INTEGRATED MANAGEMENT OF FUSARIUM WILT AND ANTHRACNOSE OF VEGETABLE COWPEA (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) USING NEW GENERATION FUNGICIDES

by

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(2011 – 21 - 112)

THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Integrated management of Fusarium wilt and anthracnose of vegetable cowpea (*Vigna unguiculata* subsp. s*esquipedalis* (L.) Verdcourt) using new generation fungicides"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Integrated management of Fusarium wilt and anthracnose of vegetable cowpea (*Vigna unguiculata* subsp. s*esquipedalis* (L.) Verdcourt) using new generation fungicides"** is a record of research work done independently by Ms. Sreeja S. J. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
@	At the rate of
°C	Degree Celsius
CD	Critical difference
cfu	Colony forming units
DAS	Days after seed emergence
et al.	And other co workers
h	Hours
sec	Seconds
min	Minutes
i.e.	that is
ha	Hectares
t/ha.	Tons per hectare
l	Litre
ml	Milli litre
µl	Micro litre
cm	Centimeter
mm	Millimeter
µm	Micro meter
kg	Kilogram
g	Gram
mg	Milli gram
µg	Micro gram
ng	Nano gram
M	Molar
mM	Milli molar
rpm	Rotations per minute
sp. or spp	Species (Singular and plural)
viz.	Namely

bp	Base pairs
kb	Kilobases
PCR	Polymerase Chain Reaction
BDL	Below Detectable Level
ITS	Internal Transcribed Spacer
rDNA	Ribosomal DNA
ppm	Parts per million

Introduction

1. INTRODUCTION

Vegetable cowpea, also known as yard long bean (*Vigna unguiculata* subsp. s*esquipedalis* (L.) Verdcourt) is an important legume crop of the tropics whose centre of origin has been reported from Africa and was introduced to the Indian sub continent approximately 2000 to 3500 years ago. It may be consumed at various stages of its development; green leaves, green pod, green peas and dry grains as well as for fodder purposes. It is an inexpensive source of vegetable protein, which is easily digestible, relatively cheaper and has higher biological values. The ability to fix atmospheric nitrogen makes the crop agriculturally important. The well branched root system provides better soil binding effect and checks soil erosion. Cowpea is hence, a valuable crop and an integral component of crop rotation systems.

Due to the favorable agro climatic conditions, the crop has gained much importance in Kerala and has come to occupy a prime position among the vegetable crops raised in the State. But the production of cowpea is hindered by an array of diseases *viz.,* Fusarium wilt, anthracnose, web blight and collar rot, rust, powdery mildew, Cercospora leaf spot, Choanephora pod rot, Pythium stem rot, viral diseases *etc.* that cause growth suppression or death of plants, leading to reduced yield and productivity. Among the various diseases, Fusarium wilt and anthracnose have emerged as major fungal diseases affecting the crop in Kerala.

Fusarium wilt of cowpea which may cause total yield loss is manifested as basal stem swelling, yellowing, withering and drooping of leaves, drying up of vines and occasionally as abnormal flattening of the stem along the growing tip of the affected plants. Yield losses as high as 50 % have been recorded due to anthracnose causing brown sunken lesions on leaves, stem, branches and pods and coalescence of lesions leads to total death of plants.

Fungicide application is the easiest and reliable approach to combat diseases in commercial cultivation. However, the excessive reliance on these agro-

chemicals has led to the problems of environmental pollution, development of resistant strains, destruction of beneficial flora and fauna and also impairment of human health due to the consumption of vegetables. Recently, the Central Insecticide Board and Registration Committee has approved a number of new molecules that are ecologically safe with lower toxicological profiles and required at much lower dose rates than their earlier counterparts. The results of this study will help in locating safer options for the vegetable growers of Kerala through the screening of registered new generation fungicides against the two dreaded pathogens crippling cowpea cultivation.

Integration of cultural, biological and chemical methods in a compatible manner is essential to arrive at a long lasting solution for the management of plant pathogens. The present study envisaged integration of effective new generation fungicides with eco friendly practices to develop an IDM package for tackling Fusarium wilt and anthracnose of cowpea and to boost up the production with least disturbance to soil and environment.

Since, the arbitrary use of pesticides on any crop leads to unwanted residues, information on the nature and quantity of chemicals which remain in the original form or as degraded product on the edible parts is of paramount importance. Prior to the recommendation of any pesticide for field use, it is mandatory to study its degradation kinetics. The data generated in this study is expected to be of immense value in fixing the waiting periods, the safety milestone for any pesticide in a crop.

In the light of the above, the present study was undertaken with the following objectives,

- To isolate and characterize the pathogens associated with Fusarium wilt and anthracnose of vegetable cowpea
- To evaluate the efficacy of new generation fungicides against the pathogens associated with Fusarium wilt and anthracnose of vegetable cowpea under *in vitro* and *in vivo* conditions

- To assess the safety of these fungicides to beneficial microbes
- To develop an integrated disease management package for the control of Fusarium wilt and anthracnose of vegetable cowpea using effective fungicides compatible with beneficial microbes and eco friendly practices
- To determine the persistence and degradation kinetics of the fungicides

Review of literature

2. REVIEW OF LITERATURE

Cowpea is a widely grown vegetable crop in the wetland fallows in Kerala. An array of diseases affects the crop at various stages of its growth, of which Fusarium wilt and anthracnose pose major concern. The literature related to yield loss, symptomatology, etiology and characterization of the two pathogens associated with Fusarium wilt and anthracnose are reviewed and presented here. Review on integrated management of the two diseases especially through soil solarization, application of *Trichoderma* enriched neem cake organic manure mixture and fungicides, compatibility of fungicides to beneficial microbes and the dissipation kinetics of selected fungicides are also detailed. Since literature on these aspects pertaining to cowpea are scanty, works done in this direction on other pulse crops is also being reviewed here to supplement the information.

2.1. INCIDENCE, SEVERITY AND EXTENT OF YIELD LOSS DUE TO FUSARIUM WILT

Fusarium wilt has been reported to affect several pulse crops and the yield loss due to the disease varied with the stage at which it occurred. Fusarium wilt had been first reported in cowpea from USA (Orton, 1902), while it was first reported in India by Singh and Sinha (1955). The wilt of cowpea was noticed in farmers' fields in Thiruvananthapuram district of Kerala since 1995 - 96 (Reghunath *et al.,* 1995). Losses in seed yield due to Fusarium wilt ranged from 9.11 to 80.30 % and from 8.30 to 86.51% in cowpea cultivars BR-17 Gurgueia and IPA-206, respectively in Northeastern Brazil (Assuncao *et al.,* 2003).

Reports from Pakistan indicated that the productivity of chickpea was below world average and had been uncertain, erratic and low with about 10 % of the world's production (Auckland and Vander-Maesan, 1980). Cortes *et al.* (1998) reported that Fusarium wilt was the most important soil borne disease of chickpea particularly in the Indian sub continent, the Mediterranean Basin and California. Attacks by the pathogen destroyed the crop completely or caused significant yield losses. Annual yield losses in chickpea due to Fusarium wilt were estimated at

10 % in India and Spain and 40 % in Tunisia. Fusarium wilt reduced chickpea yield by decreasing both seed yield and seed weight. These effects were related to sowing date, chickpea cultivar and virulence of the prevalent *Fusarium oxysporum* f. sp. *ciceris* race (Cortes *et al.,* 2000).

Losses due to wilt in pigeonpea in Kenya varied from negligible amount to 100 % depending on the stage of the crop and environmental factors (Kannaiyan and Nene, 1981). Kannaiyan *et al*. (1984) noticed an incidence of 15.9 %, 36.6 % and 20.4 % due to Fusarium wilt in pigeonpea in Kenya, Malawi and Tanzania respectively with annual loss estimated at US $ 5 million in each of the countries. In India, the annual loss due to Fusarium wilt of pigeonpea was estimated at US $ 71 million in susceptible cultivars (Reddy *et al*., 1993). In Tanzania, incidence of Fusarium wilt as high as 96 % had been observed in pigeonpea (Mbwaga, 1995). Fusarium wilt caused economic loss in pigeonpea of about 470000 t of grain in India and 30000 t of grain in Africa (Joshi *et al*., 2001). Kiprop (2001) reported wilt incidence of 0 to 96.1 % in pigeonpea during a field survey carried out in Eastern, Coastal, Central and Nairobi provinces in Kenya during the flowering, pod setting and dry pod stages. Saxena *et al.* (2002) observed that the total loss due to pigeonpea wilt was approximately 97000 t/year in India.

Fusarium wilt and root rot caused great damage and reduced the average yield of soybean by up to 59 % (Sinclair and Backman, 1989). Yield losses from soybean Fusarium root rot ranged from slight to nearly 50 %. With the increase in continuous cropping, root rot became more and more severe in soybean, affecting the yield as well as quality (Li *et al.,* 2010).

