical properties of the soil, environmental conditions, host plant species, presence of non-target plant pathogens, and inter-actions with the soil micro-biota, affected the ability of applied beneficial organisms to colonize, disperse and produce necessary compounds inhibitory to plant pathogens (Duffy and Defago 1997, 1999). Various isolates of *Trichoderma*, including *Trichoderma harzianum* isolate T-39 of the commercial biological control product Trichodex, were effective in controlling anthracnose (*Colletotrichum acutatum*) and grey mould (*Botrytis cinerea*) in strawberry, under controlled and greenhouse conditions (Freeman *et al.*, 2004). *Pseudomonas fluorescens* applied at two per cent concentration by foliar spray (two times) was found to be best antagonistic microbe in controlling *Colletotrichum capsici* infecting *Curcuma longa* (Ramkumar *et al.*, 2012). However, the inhibitory effects of the bacterial and fungal antagonists on the growth of the mycelia of the pathogen were not exemplified in the present greenhouse studies conducted. One factor limiting commercial utilization of biological control organisms is their inconsistent performance in the field. So a concerted effort is required to undertake preliminary investigation on the nature and stability of the metabolites involved in their antagonism towards the anthracnose leaf spot pathogen *C. fructicola* and also their survivability on culinary melon plants so as to improve the performance of these bio-control agents in controlling diseases of the crop.

Four treatments that most effectively controlled anthracnose leaf spot of culinary melon, screened from the greenhouse experiment, were further evaluated by conducting two field experiments at IF of COA, Vellayani, during January 2015 -March 2015 and April 2015 - June 2015. Results of the experiment indicated that application of a combination of the foliar fertilizer NPK-19-19-19 with either of the fungicides *viz.,* mancozeb or azoxystrobin, effectively controlled the disease compared to all other treatments. The effect of the fungicide mancozeb in inhibitory the anthracnose pathogen had been indicated, earlier pertaining to the *in vitro* and greenhouse experiments. Azoxystrobin belonging to the strobilurin class fungicide, has a broad spectrum of activity against the major classes of plant pathogenic fungi *viz.,* Ascomycotina, Basidomycotina, Deuteromycotina and Mastigomycotina (Wong and Wilcox, 2001). Curative and eradicative activity of strobilurin fungicides against several airborne pathogens have been reported (Anesiadis *et al.,* 2003). Azoxystrobin at 0.3g/l significantly reduced the lesion length (2.75 mm) compared to control (26.4 mm) caused by *Didymella bryoniae* (stem blight) of cucumber (Utkhede and Koch, 2006). According to (Yamaguchi and Fujimura, 2005) inhibition of mitrochondrial respiration of the fungal pathogen is the main mechanism of action of this fungicide. Chemical fungicides often induce systemic resistance to pathogens in plants as indicated by Anand *et al* (2007). Sundravadana *et al.,* (2007) observed the remarkable changes in lignifications related enzymes like peroxidase, poly phenol oxidase, pal β-1,3 glucanase, chitinase, catalase and total phenols due to spraying plants with azoxystrobin. The present field studies revealed that although the *in vitro* performance of azoxystrobin was not very effective, its field performance was very remarkable and significant. This may be due to the induction of systemic resistance by this fungicide as highlighted in many of the earlier reports (Wong and Wilcox, 2002).

There was no special advantage in mixing calcium nitrate with foliar fertilizer as the resultant disease suppression was lower than that observed in plants sprayed

merely with foliar fertilizer or those cultivated as per POP recommendations of KAU (2011). Disease incidence (DI) and percentage disease index (PDI) recorded in plants raised by adopting farmers' management practices of crop management and plant protection were high (treated check) and next only to the absolute control or untreated check.

Fruit dimensions (fruit length and breadth) and fruit weight were also consistently higher in plants sprayed with foliar fertilizer NPK 19:19:19 (0.5 per cent) mixed with either of the fungicides *viz.*, mancozeb (0.4 per cent) or azoxystrobin (0.15 ml/l) during both the cropping seasons. Total yield of fruits recorded per pit consisting of two plants each, was highest in plants sprayed with combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and the fungicide azoxystrobin (0.15 ml/l). This was followed by yield from plants sprayed with combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and the fungicide mancozeb (0.4 per cent). Total yields in the remaining treatments were comparable except for those from absolute control which produced the lowest yield. Sendhilvel (2003) recorded 43.06% and 33.98% increases in yield of grapes over the farmers' management practices during the first and second seasons respectively Anand *et al.* (2009) reported maximum fruit yields of (10.54 t/ha and 10.35t/ha) cucumber by spraying azoxystrobin (250 ml/ha) for the first and second seasons respectively when compared to yields from control plots (4.08t - 4.63t/ha). Sundravadana *et al.* (2007) reported that spraying azoxystrobin at the concentrations of 2-4ml/litre on mango trees for anthracnose disease control resulted in greater yield of fruits when compared to conrol plants. Fitsum *et al.* (2014) reported that yield losses due to bean anthracnose (*C. lindemuthianum*) were highly reduced by (27.30 per cent) when compared to control, by spraying plants with the fungicide mancozeb. In spite of the adequate control of anthracnose disease by mancozeb, Mc Millan (1972) had cautioned the constant use of this fungicide due to the production of ethylene as by-product.

Confirmation trials of the more effective treatments (foliar fertilizer NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) and foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) screened from the greenhouse and field experiments conducted at CoA, Vellayani, were laid out in farmers' fields at three different locations *viz.*,Venganoor, Vavamoola and Venjaramoodu during (July 2015 - October 2015) comparative performance of these treatments with treated check (package of practices recommendations of (POP) KAU (2011), adoption of farmers' disease management practices and absolute control) in controlling anthracnose leaf spot disease of culinary melon, were evaluated in these trials. Yield parameters, physiological parameters as well as microbial population of rhizosphere and phyllosphere of culinary melon plants that were applied with these treatments, were also assessed.

In all the three locations of farmers' fields chosen for the confirmation trial culinary melon, plants sprayed with foliar fertilizer NPK 19:19:19 (0.5 per cent) and azoxystrobin (0.15 ml/l) recorded the least DI and PDI of anthracnose leaf spot and among the three locations the lowest ratings were observed at Venganoor (Disease incidence and percentage disease index). This was followed by plants sprayed with foliar fertilizer NPK 19:19:19 (0.5 per cent) and mancozeb (0.4 per cent) which recorded comparatively lower DI and PDI than those recorded in the remaining treatments, at the locations of Venganoor and Vavamoola. In Venjaramoodu efficacy of this treatment was comparable with that of foliar fertilizer NPK 19:19:19 (0.5 per cent) application. With respect to the remaining treatments, efficacies of application of foliar fertilizer NPK 19:19:19 (0.5 per cent) and package of practices recommendations of (POP) KAU (2011), were comparable at the locations Venganoor, whereas at Vavamoola application of foliar fertilizer NPK 19:19:19 (0.5 per cent) was more effective than package of practices recommendations of (POP) KAU (2011). Culinary melon plants raised by adopting farmers' management practices of cultivation and plant protection had higher level of DI and PDI compared

to all the treatments except for the control. Results of the farmers' trials therefore confirmed the efficacies of those treatments that were observed in the greenhouse as well as field trials which were authenticated by several reports that have been discussed in these previous experiments. Foliar fertilizer NPK 19:19:19 (0.5 per cent) in combination with azoxystrobin (0.15 ml/l) has been proved to be very efficient in combating anthracnose leaf spot of culinary melon for the first time in Kerala. Previous studies conducted at CoA, Vellayani have also indicated the efficiency of this fungicide in controlling diseases of other crops such as Sigatoka leaf spot of banana (Shinde, 2013). However it was observed that many of the reports mainly pertain to the separate effects of the fungicide azoxystrobin (0.15 ml/l) /mancozeb (0.4 per cent) in the management of plant diseases whereas in the present study, combinations of these fungicides with foliar fertilizer were evaluated for their management of anthracnose disease of culinary melon.

With regard to yield parameters, maximum no. of harvests were recorded in plants sprayed with combination of foliar fertilizer and azoxystrobin (8.62) at Vavamoola and (7.69) at Venganoor. At Venjaramoodu the number of harvests was comparatively low for all treatments and ranged from (1.92 to 2.33). The highest daily yield of fruits was also obtained in plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) and azoxystrobin (0.15 ml/l) at Venjaramoodu followed by yield obtained in the same treatment at Vavamoola. Lowest yield (T5) control plot (1.31) was recorded at Venjaramoodu. The daily yields from plants treated with foliar fertilizer mixed with fungicides azoxystrobin (0.15 ml/l) were almost comparable in all the locations. Pooled analysis of total yield of culinary melon from all three locations indicated that highest yield was obtained from plants treated with a combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and azoxystrobin (0.15 ml/l) and this was followed by foliar fertilizer NPK 19:19:19 (0.5 per cent) and mancozeb (0.4 per cent). Yield obtained from plants raised according to farmers' management practices was lower than those of plants managed by package of

practices recommendations of (POP) KAU (2011) and lowest yield was obtained from absolute control. There are many reports on the remarkable increases in yield of several crops due to application of the fungicide azoxystrobin (0.15 ml/l). The present pioneer study on the influence of foliar fertilizer and its combinations with fungicides, in the management of anthracnose leaf spot of culinary melon indicated the efficacy of combination of foliar fertlizer with either of the fungicides azoxystrobin or mancozeb in controlling the disease. Foliar fertilizer without any combination with fungicides also resulted in moderate control of the disease though not as effective as the combinations. Relatively few studies have been undertaken in India to evaluate the impact of foliar fertilizer in the management of plant diseases. Experiments conducted in phases at CoA, Vellayani, followed by the confirmation trials conducted in farmers' fields at three different locations indicated the efficiency of foliar fertilizer and its combinations with fungicides in the suppression of anthracnose leaf spot disease of culinary melon. The efficacies of the combinations of foliar fertilizer with fungicides in controlling anthracnose leaf spot disease of cucumber were also reflected on yield increase. Application of NPK foliar fertilizer combined with the fungicides *viz.,* azoxystrobin gave highest gross income (Net revenue) and marginal revenue (benefit–cost ratio) in two locations (Venganoor and Vavamoola) and was closely followed by its combination with the fungicide, mancozeb. Yield obtained from the third location *viz.,* Venjaramoodu was comparatively lower from those recorded in the other two locations as some of the plots in the experimental area became partially flooded towards the end of the experiment on account of the incessant rains during the final stages of the crop. However the impact of the effective treatments on disease management were discernible in this location also. Although the disease suppression by mere spraying with foliar fertilizer was not as effective as those recorded in the combination of the foliar fertilizer and fungicides, the marginal revenue was comparatively higher than those observed in the remaining treatments. This present study has therefore indicated the impact of foliar spray of NPK 19:19:19 (0.5 per cent) combined with

fungicides on disease suppression and consequent increase in marginal revenue. Nwogbaga and Iwuagwu (2015) foliar fertilizer NPK controlled fungal diseases of cucumber (*Cucumis sativus*) though not better than the fungicide, Hexaconazole, the application of the foliar fertilizer gave higher marginal revenue (B:C ratio) compared to the fungicide. However they had not ascertained the effect of combining foliar fertilizer with fungicides on disease suppression and marginal returns.