According to Maheswari *et al.* (1981), wilt complex disease caused yield loss of 13.9 - 95 % in pea fields in North India Fusarium wilt caused complete crop failure under favorable conditions for disease development and became a major limiting factor for lentils cultivation where the crop was grown (Chaudhary and Amarjit, 2002). According to Zian (2005), Fusarium wilt was one of the most

destructive diseases of white lupin causing severe losses in its seed yield and quality.

Naseri (2008) reported that Fusarium wilt or Fusarium decline of dry bean was a major economic problem in Northwest Iran which showed up to 70 % yield loss. Seed yield losses due to Fusarium root rot in susceptible kidney beans was found to be greater than 50 % (Akrami *et al*., 2012). According to Sivaprasad *et al.* (2013), the productivity of black gram in India got severely hampered due to diseases caused by bacteria, fungi and viruses. The percentage yield loss due to various biotic factors was in the tune of 40 – 60 %, out of which the fungal pathogen *Fusarium oxysporum* causing wilt disease alone accounted for 10 – 25 %.

2.2. INCIDENCE, SEVERITY AND EXTENT OF YIELD LOSS DUE TO ANTHRACNOSE

Yield losses as high as 50 % had been recorded due to cowpea anthracnose in mono crops in humid regions of Nigeria (Williams, 1974) while, losses were probably low on mixed crop farms. Emechebe and Lagoke (2002) found that anthracnose due to *Colletotrichum destructivum* in susceptible cowpea cultivars attacked all parts, including seedlings, hypocotyls, stem, peduncles, flowers, leaves and pods and lead to yield loss up to 50 %. A survey on cowpea anthracnose conducted at 17 locations in Himachal Pradesh showed that the disease severity ranged between 19.7 - 53.5 % being maximum at Palampur (Banyal *et al.,* 2012). Enyiukwu and Awurum *et al.* (2013) reported that cowpea anthracnose resulted in 50 % grain yield reduction in the affected crop.

Laxman (2006) observed greengram anthracnose severity to the tune of 18.2 - 86.57 in northern parts Karnataka. According to Kulkarni and Benagi (2012), the yield losses due to greengram anthracnose was proportional to disease severity and varied remarkably depending on the stage of infection, genotypes and environmental conditions. Field surveys conducted in Karnataka during *kharif*

2006 and 2007 revealed that severity of greengram anthracnose was found highest in Bidar district (49.43 %) followed by Gulbarga district (48.12 %) and least in Bijapur (23.86 %). The congenial weather conditions like frequent rains, moderate temperature coupled with higher humidity helped in the building up of high disease pressure in Bidar and Gulbarga districts (Kulkarni and Benagi, 2013).

Seedling losses of 30 - 100 % was recorded in soybean stands in Asia (Buddenhagen *et al.,* 1987) while, anthracnose of mature soybean plants reduced grain yield by 16 - 26 % in USA, 30 - 50 % in Thailand and up to 100 % in Brazil and India (Sinclair and Backman, 1989). Khan and Sinclair (1992) stated that anthracnose and pod blight of soybean cause yield losses up to 30 % in India.

Schwartz *et al.* (1982) noted that leaf, stem and pod infections due to common bean anthracnose resulted in premature defoliation, shriveled pods and shrunken seeds and recorded yield losses up to 80 %. Allen (1983) reported yield loss of 90 - 100 % due to bean anthracnose in many countries throughout tropical America and Africa. Bassanezi *et al*. (2001) observed that on dry beans grown for seed purpose, anthracnose could bring about complete plant defoliation and extensive yield losses, if plants became diseased prior to and/or during pod filling stage.

Deeksha and Tripathi (2002a) reported loss in yield due to urdbean anthracnose ranging from 24 - 67 % in India. Studies conducted in Manitoba demonstrated that infection of 7 % of the total viable seed of the navy bean cultivar Navigator due to anthracnose resulted in yield losses ranging from 15 - 32 % (Conner *et al.,* 2004). Survey on major dolichos bean growing areas of southern Karnataka during *kharif* 2010 and 2011 indicated that the occurrence of disease ranged from 23.36 - 47.54 %. The highest mean foliar infection and pod infection was recorded in Mysore followed by Chamarajanagar and the least was observed in Ramanagar district (Manjunath *et al.,* 2012).

2.3. SYMPTOMATOLOGY STUDIES

2.3.1. Symptomatology of Fusarium wilt

Singh and Sinha (1955) observed that Fusarium wilt of cowpea caused by *F. oxysporum* f. sp. *tracheiphilum* showed sudden, outright wilting of the whole young plants and in mature plants, the wilt was often preceded by yellowing of leaves, which soon withered, leaving a dry naked stem. Senthil (2003) described that symptoms of Fusarium wilt of cowpea started as yellowing of foliage followed by defoliation. Roots as well as the lower stem portions rotted leading to drying up of vines above. In severe cases, the lower stem portion and the upper part of the tap root together formed a swollen tuber like structure which gradually became shredded and disintegrated.

Khare (1980) reported that symptoms of vascular wilt of lentils appeared as wilting of top leaves that resembled water deficiency, stunting of plants, shrinking and curling of leaves from the lower part of the plants that progressively moved up the stem of the infected plant. Plants finally became completely yellow and died. Root symptoms included reduced growth with marked brown discoloration, tap root tips were damaged and proliferation of the secondary roots above the tap root. Discoloration of vascular tissue in the lower stem was not always visible. General symptoms at the seedling stage included seed rot and sudden drooping more like wilting and damping off.

As per the symptom description by Nene *et al.* (1991) Fusarium wilt infected chickpea seedlings collapsed and fell on the ground retaining their dull green color. Adult plants showed typical wilt symptoms of drooping of petioles, rachis and leaflets. The roots of the wilted plants did not show any external rotting but when split opened vertically, dark brown discoloration of internal xylem was seen. Pods from the wilted plants looked normal but seeds were generally smaller, wrinkled and discolored.

Symptoms of Fusarium wilt in pea appeared as downward turning of the leaves and stipules, with older leaves of infected pea plant becoming dry and brittle. The basal portion of an infected plant became brown and discolored before the apical portion due to the upward progression of the fungus in the vascular tissue (Haglund and Kraft, 2001).

Kurmut *et al.* (2002) observed that root rot of faba bean caused by *F. nygami* showed black root rot and decay of the lateral root system. Severely infected plants showed black neck canker at the soil level. These symptoms were usually accompanied by loss of the leaf turgor, followed by browning and death of intact leaves.

According to Jasnic *et al.* (2005) the symptoms of Fusarium wilt in soybean included leaf chlorosis, wilt of the apical portion of the plant, necrosis of the root and lower stem, and wilting of the whole plant. The pods were often poorly developed. The seeds were smaller and lighter in weight and infected.

Karimi *et al*. (2012) described the symptoms in pigeonpea as progressive chlorosis of leaves, interveinal clearing, partial wilting and collapse of the root system. The most characteristic internal symptom was a purple band extending upwards from the base of the main stem. The xylem developed black streaks and resulted in brown band or dark purple bands on the stem surface of partially wilted plants extending upwards from the base and visible when the main stem or primary branches were split open. The intensity of browning or blackening decreased from the base to the tip of the plant. Sometimes, lower branches had die-back symptoms with a purple band extending from tip downwards with intensive internal xylem blackening.

2.3.2. Symptomatology of Anthracnose

According to Onesirosan and Barker (1971), the initial symptoms of cowpea anthracnose appeared as pinkish lesions girdling the stem at ground level and distinct lens shaped, tan pink, sunken lesions with dark red margins on the stem,

causing necrosis. On the leaves, elongated vein lesions and tan red spots with chlorotic halo were formed. Allen (1983) described the symptoms of cowpea brown blotch, caused by *C. capsici* and *C. truncatum* as purplish or reddish brown blotches on petioles, leaf veins, stem, peduncle and pods without the formation of definite lesions. Girdling resulted in stem collapse. Necrosis of peduncle resulted in floral abortion, distortion as well as shriveling of immature pods. On pods, sporulation appeared as alternating black and brown bands, diagnostic of the disease.

In urdbean and mungbean, the symptoms appeared as water soaked greenish lesions which assumed the shape of a horse shoe and turned straw colored with narrow reddish brown to dark brown margins. Later, necrotic areas were shed leaving behind the reddish brown margin intact. In the case of mungbean, the margin around necrotic areas became irregular in shape as a result of extension of infection to the lower leaves. The spots occasionally coalesced (Bharadwaj and Singh, 1986).

Singh and Shukla (1988) opined that *C. truncatum* causing serious leaf spotting in green gram, manifested mainly on the leaves. The leaf spots later formed 'shot holes' leading to pre mature defoliation. Under severe conditions small, reddish brown, one to four mm diameter spots appeared on petioles. On stem light grey, water soaked, irregular, rustic brown, 10 - 14 mm diameter spots developed while, small reddish blotches were seen on pods. Rathaiah and Sharma (2004) noted the appearance of characteristic blood red ring like spots of 8 – 11 mm diameter on the upper surface of greengram leaves. On the lower surface, large patches of bright blood red spots were noted and their corresponding upper surface became chlorotic. Blood red stains measuring up to 2.5 cm appeared on pods and petioles too.

Buchwaldt *et al.* (1996) observed that the first symptoms of lentil anthracnose in the field were insignificant lesions on leaves and stem of young plants and the crop remained apparently healthy till the early flowering stage. At

this stage, more lesions developed on lower leaflets leading to premature leaflet abscission. This was followed by development of severe lesions on the stem base that progressed upwards. During rainy periods, acervuli were formed in the lesions on the plant and also on fallen leaves.

The incidence of anthracnose on scurf pea showed wilting of branches and light brown lesions on the leaves. The characteristic symptom was a bluish black discoloration on the infected leaves and stems. In some plants, the infection spread across the entire plant leading to the death of the plant. (Nair *et al.,* 2010).

In soybean pinkish brown lesions were observed on the pods and the formation of dark lesions on leaves and stem was followed by stem girdling, dieback, and distorted growth. At later stages, numerous epidermal acervuli developed on the lesions and mucilaginous conidial masses appeared during periods of high relative humidity (Mahmodi *et al.,* 2013b).

Mahmodi *et al.* (2013a) noticed that in chickpea the symptoms of anthracnose appeared as tan lesions with darker brown borders on leaves and were associated with premature leaf drop. On stem, lesions initially appeared on the lower parts which later progressed vertically upwards resulting in girdling of stem leading to dieback.

2.4. ETIOLOGY

2.4.1. Etiology of Fusarium wilt

Fusarium spp. cause seed rot, damping off and vascular wilts of vegetables, pulses, ornamentals and fibre crops. Cotton wilt noticed in desi cotton was identified as due to infection of *F. oxysporum* (Butler, 1926). *F. oxysporum* f. sp. *psidii* was reported to be the causal agent of wilt of guava (Prasad *et al.,* 1952) which occurred as a severe problem in Uttar Pradesh. Singh and Sinha (1955) reported the involvement of *F. oxysporum* f. sp. *tracheiphilum* with cowpea in Uttar Pradesh.

Booth (1971) identified the pathogen involved in wilt of chickpea as *F. oxysporum* f. sp. *ciceri*. The yellow disease of ginger caused by *F. oxysporum* f. sp. *zingiberi* was reported for the first time by Haware and Joshi (1973).

F. udum incited pigeonpea wilt occurred as a serious disease in vertisols of Central India (Kannaiyan *et al.,* 1984). Narain *et al.* (1989) reported the incidence of groundnut seed rot caused by *F. pallidoroseum* which occurred in Orissa during 1983-88.

Fusarium wilt (Panama wilt) of banana had been reported from most of the banana growing regions in India. Four races of *F. oxysporum* f. sp. *cubense* were reported to be involved in this disease (Ploetz, 1992).

F. solani had been reported to be involved in the root rots of chickpea, soybean, sunflower, cucurbits and melon in India. Fusarium wilt caused by *F. solani* was identified as new threat to the potato cultivation in Brazil by Lopes and Ventura (1996).

Severe vascular wilt of common bean (*Phaseolus vulgaris*) caused by *F. oxysporum.* f. sp. *phaseoli* in Central Africa was reported for the first time by Buruchara and Camacho (2000). Tosi and Cappelli (2001) reported the incidence of wilt in lentil due to infection of *F. oxysporum* f. sp. *lentils* in every continent where lentil was grown except Australia.

Sharma (2001) reported the involvement of *F. pallidoroseum* in wilt disease of horse gram. Senthil (2003) noted the association of *F. pallidoroseum*, *F. oxysporum* and *F. solani* with wilt of cowpea in Thiruvananthapuram district of Kerala.

Zian (2005) reported the association of *F. oxysporum.* f. sp. *lupini* with the wilt of white lupin, one of the most destructive diseases causing severe losses in seed yield and quality. Sharma and Muehlbauer (2007) reported the involvement

of eight physiological races (0, 1A, 1B/C, 2, 3, 4, 5 and 6) of *F. oxysporum* f. sp. *ciceris* associated with chickpea wilt in India.

Polizzi *et al.* (2010) mentioned the first occurrence of Fusarium wilt of *Bougainvillea glabra* caused by *F. oxysporum* in Italy. Perveen and Bokhari (2010) published first report of Fusarium wilt of *Lavandula pubescens* caused by *F. oxysporum* in Saudi Arabia. Felgueiras *et al*. (2010) reported Fusarium wilt caused by *F. oxysporum* f. sp. *basilici* on *Ocimum minimum* in Portugal. Karaca and Kahveci (2010) published first report of *F. oxysporum* f. sp. *radicis-cucumerinum* on cucumbers in Turkey.

Li (2011) identified the association of *F. semitectum* with soybean root rot, a widespread and destructive soybean disease.

2.4.2. Etiology of Anthracnose

The principal *Colletotrichum* species that affect grain legumes are *C. lindemuthianum*, which has a worldwide distribution affecting common bean, cowpea, soybean and pea; *C. capsici* which is pan tropical on cowpea, chickpea, soybean, winged bean and peanut; *C. truncatum*, whose host range includes soybean, cowpea, lima bean, pigeonpea, peanut and lentil; *C. destructivum* and its teleomorph *Glomerella glycines*, affecting soybean and lentil in USA and Asia; *C. gloeosporioides* and its teleomorph *G. cingulata*, reported from pigeonpea, soybean and peanut in Asia and America (Lenne, 1992).

Three *Colletotrichum* species are important pathogens of cowpea, *Vigna unguiculata*. Anthracnose, a wide spread disease in cowpea throughout Asia and Africa is principally a stem disease and has been reported to be caused by *C. lindemuthianum*, while the two other species, *C. capsici* and *C. truncatum*, were reported to cause brown blotch of cowpea throughout the African savannas (Allen, 1983). Cowpea seed infection due to *C. lindemuthianum*, a primary source of disease spread was first reported in India by Prasanna (1985).

The principal *Colletotrichum* species that affect pasture legumes include *C. trifoli* on lucerne, red clover and pasture species of the genera *Medicago* and *Trifolium*, and *C. gloeosporioides* on lupin and tropical pasture legumes of the genera *Stylosanthes* (Lenne and Calderon, 1984).

Stover and Simmonds (1987) reported the involvement of *C. musae* with wounded and green ripe banana fruits. *C. truncatum*, *C. gloeosporioides* and *C. destructivum* were found in association with anthracnose of soybean (Sinclair and Backman, 1989). Soybean was susceptible to *C.truncatum* at all stages of development, while *C. gloeosporioides* and *C. destructivum* were found to infect mature plants and seedlings.

Thakur and Khare (1989) reported the association of three different species of *Colletotrichum viz., C. truncatum*, *C. lindemuthianum* and *C. dematium* with anthracnose of mungbean in India. Waller *et al*. (1993) reported the involvement of *C. kahawae* with coffee berry disease.

Sharma *et al*. (2005) found that anthracnose of chilli was caused by *C. capsici* and *C. gloeosporioides* in India. Jayasinghe and Fernando (2009) mentioned the first report of *C. acutatum* on mango in Sri Lanka.

C. gloeosporioides, *C. acutatum*, *C. coccodes*, and *C. dematium* are the four main species of *Colletotrichum* that cause tomato anthracnose (Zivkovic *et al.,* 2010). *C. capsici* and *C. gloeosporioides* had been reported to be involved in the leaf spot of turmeric in India (Chawda *et al.,* 2012).

2.5. CHARACTERIZATION OF PATHOGENS

2.5.1. Morphological and Cultural Characterization of *Fusarium* spp.

In nature, plant pathogens exist as different strains that exhibit variation in their morphological and cultural characters, pathogenicity and virulence. To understand the present plant disease situations and to predict the possible future

development it is essential to learn as much as possible about the variability in fungi that are pathogenic to plants.

Madhukeshwara (2000) studied morphological and cultural variability among six isolates of *F. oxysporum* f. sp. *udum* causing wilt of pigeonpea. All the isolates varied each other in terms of growth, mycelium, pigmentation and sporulation. Most of the isolates produced cottony white raised mycelium, pale yellow to dusky red color pigmentation and moderate to profuse sporulation on PDA medium. The sizes of micro and macro conidia were 6 - 8 x 2 - 3 µm and 19 - 26 x 3.5 µm respectively. Septation ranged from 2 - 5 in macro conidia and 0 - 1 in microconidia. Macroconidia was sickle shaped with pointed ends and hyaline. The micro conidia were oval shaped and hyaline.

Desai *et al.* (2003) observed that the size of micro and macro conidia of *F. oxysporum* f. sp. *ricini* causing wilt of castor ranged from 5.25 - 14.00 µm x 3.50 -7.00 µm and 17.5 - 70.00 µm x 3.50 - 5.25 µm, respectively. The isolates which were highly virulent produced abundant sporulation, while moderately virulent isolates were having poor sporulation.