During the course of trials conducted in farmers' fields for confirming the efficacies of the screened treatments in the management of anthracnose leaf spot of culinary melon, susbsidiary observations were taken to elucidate changes in the microbial flora of the plants, and also on the biochemical as well as physiological changes, in both treated and untreated plants

Comparative assessment of the effects of various treatments on the microbial population in rhizophere and phyllosphere of culinary melon plants, were made in the trials conducted in farmers' fields. In the present study, the fungal populations of rhizosphere were higher in the plants raised in control plots as well as in those raised according to package of practices recommendations of (POP) KAU (2011) and also farmers' management practices, whereas fungal populations in the rhizosphere of the plants sprayed with foliar fertilizer NPK 19:19:19 (0.5 per cent) and its combination with fungicides, were lower. Foliar fungicides applied as spray may result in high proportions of the fungicide being deposited in the soil. Up to 55 per cent of sprayed fungicides can be deposited in soil especially if applied in the early growth stages of crop cultivation especially with reduced crop cover (Jensen and Spliid, 2003). Fungicides like azoxystrobin have been reported to inhibit both mycelia and spore germination of fungi in the rhizosphere (Bartlett *et al.,* 2002; Slawecki and Young, 2002); Demirci *et al.,* 2003 and Ma and Michailides, 2004).

The fungal population of the phyllosphere, in general, was higher in the control plants as well as in those raised according to management practices of KAU (2011) and also in plants raised by farmers' management practices. In all other treatments, which comprised of foliar spray with fertilizer with and without combination of fungicides, a comparative decline in the fungal populations of the phyllosphere was observed in all the locations where the trials were conducted which is in accordance to the findings of Lindow and Brand, (2003), that fungicides reduced the abundance of yeast-like fungi on leaf surface. Karlsson (2014) observed that fungicide-use was associated with moderate but significant changes in fungal community composition on wheat leaves and advocated further research to identify the mechanisms behind fungicide-fungi interactions in the phyllosphere of agricultural crops. Identification of the interactions between pathogenic and saprotrophic phyllosphere fungi and management practices has the potential to guide the development of sustainable disease control strategies.

In two locations (Venganoor and Vavamoola), the bacterial populations were higher in rhizosphere of the plants in control plot. The bacterial populations were lower in all other treatments. This indicated that in the plants which did not receive application of chemical fertilizers, the bacterial populations of rhizosphere was high. With regard to bacterial population of phyllosphere, it was lower in the control plants in two locations (Venganoor and Vavamoola) whereas the foliar fertilizer NPK 19:19:19 (0.5 per cent) seemed to induce bacterial population in the phyllosphere which was further enhanced by the addition of azoxystrobin. Plants sprayed with combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and mancozeb (0.4 per cent) had lower bacterial population in the phyllosphere. Similarly the bacterial population in the phyllosphere of the control plants of two locations (Venganoor and Vavamoola) were low.

Results of the rhizosphere populations were not consistent and there were not much demarcations in the impact of various treatments on the fungal and bacterial populations. However in the phyllosphere, the general trend observed that the fungal population had declined due to the application of fungicides, foliar fertilizer and combination of these chemicals whereas these chemicals seemed to have increased the population of bacteria in the same region.

The present results are based only on preliminary observations on the impact of foliar fertilizer and their combinations with fungicides in comparison to plants treated with basal application of foliar fertilizer as well as control. Therefore more systematic studies on the microflora of culinary melon have to be conducted separately in order to determine the actual impact of various treatments tested in the present investigation, during the entire growth period of the crop.

Chemical fungicides often induce systemic resistance to pathogens infecting the plants. A superficial inquest for the detection of induced systemic resistance (ISR) due to the application of various treatments in the confirmation trials conducted in farmers' fields revealed that azoxystrobin (0.15 ml/l) brought about significant changes in PAL, PO, PPO, β-1, 3 glucanase, SOD and phenols. These results corroborate with the findings of SendhilVel *et al.,* (2003) who observed that activity of the PAL, PO, PPO, β-1, 3 glucanase, SOD and phenols was higher in azoxystrobin treated grapevine plants. Hewitt (1988) had indicated that the disease controlling effect of azoxystrobin was caused by host mediated reaction. The accumulation of PAL in tomato leaves treated with Fosetyl-Al to control *Fusarium* wilt has also been reported (Bompeix *et al.,* 1981).

PAL activity increased gradually in the culinary melon leaves following treatments with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant, NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant when compared to

control in all three locations and reached maximum peak on the 10th day after treatment and thereafter declined. Archana *et al.* (2011) observed that application of azoxystrobin enhanced the PAL activity in different treatments. The PAL activity was induced from first day of inoculation and reached maximum at three days after inoculation with *Plasmopara viticola*. Among the treatments, PAL activity was maximum with azoxystrobin 23SC and was almost statistically on par with *P. fluorescence* sprayed seedlings. There was almost two fold increase in PAL activity in the above treatment over pathogen inoculated and healthy control.

Peroxidase (PO) is one of the key enzymes involved in phenylpropanoid pathway and it is associated with disease resistance in plants (Hammerschmidt and Kuc, 1982). Peroxidase (PO) is a component of an early response in plants to pathogen infection and plays a major role in the biosynthesis of lignin which limits the extent of pathogen spread (Bruce and West, 1989). In the present study the activity of PO uniformly increased from 15th day in all the treatments in all three locations, and there was not much variations in induction of the enzyme in plants applied with various treatments as well as in untreated plants. However the highest activity was observed in plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant followed by the activity in plants treated with combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant. Maximum activity of PO was recorded on the 15th day in all the treatments of all the three locations. Generally variation in PO activity is correlated with innate disease resistance in many plants (Vidhyasekaran, 1988). Bradley *et al.* (1992) reported that increased PO activity has been correlated with resistance in many species including barley, cucurbits, cotton, tobacco, wheat and rice and these enzymes are involved in the polymerization of proteins and lignin or suberin precursor into plant cell wall, thus constructing a physical barrier that could prevent pathogen penetration of cell walls or movement through vessels.