Honnareddy and Dubey (2007) described the morphological and cultural features of different isolates of *F. oxysporum* f. sp. *ciceris* causing chickpea wilt collected from major chickpea growing areas of India. The isolates had variable pigmentation of medium from normal white to violet, brown, reddish violet, greenish violet, yellowish pink and dark green. The intensity of color varied with age and temperature. Most of the isolates produced aerial and fluffy mycelium and a few produced flat and suppressed mycelium. Sporulation count ranged from 0.4×10^6 to 2.3×10^6 conidia/ml. Chlamydospores were observed in ten day old cultures of all the isolates. They were either terminal or intercalary and formed singly or in pair, but rarely in chains. All the isolates produced abundant microconidia and macroconidia. Kumar *et al.* (2012) opined that isolates of *F. oxysporum* f. sp. *ciceri* collected from the Eastern plateau region of India appeared to differ from each other with regard to their morphological and

pathogenic features. Chlamydospores produced were either terminal or intercalary and the sporulation count ranged from 0.4×10^6 to 1.0×10^6 conidia/ml.

Chavan (2007) reported that isolates of *F. solani* causing wilt of patchouli showed moderate growth, covering the agar plate within 6 - 10 days. The colony was sparse to dense, greyish white to pinkish in color. The pathogen produced three kinds of spores *viz.*, macroconidia, microconidia and chlamydospores. Microconidia were abundant, hyaline, cylindrical, single or two celled and measured 6.60 - 19.80 µm x 3.30 - 6.60 µm. Macroconidia were 3 - 4 septate and measured 29.70 - 47.85 µm x 4.95 - 6.60 µm. Chlamydosproes were hyaline, spherical and one celled and measured 8.25 - 11.5 µm x 6.60 - 9.90 µm. They were produced singly or sometimes in chains.

Mwangombe *et al.* (2008) reported that the Kenyan isolates of *F. solani* f. sp. *phaseoli* from common bean exhibited high variability in colony characteristics on PDA media. The mycelial texture was either fluffy or fibrous and the colony colors observed were purple, pink and white. The hyphae of the cultured isolates were highly branched, slender, septate and produced conidia and chlamydospores. The microconidia observed on the isolates were 0 - 1 septate, and their length ranged from 6.0 to 13.0 µm, while the width ranged from 2.8 to 3.6 µm. The most commonly observed macroconidia from all the isolates ranged from 3 - 7 septate. The mean macroconidial length x width was 36 x 4.0 µm.

Chandran and Kumar (2012) studied the cultural and morphological variability among the isolates of *F. solani*, an incitant of dry root rot in citrus. Most of the isolates grew more than 85 mm after seven days of inoculation. White dense, fluffy mycelium with concentric rings or raised mycelium with smooth margins was observed for the isolates. Moderate to profuse sporulation was noted. The pigmentation of isolates varied from pale pink to dusky red or pale yellow to dark yellow. The size of macro conidia ranged from 13 - 15 µm x 3 - 4 µm to 27 - 29 µm x 4 - 5 µm and the size of micro conidia ranged from

3 - 4 µm x 1- 2 µm to 9 - 10 µm x 1 – 3 µm. The number of septa in macro conidia and micro conidia was 3 - 5 and 0 - 1 respectively and conidia were hyaline. The macro conidia were sickle shaped with blunt end and the micro conidia were round to oval in shape. Intercalary and terminal chlamydospores were observed in all the *F. solani* isolates.

Motlagh (2010) observed the characters of *F. equiseti* associated with *Echinochloa* spp., as abundant mycelium initially white becoming brown with age. Pale to dark brown pigmentation was noticed. Macroconidia were long and slender with a dorsiventral curvature. Apical cell was tapered and elongated or whip-like. Basal cell was foot shaped and elongated in appearance. Number of septa was usually 5 - 7. Microconidia were absent. Zainudin *et al.* (2011) noted that among the *Fusarium* species associated with Fusarium ear rot of corn; *F. equiseti*, *F. longipes*, and *F. pseudograminearum* produced only macroconidia without microconidia.

2.5.2. ITS – Sequence Based Molecular Identification of *Fusarium* spp.

The ribosomal RNA genes (rDNA) possess characteristics that are suitable for the detection of pathogens at the species level. The rDNA repeat unit contains genic and nongenic or spacer regions. Each repeat unit consists of a copy of 18S, 5.8S and 28S like rDNA and two spacers, the internal transcribed spacer (ITS) and intergenic spacer (IGS) (O'Donnell, 1992). The rDNA genes have been employed to analyze major evolutionary events because it is highly conserved, whereas the rDNA internal transcribed spacer (ITS 1 and ITS 2) is more variable so that it has been used for the investigation of the species level relationship (Bruns *et al.,* 1991) and has been used in classifying fungal species due to its systematic and taxonomic usefulness (Chillali *et al.,* 1998).

Mishra *et al.* (2000) reviewed that ITS sequence comparison could be used for grouping *Fusarium* isolates in to two sections, one comprising of *Discolor, Sporotrichiella* and *Gibbosum* and the other comprising *Elegans, Liseola,*

Martiella and *Roseum* and it resolved the identification and taxonomic problems of *Fusarium* spp. especially at sectional level. Shahnazi *et al*. (2012) identified *F. solani* and *F. proliferatum* isolates responsible for yellowing disease of black pepper based on sequencing of ITS 1 and ITS 2 and 5.8S ribosomal DNA regions and confirmed that this molecular technique enabled identification of *Fusarium* at the species level.

2.5.3. Morphological and Cultural Characterization of *Colletotrichum* spp.

Morphological and cultural characters pertaining to principal *Colletotrichum* species affecting grain legumes *viz., C. lindemuthianum, C. truncatum, C. dematium* and *C. gloeosporioides* are reviewed here.

Quimio (1975) described the morphological and cultural characters of an isolate of *C. lindemuthianum* from green gram. The mycelium was branched, septate, hyaline becoming dark with age. The acervuli were saucer shaped, sub-cuticular and became erumpent. The conidia were borne acrogenously on short conidiophores and appeared pink in mass. They were one celled, hyaline, oblong, cylindrical with rounded ends or with one end slightly pointed. Conidia measured 10 - 20 × 3 - 6 µm in size. The appressoria were sparse, pale to dark brown, clavate or circular in out line, regular and 8 × 6.7 µm in size.

Sinclair and Backman (1989) reported the morphological characters of *C. truncatum* causing soybean anthracnose. They found, black acervuli borne on well developed stomata. The acervuli were oval to elongate, hemispherical to truncate, conical and erumpent with numerous black, needle like intermixed long and short setae, 60 - 300 × 3 - 8 µm. Conidia were bluntly tapered, curved unicellular and hyaline and measuring 17 - 31 × 3 - 45 µm. Germ tube in contact with solid surface produced dark, sticky appressorium, which penetrated directly.

Murthy (1997) observed the morphological features of *C. dematium* inciting anthracnose of horsegram. Acervuli were black in color and oval to conical in shape measuring 145.0 × 210.7 µm. Conidia were single celled hyaline, curved

and measured 20.5 - 24.5 × 3.5 - 4.6 µm. Setae were black in color, longer than conidia, broader at base, tapering at apex and measured 80.0 - 190.0 × 3.7 - 5.8 µm.

Photita *et al.* (2005) reported that isolates of *C. gloeosporioides* from herbaceous plants had a sparse, cottony, white to pale grey mycelium with abundant mycelia containing bright orange conidial masses produced in concentric rings on the colonies. Than *et al.* (2008) observed that isolates of *C. gloeosporioides* from chilli had pale grey to black zonated colonies with abundant orange conidial masses near the centre. Conidia were cylindrical and measured 13.5 x 4.5 µm. Appresoria produced were irregular in shape and measured 9.0 x 6.3 µm.

The isolates of *C. gloeosporioides* from orchid, varied from white to grey, to dark orange or pinkish grey, while the reverse side of the colonies was of white, dark grey, orange or a mixture with regular colony margins. The mycelium was hyaline, brown or both, abundant/sparse with floccose, loose or compact growth. The conidia were cylindrical with both apices rounded; or with one apex rounded and the other end pointed. The conidial sizes varied from 7.57 - 15.50 × 3.38 - 7.52 µm (Chowdappa *et al.,* 2012).

2.5.4. ITS – Sequence Based Molecular Identification of *Colletotrichum* spp.

Sequence analysis from ITS region has made progress towards a better understanding of the taxonomy of *Colletotrichum* spp. (Cunnington *et al*., 2004). Molecular analysis based on sequences of rDNA internal transcribed spacers (ITS 1 and ITS 2) of *Colletotrichum* spp. paralleled the morphological and cultural groupings too (Morlwakll *et al.*, 2002 and Photita *et al.,* 2005). Confirmation of the identity of *Colletotrichum* species using sequence data from ITS-rDNA region had been attempted by other workers as well (Forseille *et al*., 2011; Arzanlou and Torbati, 2013 and Raj *et al*., 2013b).

2.6. INTEGRATED DISEASE MANAGEMENT OF FUSARIUM WILT

Control of plant diseases is successful when all available information regarding the crop, its pathogen, environmental conditions, control measures and their costs are taken into account for controlling the disease. Integrated disease management involves the selection and application of a harmonious range of control strategies that minimize losses and maximize returns.

Soil amendment of cabbage leaf residues in combination with soil solarization with transparent polyethylene mulch (25 μm) for 40 days resulted in an increase of 9.8°C in mean maximum temperature and complete elimination of *F. oxysporum* f. sp. *gladioli* at 5 cm soil depth (Raj *et al.,* 2005). Soil solarization alone or in combination with seed treatment with thiram + benomyl (1:1) @ 3 g/kg seed reduced pigeonpea wilt to the extent of 22.8 % and 22.6 % during the first year and 16.3 % and 15.7 % during the second year respectively (Gade *et al.,* 2007). Integration of soil solarization for 15 days in summer followed by growing of sorghum in *kharif* and application of either carbendazim granules @ 10 kg/ha one month after sowing or application of *T. viride* in organic carrier @ 62.5 kg/ha was highly effective for the management of cumin wilt (Jadeja and Nandoliya, 2008). Soil solarization for a period of 40 days in combination with dipping of corms in carbendazim + iprodione followed by two times soil drenching of the same fungicide recorded the lowest incidence of Fusarium wilt of gladiolus (Chandel and Tomar, 2011). Integration of soil solarization with application of *T. harzianum*, neem extract and captan (0.01 %) resulted in 100 % reduction of Fusarium wilt of tomato in the vegetable fields of West Bengal (Ojha and Chatterjee, 2012).

Studies conducted by Senthil (2003) revealed that combination of seed treatment (4 g/kg seed) and soil application (2.5 kg/ha) of *T. viride*, soil application of neem cake 150 kg/ha and soil drenching of mancozeb (0.3 %) effectively suppressed Fusarium wilt of cowpea in Kerala and also appreciably increased the biomass and pod yield of crop. Madhavi and Bhattiprolu (2011)

indicated that integration of seedling root dip with carbendazim (0.1 %), addition of vermicompost @ 100 g/kg soil, drenching of the combination fungicide carbendazim + mancozeb (0.2 %) along with soil application of *T. viride* @ 100 g/pot was found to be most effective against *F. solani*, incitant of wilt in chilli with minimum mortality of plants (5.83 %). Trials conducted by Hossain *et al.* (2013) revealed that the integration of soil treatment with *T. harzianum* isolate T-75, *Azadirachta indica* leaf extract and seed treatment with Provax-200 appeared to be significantly superior in reducing wilt and improving seed yield of chickpea compared to any single or dual application of them in the field.

Experiments conducted at Tamil Nadu Agricultural University for the integrated management of Fusarium wilt of banana revealed that basal application of neem cake at 0.5 kg/plant + sucker dipping in spore suspension of *P. fluorescens* for 15 min + soil application of *P. fluorescens* at 10 g/plant at 3.5 and seven months after planting showed the greatest suppression of wilt disease (Saravanan *et al.,* 2003). Zote *et al*. (2007) reported that soil/seed application of *T. viride* recorded the lowest chick pea wilt incidence (19.04 - 33.33 %), highest wilt reduction (66.67 - 80.86 %) and maximum seed germination (86.73 - 90.00 %) followed by soil applications of neem cake and castor bean cake, which recorded 86.60 % and 85.40 % seed germination and 38.09 % and 47.60 % wilt incidence and 61.91 % and 52.40 % wilt reduction, respectively. Field trials conducted by Bhatnagar *et al.* (2012) revealed that a combination of vermicompost as soil application @ 1.5 t/ha + neem cake as soil application @ 0.5 t/ha and carbendazim as seed treatment @ 2 g/kg seed recorded the minimum cumin wilt incidence of 5.6 % and highest yield of 6.25 q/ha.

Chattopadhyay and Sastry (1999) reported that seed treatment with carbendazim (0.1 %) and a fungicide tolerant strain of *T. viride* (*Tv* Mut) integrated with soil application of potassium chloride @ 20 kg/ha resulted in highest yield and maximum (82.7 %) reduction in the incidence of safflower wilt and rhizosphere soil population of *F. oxysporum*. Combined application of manganese sulphate (12.5 mg/kg) + *T. harzianum* (1.25 mg/kg soil) significantly

reduced the alfalfa wilt incidence accompanied by improved plant growth and yield in pot culture (Adhilakshmi *et al*., 2008). A combination of seed treatment with carbendazim @ 2 g/kg seed + soil application of *P. fluorescens* and *T. viride* each @ 2.5 kg/ha in FYM @ 50 kg/ha recorded least mean chickpea wilt incidence of 7.25 % with a mean yield of 1203.17 kg/ha (Mahesh *et al.*, 2010). Significant reduction (13.81 %) in pigeon pea wilt incidence was observed in combined seed treatment of metiram (0.1 %) + *T. viride* which was on par with *T. viride* alone (20.26 %) as compared to (52.23 %) control (Ram and Pandey, 2011).

2.6.1. Soil Solarization

Soil solarization is a simple, safe, and effective method of heating soil by covering it with transparent polythene sheet during hot periods to control soil borne diseases.

Desai and Dange (2003) evaluated the effect of soil solarization on Fusarium wilt of castor and reported that solarization increased mean soil temperature by 8.53ºC at 10 cm soil depth and reduced the pathogen population by 67.25 %, wilt incidence by 38.43 % and increased castor seed yield by 124.68 % as compared to non-solarized plots. Tamietti and Valentino (2006) observed that semi-solarization and full solarization for five consecutive years increased the mean soil temperature at 25 cm depth by 8.6 - 12.6°C and 12.6 - 16.3°C, respectively, reduced the native *Fusarium* spp. population from 2 - 7 × 10^3 to 0 - 25 cfu/g soil and wilt incidence by 82 – 90 %.

Soil solarization with 25 µm LLDPE plastic cover for 15 days in summer proved most effective in reducing cumin wilt incidence to 26.27 % as against 44.09 % in non-solarization and increased yield to 396 kg/ha against 286 kg/ha in non – solarized plots (Jadeja and Nandoliya, 2008). The effects of soil solarization against linseed wilt indicated that the average reduction in wilt incidence was 58.7 % in four weeks after solarization followed by three weeks

(41.0 %), two weeks (25.5 %) and one week (18.5 %). The incremental yield was 109.0 %, 66.9 %, 58.0 % and 18.4 % respectively (Kishore *et al*., 2008).

Solarization with transparent polyethylene sheet and biodegradable plastic sheet of 25 µm thickness for 40 days resulted in an increase in average maximum soil temperature by 5.6 and 3.0°C, respectively inside polyhouse and reduced carnation wilt incidence by 81.82 and 63.63 % respectively (Negi and Raj, 2013). Saremi and Saremi (2013) attempted soil solarization for the management of *F. pseudograminearum*, *F. solani* and *F. oxysporum*, the causal agents of wilt disease of wheat, bean and date palm, respectively. After six weeks of solarization, the population densities of these species decreased from 900 - 100 cfu/g in *F. solani*, from 600 to 50 cfu/g in *F. oxysporum* and from 550 - 0 cfu/g in *F. pseudograminearum* showing a promising result in controlling soil borne pathogens.

Literature on the effect of soil solarization in improving growth and yield parameters of plants are also reviewed here. Kumar *et al.* (2002b) noted that solarization of tomato fields using 0.05 mm transparent polythene sheet resulted in tallest plants (78.4 cm), large sized fruits (0.893 kg/plant), highest number of branches per plant (8.20 per plant), leaf area index (2.563), crop yield (21.6 t/ha), gross income (Rs 10,0306 /ha), net income (Rs 80,451 /ha) and benefit : cost ratio (1: 4.05). The effects of period of soil solarization on productivity of soybean revealed that solarization for four and five weeks with a transparent polyethylene markedly increased the yield attributes such as number of pods per plant, 1000 seed weight, number of seeds per pod and seed yield of soybean while, the highest seed yield (1645 kg/ha) was obtained with solarization for five weeks, which was higher by 110 % than the seed yield of non-solarized plots (Singh *et al.,* 2004).

Patel *et al.* (2008) opined that maximum plant height, number of branches, number of pods per plant, total dry matter accumulation as well as pod and haulm yields of groundnut were registered when transparent polyethylene (TPE) sheet of 0.025 mm was kept for 45 days. Thankamani *et al.* (2008) reported that black

pepper cuttings raised in solarized potting mixture with recommended nutrients showed significant increase in number of leaves (5.3), length of roots (20 cm), leaf area (177 cm^2), nutrient contents and biomass (3.7 g/plant).

Jimenez *et al.* (2012) noted that pre-planting soil solarization enhanced growth, nutrition and yield in dry beans and the results indicated that 60 days of solarization produced yields of 3.7 t/ha while no solarization produced yields of 2.1 t/ha. Soil heat units were positively correlated with yield due to an increase in heat accumulation during solarization periods in addition to an increase of leaf area and to an enhancement of plant nutrition.