Polyphenol oxidase (PPO) is an enzyme which uses molecular oxygen to catalyze the oxidation of monophenolic and orthophenolic compounds. In the present study, significant increases in activity of PPO was observed in plants treated with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant followed by NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant on 15th day. On 15th day, plants treated with foliar fertilizer without any combination with fungicides, also exhibited higher induction of PPO along with plants treated with T1 and T2. Kalim *et al.* (2000), reported that there was increase in activity of PPO in roots of plants raised from seeds treated with the systemic fungicide carbendazim. Ahmed (2016) observed that the best inducer treatments in increasing the activities of PPO in cucumber plants were amistar fungicide (azoxystrobin), potassium silicate and humic acid respectively while, the least effective treatment was propolis extract (bee glue).

Initially, the β-1, 3 glucanase activity increased gradually in the culinary melon leaves following treatments with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant, NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant when compared to control in all three locations and reached maximum peak on the 15th day. Amistar fungicide was the least effective treatment in the induction of β-1,3 glucanase activity at all three incubation periods (Ahmed, 2016).

Superoxide dismutases (SODs: EC.1·15·1·1) are metal-containing enzymes that catalyze the dismutation of super-oxide radicals to oxygen and hydrogen peroxide. SODs are localized in different subcellular compartments which can scavenge the superoxide radical at the site of formation, thus minimizing the damage to cellular components (Bowler *et al.,* 1992). In the present study revealed that SOD were observed in the leaves treated with combination of foliar fertilizer NPK19:19:19 (0.5 per cent) and the fungicide azoxystrobin (0.15 ml/l) + adjuvant followed by combination of foliar fertilizer NPK19:19:19 (0.5 per cent) and the fungicide mancozeb (0.4 per cent) + adjuvant. Maximum SOD content was observed on 15th

day in all treatments, when compared to control. Superoxide is a reactive substance that can cause the modification of cellular proteins, nucleic acids and lipids in membranes, and it is therefore toxic (Roskoski, 1996). The enzyme dismutation is a reaction in which two identical molecules are converted into different substances. Superoxide dismutase catalyzes the conversion of two superoxide anions (and two protons) to oxygen and hydrogen peroxide. Here, one molecule of superoxide is oxidized and the other is reduced. Superoxide dismutase plays a pivotal role in protecting plants against oxygen toxicity. It has been assumed that SOD has a central role in the defense against oxidative stress (Scandalias, 1993). However superoxide dismutase activity did not confer protection against oxidative damage in salt-stressed cowpea leaves (Cavalcanti *et al.*, 2004).

Phenolics are fungitoxic in nature and increase the physical and mechanical strength of the host cell wall. Plant phenolics and their oxidation products such as quinones are highly toxic to invading fungi thereby offering resistance against a wide range of pathogens (Cahill and Mccomb, 1992). Some phenolics may act as signal molecules or antioxidants and thus induce resistance (Malamy *et al.*, 1990). In the present study, increases in phenolic content were observed in culinary melon leaves applied with all the treatments when compared to control on 15th day of estimation. Significantly higher levels of phenolic content were observed in the leaves treated with combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and the fungicide azoxystrobin (0.15 ml/l) + adjuvant followed by combination of foliar fertilizer NPK19:19:19 (0.5 per cent) and the fungicide mancozeb (0.4 per cent) + adjuvant. Maximum phenolic content was observed on 15thday in all treatments, when compared to control. The same trend in the content of this defense related compound was observed in all the three locations where the trials were conducted Although phenolic content was significantly lower in the untreated plants (control) a gradual increase in the level of the fungitoxic compound was observed during the three days of observation, reaching a peak on the 15th day as observed in all the other treatments.

In all three locations where farmers' fields trials were conducted, highest content of the three nutrients N, P and K were observed in culinary melon leaves that were collected from plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant, followed by foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant. Percentage content was highest for the nutrient potassium followed by nitrogen and phosphorous and the nutrient contents were almost similar in all the three locations. Studies conducted by (Shankar *et al.,* 1994) revealed that the increased potassium content in mulberry leaf was due to availability of adequate K in soil as well as through the foliar spray of nutrients. Further, the increase in K content may also be attributed to the increased nitrogen content of the leaves which had a synergistic effect on the K in the leaves. Increased N and P content was observed by foliar application of seriboost to mulberry (Singhvi *et al.*, 2000 and Rajegowda *et al.*, 2000).

Foliar application is considered to be part of the agronomic techniques carried out during the period of cultivation as an opportunity to increase plant productivity (Bileva and Babricov, 2007; Fawzy *et al.,* 2010). In all three location, nutrient use efficiency (NPK) was highest in leaf samples obtained from plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (1.89%) and was significantly different from other treatments. Thimmaiah (2015) stated that the nutrient use and agronomic use efficiencies were highest in NPK + FYM at 7.5 t ha^{-1} + PGPR at 2 kg ha^{-1} + Frond compost at 3.75 t ha^{-1} as top dress at 25 DAT. The highest available NPK and physiological use efficiency was recorded in NPK + FYM at 7.5 t ha^{-1} + PGPR at 2 kg ha^{-1} + Vermicompost at 3.75 t ha^{-1} as top dress at 25 DAT. The highest partial factor productivity was recorded in NPK + FYM at 7.5 t ha^{-1} + PGPR at 2 kg ha^{-1}. Similarly, higher net returns (Rs. 66,728 ha^{-1}) was recorded in recommended NPK + FYM at 7.5 t ha^{-1} + PGPR at 2 kg ha^{-1} + Frond compost at 3.75 t ha^{-1} as top dress at 25 DAT. Thus the study reveals that a combination of foliar fertilizer NPK 19:19:19 and azoxystrobin (0.15 ml/l)along with the adjuvant applied

twice at 15 days' interval, was found to have both disease suppressing and growth promoting effects and thereby delivers a promising component for the integrated management of anthracnose leaf spot disease of culinary melon.