2.6.2. Soil Amendment with *Trichoderma* Enriched Neem cake Organic manure Mixture

Soil amendment with decomposable organic matter helps in alteration of the physical, chemical and biotic conditions of the soil and improves soil structure promoting the root growth of the host. In addition, enrichment with antagonistic microbes reduces the inoculum potential of the soil dwelling pathogens.

Experiments conducted at Kerala Agricultural University revealed that a combination of organic manure + neem cake + arbuscular mycorrhizal fungi + *Trichoderma* produced significantly higher yield in ginger and a buildup of soil nutrients *viz.,* nitrogen, phosphorus and potassium (Sreekala and Jayachandran, 2006). Kulkarni and Anahosur (2011) reported that pre sowing application of organic manure + neem cake + *T. harzianum* + *T. viride* was most effective in avoiding stalk rot infection in maize and recorded the maximum plant stand (97.33 %) and highest grain yield (1363.14 kg/ha). Latha (2013) observed that least collar rot incidence (20.4%) and maximum groundnut pod yield (1321kg/ha) was recorded in the combined application of *P. fluorescens*, *T. viride*, neem cake and farmyard manure (FYM).

Seed treatment @ 4 g/kg seed + soil application (on 0 and 30 days after sowing) @ 100 g/m^2 of *T. virens* combined with FYM showed maximum

reduction of urdbean dry root rot incidence, increased the rhizosphere population of antagonists, inhibited the propagules of *M. phaseolina* and improved growth parameters (Christopher *et al.*, 2008). The efficacy of *T. harzianum* and *T. viride* as seed treatment and soil application with or without FYM in controlling Fusarium wilt of cumin revealed that highest reduction in wilt incidence was recorded when *T. harzianum* was used as seed treatment @ 4 g/kg seed + soil application @ 5 g/kg soil along with soil amendment of farm yard manure @ 10 g/kg soil and also resulted in highest dry weight of cumin plants (Gangopadhyay and Gopal, 2010). Godhani *et al.* (2010) noted that significantly least (5.35 %) incidence of chickpea wilt disease, highest (1247 kg/ha) grain yield and maximum (80 cm) plant canopy perimeter in seeds treated with *T. harzianum* @ 8 g/kg seed + soil application of FYM (5 t/ha) colonized with *T. harzianum* (2 x 10^{12} spores /ha).

Saravanan *et al*. (2003) noted that basal application of neem cake @ 0.5 kg/plant along with either *P. fluorescens* or *T. viride* @ 10 g/plant had a significant reduction in the incidence of Fusarium wilt of banana. Studies conducted at Annamalai University revealed that combination of *T. viride*, *P. fluorescens*, *P. lilacinus* and neem cake as seed treatment along with soil application recorded minimum wilt incidence and significantly increased the shoot length, root length, biomass and fruit yield in tomato (Sivakumar *et al*., 2008).

Patel and Patel (2012) evaluated the efficacy of *Trichoderma* based organic amendments in the management of Fusarium wilt of pigeonpea and found that significantly lower wilt incidence of 13.47 % was observed in sesamum cake, which was on par with neem cake (13.93 %). Sumanal *et al.* (2012) reported that neem cake formulation of *T. viride* was found to be more promising than talc formulation against Fusarium wilt and root knot complex disease in tobacco and affected 60.9 % disease control over check when applied at 60 and 70 days after transplanting.

Bhaskar *et al.* (2007) revealed that *T. harzianum* in combination with either FYM or neem cake proved to be the most effective treatment in reducing root rot disease complex in berseem and recorded the highest green fodder yields. Chawla and Gangopadhyay (2009) observed that antagonistic potentiality of *T. harzianum* and *P. fluorescens* against *F. oxysporum* f. sp. *cumini* was relatively better in the presence of farm yard manure or mustard cake.

2.6.3. Fungicides

Fungicide application remains as one of the easiest and best means to mitigate the losses due to diseases in commercial cultivation.

The growth and sporulation of *F. oxysporum* f. sp. *lini* were completely inhibited by carbendazim, benomyl and copper oxychloride (500, 1000 and 1500 ppm) at all concentrations tried *in vitro* (Sharma *et al.,* 2002). Maximum inhibition of the mycelial growth of *F. oxysporum* f. sp. *cumini* at the lowest concentration of fungicide (10 ppm) *in vitro* was shown by carbendazim and thiophanate-methyl (Bardia and Rai, 2007). Carbendazim (0.1 %), thiram (0.15 %) and captan (0.1 %) alone and in combination tested against *F. oxysporum* f. sp. *ciceris in vitro* revealed that chickpea seeds treated with thiram (0.15 %) + carbendazim (0.1 %) effectively inhibited the growth (90 %) of pathogen (Nikam *et al.,* 2007).

Among the fungicides tested *in vitro* against *F. oxysporum* f. sp. *ciceris*, carbendazim, copper hydroxide, copper sulphate, captan and thiram was effective as they checked the growth of the fungus completely (Tripathi *et al.*, 2007). *In vitro* evaluation of the fungicides against *F. oxysporum* f. sp. *cubense* indicated that carboxin + thiram (500, 1000, 2000 and 3000 ppm) and azoxystrobin (0.01, 0.1, 1, 2, 3 and 4 ppm) had the best effect in reducing fungal colony (Araujo *et al*., 2008). *In vitro* efficacy of fungicides against *F. oxysporum* f. sp. *lentils* showed that carbendazim and carboxin completely inhibited the growth of pathogen

whereas, thiram and captafol inhibited 87.5 % and 83.1 % of the mycelial growth respectively (Singh *et al.*, 2010).

Madhavi and Bhattiprolu (2011) observed that among the fungicides tested *in vitro* against *F. solani*, carbendazim + mancozeb (0.2 %) was found to be highly effective in inhibiting the mycelial growth (93.6 %) followed by carbendazim (0.1 %) (92.4 %) and benomyl (0.1 %) (91.34 %). Tebuconazole (0.5 %) (83.1%) and thiophanate-methyl (0.1 %) (80.1 %) were also effective whereas pencycuron (0.1 %) (4.1 %) was ineffective for the control of *F. solani*. Carbendazim (500 µg/ml), difenoconazole (100 µl/ml), hexaconazole (200 µl/ml), captan + hexaconazole (250 pg/ml), and carbendazim + mancozeb (500 µg/ml) completely inhibited the mycelial growth of *F. udum in vitro* (Ram and Pandey, 2011).

Different concentrations (35, 70, 105 and 140 ppm) of tebuconazole and thiophanate-methyl evaluated against *Macrophomina phaseolina, F. oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii* isolated from cowpea, tomato and chickpea indicated that all the concentrations of tebuconazole completely arrested the growth of three fungal species while, thiophanate-methyl was ineffective against all fungal species (Kanwal *et al.*, 2012). Benomyl and captaf at 400 µg/ml completely inhibited the growth of *F. oxysporum* f. sp. *lentils*, *in vitro* (Garkoti *et al.*, 2013).

In vivo efficacy of seed treatment with carbendazim and carboxin against *F. oxysporum* f. sp. *lentils* indicated that carbendazim and carboxin improved seed germination (90.0 % and 89.0 %), root length (10.1 cm and 10.0 cm.), shoot length (4.8 cm and 4.8 cm) and vigour index (1342.0 and 1317.0) of lentils. Similarly, foliar spray with these two fungicides separately gave best results in reducing wilt incidence from 37.5 to 5.0 % (Singh *et al.*, 2010). Seed treatment with carbendazim gave maximum germination (94.5 %) in musk melon and least incidence (16.5 %) of wilt followed by difenoconazole and propiconazole which were on par in both increasing seed germination (90.2 and 90.0 % respectively)

and reducing wilt incidence (22.5 and 25.2 % respectively) (Gurjar and Shekawat, 2012).

Seedling dip with carbendazim (1 g/l water) against *F. oxysporum* f. sp. *lycopersici* significantly reduced tomato wilt incidence by 73.1 % (Musmade *et al.*, 2009). Prochloraz and bromuconazole @ 10 µg/ml completely reduced wilt of tomato both prior and after pathogen inoculation (Amini and Sidovich, 2010).

Soil drenching of carbendazim + mancozeb and carbendazim alone, recorded 100 % inhibition of mycelial growth of *F. solani* at 1000, 2000 and 3000 ppm at 10 and 15 cm inoculum depths whereas, tebuconazole and thiophanate-methyl were effective at 3000 ppm when applied at both depths (Madhavi and Bhattiprolu, 2011). Soil drenching with carbendazim 50 WP @ 40 g/m^2 was most effective exhibiting wilt incidence of 29.17 % and fruit yield of 68.87 q/ha in chilli (Najar *et al.*, 2012). Carbendazim and propiconazole were effective in controlling tobacco wilt to 61.47 % and 60.29 % and an increased yield of total cured leaf to 24.53 % and 31.77 % respectively (Sumanal *et al.*, 2012).