Chlorophyll status of the leaves analysed from plants applied with various treatments differed for the three locations. However in the same location the status of each pigment (chlorophyll a, b and total chlorophyll) was consistent for each treatment. In Venganoor, chlorophyll a, b and total chlorophyll content was highest in leaves obtained from plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant, foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant as well as those raised by farmers' management practices. In Vavamoola, consistent results were observed on analyzing leaves collected from plants raised according to package of practices recommendations of (POP) KAU (2011) and from plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant. Although the general pigment status of plants in the trials at Venjaramoodu was comparatively low, in this location also, the highest pigment status was observed in leaves analysed from plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant as well as in those raised by farmers' management practices as observed in Venganoor. Eleiwa *et al.* (2012) observed foliar application of foliar fertilizer (22% N; 21% P_2O_5; 17% K_2O with microelements) significantly increased concentration of chlorophyll *a,* chlorophyll *b* and carotenoids in potato leaves compared to control treatment. The fungicide azoxystrobin alleviated the decrease of chlorophyll caused by drought in the leaves of wheat plant (Baranyiova and Karel, 2014).

RWC was comparatively high in almost all three locations ranging from 85 per cent - 93 per cent. RWC was comparatively high in plants raised by farmers' management practices and plants treated with foliar fertilizer NPK 19:19:19

(0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant. Jiang *et al.* (2003) stated that the water requirement of any crop depends on factors such as variety, growth stage, growth duration, growing season conditions and plant population as well as soil and climate factors and crop management practices. The most significant effect on increasing water use efficiency (WUE) was found, following the application of azoxystrobin both in dry and wet variants. Besides, the negative impact of drought on yield was also mitigated mainly by application of azoxystrobin (Baranyiova and Karel, 2014).

Epicuticular wax content was also variable for the different treatments in different locations. Wax content in the leaves of plants raised according to package of practices recommendations of (POP) KAU (2011) and that in plants sprayed with foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant was comparatively higher in two locations. Similar findings revealed that increased tolerance of the epidermis to high spray solution concentrations is caused by the presence of the wax layer and the cuticular membrane Knoche *et al.* (1994). Santos *et al.* (2013) observed that fungicides reduce wax content and also alter its morphology. However in the present study no leaf injury or phytotoxicity was observed due to the application of plants with any of the treatments.

Stomatal frequency was highest on upper and lower surface of leaves in plants treated with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant followed by control. Lowest frequency was noticed in farmers' management practices in all three locations. Ahmed *et al.* (1983) reported effect the systemic fungicide benomyl upon growth and related activities of sunflower (*Helianthus annuus*), cotton (*Gossypium barbadense*) and cowpea plants (*Vigna sinensis*). Application of azoxystrobin in soybean reduced the water conductance through stomata closure resulting in lower rates of intercellular CO2, transpiration, and net

photosynthesis. However, azoxystrobin reduces photosynthesis regardless of the effect on stomata (Nason, 2004).

Summary

6. SUMMARY

The study entitled "Integrated management of foliar fungal disease of culinary melon (*Cucumis melo* L. var. *acidulus* Naudin)," was conducted at the College of Agriculture, Vellayani as well as in farmers' field during the period 2013-2016, encompassing the major objective to make a comparative evaluation of the efficacies of foliar application of foliar fertilizer, nutrient, bio-control agents and fungicide for the management of Colletotrichum leaf spot (*Colletotrichum* sp.) disease of culinary melon.

Surveys were conducted in culinary melon fields located at Instructional Farm, College of Agriculture, Vellayani as well as in farmers' fields near College of Agriculture, Vellayani, during September 2013 to December 2013, in order to assess the prevalence of major diseases affecting the crop. For conducting the survey, culinary melon fields having plants in the early stage of growth (not more than 10 days after sowing) were selected from ten different locations located in the panchayats of Kalliyoor and Venganoor. From each of these selected fields, observations were taken at 10 days interval, starting from 15 days after sowing up to 75 days stage of the crop.

Leaf samples collected from infected culinary melon plants in the surveyed fields, were diagnosed in the laboratory by studying the symptoms in comparison with plant disease diagnostic keys as well as by their microscopic examination, in order to identify the associated pathogen in case of fungal diseases.

Incidence and severity of foliar disease affecting culinary melon in the locations surveyed were assessed from September 2013 - December 2013. Anthracnose leaf spot disease was predominately prevalent in all the locations surveyed (Palapoor, Papanchani, Punjakari, Chavadinada, Kakkamoola, Venganoor, Panangodu, Kalliyoor, Peringamala and IF, Vellayani) whereas, downy mildew was

also detected in some locations such as Chavadinada, Kakkamoola, Venganoor, Panangodu, Peringamala and IF, Vellayani. The highest (DI and PDI) of anthracnose leaf spot was recorded 75 DAS at Chavadinada (70.00 per cent and 64.44 per cent respectively). Maximum DI and PDI of downy mildew disease (36 per cent and 33.33 per cent respectively) were observed at Papanchani.

Four fungal isolates that were identical in cultural characters were consistently obtained from leaf samples collected from the surveyed locations. All the isolates produced grey mycelial growth on PDA and on examining regions of aged mycelial growth, cylindrical hyaline, conidia typical of those produced by *Colletotrichum gloeosporioides*, were observed. Thus based on morphological and cultural charcters characters the fungus was identified as *Colletotrichum gloeosporioides* at Department of Plant Pathology and the identity was later confirmed at the Institute of National Fungal Culture Collections of India (NFCCI), Pune (accession No. 3808). A comparative evaluation of the pathogenicity of the from isolates tested on healthy culinary plants based on IP and DDT revealed that the IF, Vellayani isolate was most virulent among the four isolates obtained.

Molecular characterization of the virulent isolate of the pathogen (IF, Vellayani isolate) was conducted at NFCCI, Pune, by partial sequencing of internal transcribed spacer region (ITSR) of rDNA. In the sequence analysis of ITS_4 and ITS_5 the virulent IF, Vellayani isolate showed (100 per cent) sequence similarity with *Colletrotrichum fructicola* and the fungal pathogen was thus identified as *C. fructicola,* which forms there first report of the pathogen *Colletotrichum fructicola* causing anthracnose leaf spot disease of culinary melon in India.

Maximum inhibition of mycelia growth and spore germination of *C. fructicola* (100 per cent) was observed on PDA amended with a combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) and mancozeb (0.4 per cent) under *in*

vitro conditions. Amendment with the bacterial antagonist, *Pseudomonas fluorescens* resulted in (87.14 per cent) reduction over control which was on par with the inhibition induced by the fungal antagonist *Trichoderma viride* (86.33 per cent) by dual culture technique under *in vitro* conditions.

Pooled analysis of data obtained from two pot culture experiments revealed that the minimum DI (20.00) and PDI (12.22) were recorded in (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + (mancozeb 0.4 per cent) + adjuvant as compared to all other treatments. Growth parameters such as plant height, leaf length, leaf breadth, number of branches, number of leaves, number of fruits were also maximum in plants applied with this treatments.

Pooled analysis of results of two field experiments revealed that lowest DI and PDI were recorded in T1 plants sprayed with NPK 19:19:19 (0.5 per cent) + (mancozeb 0.4 per cent) + adjuvant (40.00 and 13.47 respectively) and (T2) plants sprayed with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant (40.00 and 13.05 respectively) and the 2 treatments were on par for both parameters and significantly superior to other treatments.

Field experiments were conducted in farmers' fields at three locations *viz.*, Venganoor, Vavamoola and Venjaramoodu and pooled analysis of the results revealed that lowest PDI (12.22) and DI (28.50) were recorded in plants treated (T2) NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant which was significantly superior to the other treatments. Economic analysis of cultivation using the treatments tested in the field trials conducted at Venganoor, Vavamoola and Venjaramoodu revealed that the returns from treatment (T2) foliar spray of plants with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant were high being Rs. 2.98, 2.57 and 0.62 for every rupee spent in the three locations, respectively.

In the confirmation trials the impact of the various treatments tested on the native microbial flora present on the culinary melon plants were studied. Fungal populations of rhizosphere were higher in the plants raised in control plots as well as in those raised according to package of practices recommendations of (POP) KAU (2011) and also farmers' management practices, whereas fungal populations in the rhizosphere of the plants sprayed with foliar fertilizer NPK 19:19:19 (0.5 per cent) and its combination with fungicides, were lower. Fungal population of phyllosphere was higher in the control plants as well as in those raised according to package of practices recommendations of (POP) KAU and also in plants raised by farmers' management practices. In all other treatments, which comprised of foliar spray with fertilizer with and without combination of fungicides, a comparative decline in the fungal populations of the phyllosphere was observed in all three locations.

In two locations (Venganoor and Vavamoola), the bacterial populations were higher in rhizosphere of the plants in control plot while populations were lower in all other treatments. This indicated that in the plants which did not receive application of chemical fertilizer, the bacterial populations of rhizosphere was high. Foliar fertilizer NPK 19:19:19 (0.5 per cent) seemed to induce bacterial population in the phyllosphere which was further enhanced by the addition of azoxystrobin.

Further the effects of the treatments in inducing systemic resistance of the plants were detected by analysing the changes in enzyme activities of the culinary melon plants after applying various treatments. Plants treated with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant induced the activity of the enzymes phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), β-1, 3 glucanase, super oxide dismutase (SOD) and increased the phenolic content of plants which reached maximum level on the 15th day after treatment. Significant increases in the activities of these defense related enzymes and compounds indicated the induction of systemic resistance which may be

attributed to the lower incidence and severity of anthracnose leaf spot disease observed in the plants.

The changes in physiological parameters of the plants due to the various treatments indicated that NPK content and nutrient use efficiency was highest in leaf samples obtained from plants treated with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant in all three locations, which accounts for the higher yield observed in plants applied with this treatment.

In Venganoor, chlorophyll a, b and total chlorophyll content was highest in leaves obtained from plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant. In Vavamoola, highest pigment status was observed in leaves collected from plants raised according to package of practices recommendations of (POP) KAU. In Venjaramoodu, highest pigment status was observed in leaves analysed from plants treated with farmers' management practices as well as those raised by foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant and control plants. Relative water content was comparatively high in plants raised by farmers' management practices and plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant. Epicuticular wax content of leaves of plants raised according to farmers' management practices was higher in Venganoor while the other two locations (Vavamoola and Venjaramoodu) the wax content of leaves of plants treated with package of practices recommendations of (POP) KAU and foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant was comparatively higher. Stomatal frequency was highest on upper and lower surface of leaves in plants treated with NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant in two locations (Venganoor and Vavamoola). In Venjaramoodu, the stomatal frequency was higher on upper surface and lower surface of leaves from NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant. The treatments tested in the

confirmation trials did not have consistent and discernible impact on the physiological parameters such as chlorophyll content, relative water content, epicuticular wax and stomatal frequency.