2.7. INTEGRATED DISEASE MANAGEMENT OF ANTHRACNOSE

Integration of seed treatment with alum (0.1 %) along with foliar sprays of *Lawsonia inermis* extract (1 %) + alum (0.1 %) in soybean recorded 7.0 % foliar anthracnose, 4.2 % pod blight incidence and an yield of 2191 kg/ha (Chandrasekaran *et al.*, 2000). Seed treatment with carbendazim @ 2 g/kg + two foliar sprays of carbendazim + mancozeb (0.05 %) at 15 days interval gave maximum reduction in chickpea blight incidence followed by seed treatment with carbendazim @ 2 g/kg + foliar spray of carbendazim + mancozeb (0.05 %) and *Polyalthia longifolia* (10 %) extract at 15 days interval (Varaprasad, 2000). Seed treatment followed by two prophylactic sprays of carbendazim or propiconazole @ 0.1 % each at 15 days interval showed minimum disease severity of blackgram anthracnose and maximum grain yield followed by hexaconazole (0.1 %) and mancozeb (0.2 %) sprayed plots (Deeksha and Tripathi, 2002b).

Combined effects of varieties, fungicides and sowing dates on the incidence and severity of anthracnose of sorghum showed that seed dressing with metalaxyl + carboxin (50 DS) and thiram + thiophanate-methyl (70 WP) were effective in reducing the severity of leaf anthracnose at 50 DAS and up to of 70 DAS. In the field, late sown crops and late maturing varieties, Guzama red and Guzama white had significantly lower severity of leaf anthracnose than early sown crop and the early maturing variety, Warwarabashi (Gwary *et al.*, 2008).

Integration of moderately resistant greengram genotypes (BGS-9, TM-98-50 and TM-97-55) coupled with one hexaconazole (0.1 %) spray was effective in reducing severity of anthracnose and also in enhancing the grain yield (11.46 q/ha) and stalk yield (13.83 q/ha) of greengram (Kulkari, 2009). Combined application of aqueous leaf extract of *Solanum virginianum* and *T. viride* @ 30 and 60 DAS significantly reduced the incidence of anthracnose of tomato (70 %) followed by the application of *S. indicum* and *T. viride* (68%) (Mogle, 2011).

IDM was superior to biological and chemical modules in two chilli cultivars Navjyoti and Ujwala, with lower disease incidence on foliage by 54.7 % and 65.2 %, on fruits by 51.8 % and 59.4 % and higher fruit yields by 69.7 % and 46.8 % respectively (Lydia and Zachariah, 2012)

2.7.1. Soil Solarization

Management of brown root rot of greenhouse tomatoes, a complex disease caused by *C. coccodes, F. oxysporum* f. sp. *radicis-lycopersici, F. solani, Pyrenochaeta lycopersici, Pythium debaryanum* and *Rhizoctonia lycopersici* revealed that soil when covered for 10 weeks with a transparent plastic sheet of 0.05 mm thickness reduced the number of pathogen propagules by 83.5 – 100 % compared with the control (Bourbos and Skoudridakis, 1991). Assessment of soil solarization and mouldboard ploughing against *C. coccodes* causing black rot of potato indicated that solarization reduced disease incidence by 45 % when tarping

was done for eight weeks and temperature reached up to 56°C in the top five cm of soil (Denner *et al.*, 2000).

Combined effect of *Trichoderma* application and soil solarization against *Colletotrichum* crown rot in strawberry plants showed that *Trichoderma* treated plots showed less disease incidence and the solarized plots performed better than unsolarized plots (Porras *et al.*, 2003). Chinese cabbage cultivars grew vigorously in the solarized field improving yield and reducing anthracnose incidence by 32.3 % than 71.1 % in the control plots (Ke *et al.*, 2007). Soil solarization along with application of *T. harzianum* (TH 43 and TH 39) and *P. fluorescens* (PSF 27) resulted an increase in mean maximum temperature by 54.6°C at five cm soil depth, increased plant height, collar diameter in sorghum and reduced anthracnose severity as compared to non solarized soil alone or fortified with biological control agents (Singh, 2008).

2.7.2. Soil Amendment with *Trichoderma* Enriched Neem cake Organic manure Mixture

The reports on soil amendment with *Trichoderma* enriched neem cake organic manure mixture for the management of legume anthracnose are sketchy. However, *in vitro* and *in vivo* antagonistic effect of various *Trichoderma* spp. against the pathogen causing anthracnose in pulse crops are reviewed here.

T. harzianum was found to be the best in inhibiting the mycelial growth of *C. lindemuthianum*, incitant of dolichos bean anthracnose to an extent of 73.54 % followed by *T. viride* (50.90 %) (Rajesha *et al.*, 2010). Padder and Sharma (2011) observed that *T. viride* had the maximum potentiality to suppress the spore germination, mycelial growth and seed borne infection of *C. lindemuthianum* causing bean anthracnose when compared with other bioagents like *T. harzianum*, *T. hamatum* and *G. virens*. The antagonistic effect of various fungal bioagents against *C. capsici* revealed that *T. harzianum* was superior in reducing the radial

growth of the test fungus up to 54.91 % followed by *T. viride* (47.54 %) and *T. koningii* (40.16 %) (Bal and Behera, 2012).

Among the bio-agents tested, *T. viride* recorded maximum growth inhibition (79.40 %) of *C. truncatum*, incitant of anthracnose / pod blight of soybean followed by *T. hamatum* and *P. fluorescens* with 73.74 and 69.31 % growth inhibition, respectively (Jagtap *et al.,* 2012). Manjunath *et al.* (2013) observed that *T. viride* inhibited the mycelial growth of *C. lindemuthianum* to the maximum (92.22 %) extent among the different bioagants tested *in vitro*.

Spraying *T. viride*-TH31 on inoculated cowpea plants effectively suppressed the incidence and severity of anthracnose disease and increased yield over the control (Adebanjo and Bankole, 2004). Spraying 5 % culture filtrate of *T. viride* against *C. truncatum*, incitant of soybean leaf blight was effective in reducing the disease incidence to 69.62 % against control (Guldekar and Potdukhe, 2010).

2.7.3. Fungicides

In vitro evaluation of fungicides (@ 100, 200 and 400 ppm) against *C. dematium* indicated that propiconazole gave complete inhibition of radial growth of the test pathogen at all selected concentrations. The highest concentration of carboxin inhibited 77.41 % of radial growth whereas, mancozeb and copper oxychloride were found significantly inferior towards the pathogen (Shovan *et al*., 2008). Among the fungicides (@100, 150 and 200 ppm) tested *in vitro* against *C. truncatum*, carbendazim (0.1 %) recorded the highest mean inhibition of (90.59 %) of mycelial growth of pathogen followed by propiconazole (0.1 %) (87.95 %), hexaconazole (0.1 %) (86.15 %), difenoconazole (0.1 %) (84.81 %) and chlorothalonil (0.1 %) (70.23 %) (Gawade *et al.,* 2009).

In vitro evaluation of contact fungicides against *C. lindemuthianum* revealed that, mancozeb completely inhibited the mycelial growth of the pathogen followed by propineb (48.32 %) and chlorothalonil (37.39 %) at 800 ppm. Among the systemic fungicides, carbendazim and propiconazole completely

inhibited the mycelial growth followed by difenoconazole (84.87 %) at 400 ppm (Rajesha *et al.*, 2010). Chauhan and Bhatia (2012) noted that carbendazim @ 50 ppm showed 100 % inhibitory effect on the mycelial growth of *C. lagenarium* while, copper oxychloride and mancozeb recorded complete inhibition @ 100 ppm.

Evaluation of systemic and non systemic fungicides against *C. lindemuthianum in vitro* revealed that among the systemic fungicides (@ 100, 250, 500 and 750 ppm), carbendazim and carbendazim + mancozeb were the best in inhibiting complete (100 %) growth of the pathogen at all four concentrations tested. Among the non-systemic fungicides (@ 250, 500, 1000 and 2000 ppm), mancozeb (2000 ppm) was found effective in inhibiting the growth of the mycelium up to 100 % while, least inhibition (0.00 %) of the mycelial growth was observed in copper oxychloride at 250 ppm (Manjunath *et al*., 2013).

In vivo evaluation of fungicides against *C. gloeosporioides* revealed that pyraclostrobin + epoxiconazole (0.13 + 0.05 g/l), tetraconazole (0.1 g/l), tebuconazole (0.2 g/l), chlorothalonil (2 g/l) and chlorothalonil + thiophanate-methyl (1 + 0.4 g/l) when sprayed seven times, at 15 days interval significantly reduced the anthracnose severity on peach plants, providing control up to 68 – 78 % (Mafacioli *et al.,* 2006).

Trials conducted at Kerala Agricultural University to manage cowpea anthracnose showed that seed treatment with thiram @ 3 g/kg seed, followed by three rounds of spray with carbendazim (0.1 %) @ 15, 30 and 45 days after seedling emergence recorded the lowest plant mortality (15.89 %) and highest yield (Purushothaman *et al.,* 2007). Foliar application of azoxystrobin, mancozeb or chlorothalonil during flowering and podding in lupins reduced incidence of anthracnose on pods while, tebuconazole, benomyl and carbendazim were less effective (Thomas *et al.*, 2008).