In the present study, a combination of foliar fertilizer NPK 19:19:19 and azoxystrobin (0.15 ml/l) along with the adjuvant applied twice at 15 days' interval was found to have both disease suppressing and growth promoting effects and was therefore effective in the integrated management of the foliar fungal disease, anthracnose leaf spot, affecting the culinary melon plants in the field.

Future line of work: Combining azoxystyrobin at a higher concentration with the field dose of foliar fertilizer and adjuvant in order to determine whether there is a better impact on the integrated management of anthracnose leaf spot as well as on the yield of the crop, would be very beneficial to the farmer.

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Appendices

महाराष्ट्र विज्ञान वर्धिनी

आघारकर अनुसंधान संस्था

Maharashtra Association for the Cultivation of Science

AGHARKAR RESEARCH INSTITUTE

(An Autonomous Body under the Department of Science and Technology, Govt. of India)

National Fungal Culture Collection of India (NFCCI)-A National Facility

Sender: Miss S. Narmadhavathy, Research Scholar, C/o Dr. Kamala Nayar, Department of Plant Pathology, College of Agriculture, Kerala Agricultural University, Trivendrum-695522, Kerala

Details of Fungus Identified

Sr.	Culture	NFCCI Accession	Identification Remarks	Family
1.	-	3808	*Colletotrichum fructicola* Prihast., L. Cai & K.D. Hyde	Glomerellaceae

Note: The identity was confirmed solely based on morphological characters in *in-vitro* culture.

Brief Discription of Fungal Identification

Morphotaxonomic Description *Colletotrichum fructicola* Prihast., L. Cai & K.D. Hyde **Colonies** on PDA floccose, white later becomes olivaceous grey, reverse buff to olivaceous buff. **Mycelium** of hyphae, hyaline to light olivaceous, pigmented, smooth walled, septate, upto 1.72-8.72µm wide. **Setae** rarely present and sclerotia absent. **Appressoria** produced from lateral hyphae, terminal, dark brown, globose to knob shaped to filamentous. Some appressoria repeatedly germinating to produce complex columns of several closely connected appressoria, wall irregular, upto17.12x6.9µm. **Conidia** hyaline, cylindrical to clavate, aseptate, smooth, thin walled, 31.34µm x 4.1µm.

CONDITIONS AND REMARKS:

1. THE PARTY HAS DELIVERED THE SAMPLE AT ARI.
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for PKSingh

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NFCCI/2015-08/AKC 2310-07/SKS/DKM

महाराष्ट्र विज्ञान वर्धिनी

आघारकर अनुसंधान संस्था

Maharashtra Association for the Cultivation of Science

AGHARKAR RESEARCH INSTITUTE

(An Autonomous Body under
the Department of Science and Technology, Govt. of India)

MOLECULAR IDENTIFICATION REPORT

SENDER : **Ms. S. Narmadhavathy, C/o Dr. Kamala Nayar**

INSTITUTE/ORGANIZATION :Plant Pathology Department, College of Agriculture, Kerela Agriculture University, Vellayani, Kerala - 695522

ACKNOWLEDGEMENT CODE : **2310**

JOB TITLE : Molecular identification of the fungal isolate.

PROCEDURE:

- Genomic DNA was isolated in pure form. from the culture provided by the sender.
- The **ITS region of rDNA** was successfully amplified using fungal universal primers ITS4 & ITS5.
- The sequencing PCR was set up with ABI-BigDye® Terminatorv3.1 Cycle Sequencing Kit.
- The raw sequence obtained from ABI 3100 automated DNA sequencer was manually edited for inconsistency.
- The sequence data was aligned with publicly available sequences & analyzed to reach identity.

Results of Molecular Identification:

- The tested fungal isolate showed **100%** sequence similarity with ***Colletotrichum fructicola.***
- Sequence analyses with NCBI accession number KP145431, ***Colletotrichum fructicola*** isolate CAUG18 resulted in following alignment statistics.
- Alignment statistics: Query Length – 516, Score - 931 bits (1032), Expect - 0.0, Identities - 516/516 (100%), Gaps - 0/516 (0%), Strand - Plus/ Minus

```
Query  1    TGGGGGGTTTTACGGCAAGAGTCCCTCCGGATCCCAGTGCGAGACGTAAAGTTACTACGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  530  TGGGGGGTTTTACGGCAAGAGTCCCTCCGGATCCCAGTGCGAGACGTAAAGTTACTACGC  471

Query  61   AAAGGAGGCTCCGGGAGGGTCCGCCACTACCTTTGAGGGCCTACATCAGCTGTAGGGCCC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  470  AAAGGAGGCTCCGGGAGGGTCCGCCACTACCTTTGAGGGCCTACATCAGCTGTAGGGCCC  411

Query  121  CAACACCAAGCAGAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  410  CAACACCAAGCAGAGCTTGAGGGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATG  351

Query  181  CTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  350  CTGGCGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATT  291

Query  241  ACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAA  300
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Sbjct  290  ACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTAAAA  231

Query  301  GTTTTGATTATTTGCTTGTACCACTCAGAAGAAACGTCGTTAAATCAGAGTTTGGTTATC  360
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Sbjct  230  GTTTTGATTATTTGCTTGTACCACTCAGAAGAAACGTCGTTAAATCAGAGTTTGGTTATC  171

Query  361  CTCCGGCGGGCGCCGACCCGCCCGGAGGCGGGAGGCCGGGAGGGTCGCGGAGACCCTACC  420
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Sbjct  170  CTCCGGCGGGCGCCGACCCGCCCGGAGGCGGGAGGCCGGGAGGGTCGCGGAGACCCTACC  111

Query  421  CGCCGAAGCAACAGTTATAGGTATGTTCACAAAGGGTTATAGAGCGTAAACTCAGTAATG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  110  CGCCGAAGCAACAGTTATAGGTATGTTCACAAAGGGTTATAGAGCGTAAACTCAGTAATG  51

Query  481  ATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTAC  516
            ||||||||||||||||||||||||||||||||||||
Sbjct  50   ATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTAC  15
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APPENDIX-III

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar

Peeled and sliced potatoes	- 200.00g
Agar-agar	- 20.00g
Dextrose	- 20.00g

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 molten agar and 20 g of dextrose saw dissolved into 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. King's B Broth (King *et al.*, 1954)

Protease peptone	:	20.0g
Dipotassium hydrogen phosphate	:	1.5g
Magnesium sulphate	:	1.5g
Glycerol	:	10.0ml
Distilled Water	:	1000ml
pH	:	7.2

INTEGRATED MANAGEMENT OF FOLIAR FUNGAL DISEASE OF CULINARY MELON (*Cucumis melo* L. var. *acidulus* Naudin)

S. NARMADHAVATHY

(2013-21-112)

Abstract of the

thesis submitted in the partial fulfillment of the requirement

for the degree of

DOCTOR OF PHILOSOPHY IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM - 695 522

KERALA, INDIA

2016

ABSTRACT

The project entitled "Integrated management of foliar fungal disease of culinary melon (*Cucumis melo* L. var. *acidulus* Naudin)" was undertaken with the objective of making a comparative evaluation of the efficacy of foliar application of fertilizers, micronutrients, bio-control agents and newer fungicide for the management of Colletotrichum leaf spot (*Colletotrichum* sp.) disease of culinary melon. Surveys conducted during September 2013 to December 2013, in ten culinary melon fields located at Instructional Farm (IF), College of Agriculture (CoA), Vellayani as well as in farmers' fields near, CoA, Vellayani, in order to assess the prevalence of major diseases such as Colletotrichum leaf spot and downy mildew disease affecting the crop. Highest disease incidence (DI) and percentage disease index (PDI) of Colletotrichum leaf spot were observed, 75 days after sowing, at Chavadinada (70.00 per cent and 64.44 per cent respectively). Incidence and index of downy mildew disease were recorded in four out of the ten locations surveyed (Palapoor, Papanchani, Kalliyoor and Punjakari). Maximum disease incidence and percentage disease index of downy mildew disease (36 per cent and 33.33 per cent respectively) were observed at Papanchani. The most virulent isolate of anthracnose leaf spot pathogen (IF, Vellayani isolate), obtained during the survey was identified as *Colletotrichum fructicola* by molecular characterization.

The treatment NPK 19:19:19 (0.5 per cent) combined with the fungicide mancozeb (0.4 per cent) and adjuvant was most effective in inhibiting the mycelia growth of the pathogen *C. fructicola, in vitro,* (100 per cent) over control as well as in suppressing artificially induced anthracnose disease and improving the growth parameters of the plants, in the two greenhouse experiments conducted at the CoA, Vellayani during March to June 2014 and August to October, 2014. Results of two field trials conducted at CoA, Vellayani, during January to March, 2015 and April to June, 2015 for testing four most effective treatments screened from the

greenhouse experiments, indicated that NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant (DI 40.00 and PDI 13.05 respectively) and NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant (DI 40.00 and PDI 13.47 respectively) were most effective in managing the disease and also increasing total yield of plants, when compared to the remaining treatments. Trials were conducted in farmers' fields at three locations (Venganoor, Vavamoola and Venjaramoodu) for confirming the efficacy of the two most effective treatments screened from the field trials conducted at CoA, Vellayani and pooled analysis of the results indicated that the lowest PDI (12.22) and DI (28.50) were obtained in plants treated with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15ml/l) + adjuvant, which was significantly superior to the other treatments.

Results of the microbial studies indicated that there was decline in fungal flora of the plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant, days after application of treatments whereas bacterial population was higher in plants applied with the same treatment when compared to the application of combination of foliar fertilizer NPK 19:19:19 (0.5 per cent) + mancozeb (0.4 per cent) + adjuvant. There was indication of higher induction of systemic resistance in plants treated with NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant due to the higher activity of defense related enzymes, such as phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), β-1,3glucanase, super oxide dismutase (SOD) and the compound phenol, all of which, reached maximum level on the 15th day after treatment.

Leaf samples obtained from plants treated with foliar fertilizer NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant indicated highest nutrient use efficiency in all three locations of the confirmation trials while highest pigment status due to this treatment was observed in the trial conducted at Venganoor. Relative water content was generally high in leaf samples collected from all plants irrespective

of the treatments, although it was comparatively low, in leaf samples obtained from plants of absolute control plot. Epicuticular wax content was slightly lower in the plants treated with combination of the foliar fertilizer NPK 19:19:19 (0.5 per cent) and fungicides, either azoxystrobin (0.15 ml/l) or mancozeb (0.4 per cent) + adjuvant. Stomatal frequency on the upper and lower surfaces of leaves was not much affected by application of foliar fertilizer NPK 19:19:19 (0.5 per cent) combined with the fungicides. B:C estimated ratio revealed that the highest returns were obtained from the plants treated with foliar spray of NPK 19:19:19 (0.5 per cent) + azoxystrobin (0.15 ml/l) + adjuvant, in all three locations of the farmers' field trials.

This study presents the first report of the pathogen *Colletotrichum fructicola* causing anthracnose leaf spot disease of culinary melon in India. In field conditions, combination of the foliar fertilizer NPK 19:19:19 (0.5%) and azoxystrobin (0.15 ml/l) along with adjuvant applied twice at 15 days' interval was most effective in controlling anthracnose leaf spot disease of culinary melon and also increasing the yield of the crop.