Carendazim (0.1 %) was found effective and economical in controlling soybean anthracnose, recording least disease incidence (19.43 %), mean defoliation (11.85 %), mean pod blight (9.64 %) with highest seed yield (2605 kg/ha) and economical B:C ratio (1:13.55) (Gawade *et al.,* 2009). While, Guldekar and Potdukhe (2010) reported that, in soybean, mancozeb (0.25 %) gave minimum anthracnose incidence (7.87 %) and maximum healthy pods/plant (54.66).

Studies conducted at IIHR, Bangalore revealed that triadimefon (0.01 %) was most effective in controlling three fungal diseases of chilli *viz.*, powdery mildew, leaf spot and anthracnose and also in increasing the yield followed by myclobutanil (0.04 %) (Ganeshan *et al.,* 2011). Chauhan and Bhatia (2012) reported that foliar spray with carbendazim gave the best control (89.6 %) of bottle gourd anthracnose followed by copper oxychloride (79.7 %), whereas iprobenfos was least effective (62.8 %) in controlling the disease.

Integration of seed treatment with DCT (diazinon+captan+thiophanate-methyl) and sequential application of pyraclostrobin and azoxystrobin at the fifth trifoliolate stage, first flowering, full flowering and 10 days after full flowering gave superior disease control, improved plant health, extended crop maturity and increased yield in dry beans (Gillard *et al.,* 2012). *In vivo* evaluation of fungicides against *C. capsici* on chilli revealed that copper hydroxide (0.25 %) and propiconazole (0.1 %) recorded the lowest disease severity (2.20 %) and highest mean yields per plot (2.25 kg) followed by copper oxychloride (0.20 %) (2.50 % and 2.15 kg respectively) (Goswami *et al.,* 2013).

2.8. COMPATIBILITY OF FUNGICIDES WITH BENEFICIAL MICROBES

Information on compatibility of beneficial microbes with fungicides is imperative for formulating an ideal disease management strategy. In an integrated disease management package, compatible pesticides can be incorporated along

with bioagents for effective and sustainable disease management with lesser disturbance to agro-ecosystem.

T. harzianum C52 was least sensitive to procymidone and captan and most sensitive to mancozeb, tebuconazole and thiram (Mclean *et al.*, 2001). Complete inhibition of *T. viride, T. koningii, T. harzianum* and *T. virens*, was observed in tebuconazole and hexaconazole while, tolerable growth was seen in captan and propineb (200 µg/ml) and azoxystrobin (400 µg/ml) (Pandey *et al.*, 2006). *T. viride* (TvM_1) and *T. harzianum* (ThM_1) mutants showed high compatibility with carbendazim (0.1 %) whereas, mancozeb (0.25 %) was found inhibitory to the radial growth of both. TvM_1 showed compatibility with captan (0.25 %) and copper oxychloride (0.3 %) while, ThM_1 was compatibile with mancozeb (0.125 %) (Madhavi *et al.*, 2008).

Studies conducted by Khalko and Pan (2009) revealed that propineb 70 WP tested at different concentrations (8, 16, 32, 64 and 128 ppm) appeared to be compatible with *T. harzianum*. Thiram (0.2 %), copper oxychloride (0.2 %) and mancozeb (0.2 %) were compatible with *T. harzianum* and *T. viride* but captan, tebuconazole, carboxin, propiconazole and chlorothalonil were sensitive to the fungi (Bagwan, 2010). Chlorothalonil 75 WP was compatible with *T. viride* up to 250 ppm with 44.4 % inhibition while, carbendazim 50 WP, propiconazole 25 EC, tridemorph 80 EC and hexaconazole 5 EC were inhibitory (Madhusudhan *et al.*, 2010). Sarkar *et al.* (2010) noted a progressive increase in percent inhibition of radial growth of *T.harzianum* as the concentrations of fungicides increased. The toxicity of contact fungicides was lower than systemic fungicides, among which copper oxychloride and copper hydroxide were highly compatible.

In vitro compatibility studies of *Trichoderma* spp. with fungicides showed that, carbendazim, benomyl, carboxin, propiconazole, hexaconazole, tricyclazole, tridemorph and chlorothalonil were incompatible with *Trichoderma* spp. showing 100 % inhibition of the radial growth at field concentration. Copper oxychloride, fosetyl-Al, captan, thiram and metalaxyl were least compatible showing more than

70 % inhibition. Azoxystrobin and mancozeb were moderately compatible with radial growth inhibition in the range of 20 – 45 % (Ranganathaswamy *et al*., 2012). The growth of *T. viride* was completely inhibited by carbendazim and carboxin at low, recommended and high concentrations while, mancozeb showed no inhibitory effect on the growth of *T. viride*, indicating its compatibility at all concentrations (Gaikwad *et al.,* 2011).

Compatibility studies of fungicides with *P. fluorescens* revealed that carboxin, chlorothalonil and carbendazim were least toxic to *P. fluorescens* strain PFBC-25, while captan was most inhibitory to this strain (Khan and Gangopadhyay, 2008). Anand *et al.* (2009) opined that *P. fluorescens* (Pf1) was compatible with all concentrations (100, 150, 200, 250 and 300 ppm) of azoxystrobin tested. Tricyclazole and carbendazim was compatible with *P. fluorescens* isolate 83 at all four concentrations (2000, 1000, 500 and 250 ppm) tested while, propiconazole showed compatibility at 1000, 500 and 250 ppm and chlorothalonil at 500 and 250 ppm only (Gangwar, 2013). Compatibility studies of propicaonazole (@ 250, 500,750, 860 and 1000 ppm) with bacterial biocontrol agents *viz., Pseudomonas* and *Bacillus* showed that the bacterial growth was not affected by propiconazole even at the highest concentration of 1000 ppm (Raj *et al.*, 2013a). Studies conducted by Manjunatha *et al.* (2013) on compatibility of *P. fluorescens* with fungicides indicated that carbendazim and thiram were fully compatible with *P. fluorescens* while mancozeb, captan and propiconazole were found incompatible.

Fungicide treatment of *Rhizobium* inoculated soybean seeds on subsequent nodulation and N_2 fixation indicated that metalaxyl, benalaxyl, iprodione and captafol did not stimulate or inhibit bacterial growth, apart from captafol which reduced number of viable rhizobia (Diatloff, 1986). Simultaneous treatment with carbendazim and *Rhizobium* enhanced nodulation by 16.67 % in chickpea seeds while, an enhancement of 30.27 % was recorded when *Rhizobium* was inoculated 24 h after fungicide seed dressing (Kumar *et al.*, 2002a). Compatibility studies of fungicides (@ 50, 100, 250, 500, 1000 and 2000 ppm) with *Rhizobium* spp.

revealed that carbendazim, thiophanate-methyl and metalaxyl + mancozeb did not show inhibition zones in any of the concentrations tested whereas, mancozeb, triadimefon and captan showed inhibition zones at 1000 and 2000 ppm (Singh and Mehta, 2005). *Rhizobium* and *Bradyrhizobium* strains tolerated low fungicide concentrations (< 100 µg/l) but they were sensitive to high concentrations (> 500 µg/l) with varying degrees of sensitivity and the *Rhizobium* strains were found more tolerant than *Bradyrhizobium* strains (Ahmed *et al.*, 2007).

2.9. PERSISTENCE AND DEGRADATION OF FUNGICIDES ON VEGETABLES

Agricultural crops, particularly vegetables are highly contaminated with pesticide residues on account of the extensive use of these toxicants for pest control. Bourn and Preecott (2002) found after an extensive survey on vegetables that 17 – 50 % of conventional vegetables contained pesticide residues. Information on the persistence and degradation of pesticides is essential prior to their recommendation to crops.

Persistence and dissipation studies of hexaconazole on French bean pods applied @ 525 and 1050 g ai/ha during the pod formation stage recorded an initial build up of 0.197 and 0.485 mg/kg of residues. The dissipation of residues showed half lives of 2.59 and 4.21 days and required pre harvest waiting periods of 6.5 and 13.0 days for safe bean pod consumption (Ahuja and Awasthi, 2002).

Determination of tetraconazole and diniconazole fungicide residues in tomatoes and green beans indicated that the fungicides incorporated into the plants decreased rapidly with a half life around three days for diniconazole and from 4.5 - 6.5 days for tetraconazole. No residues could be detected in the plants during the period of study of 21 days after field application (Amer *et al*., 2007).

Analysis of different samples of fresh fruits, vegetables and arable crops at the Institute of Plant Protection - National Research Institute in Poland revealed that residues of 35 pesticides, mainly fungicides were detected in 29.6 % of the

samples analyzed. Violations of MRLs were found in 1.5 % of the samples. Residues were detected in 58.1 % of fruit samples and 57.7 % of greenhouse vegetable samples. Residues of carbendazim were detected most often, in 6.9 % of the samples (Gnusowski *et al.,* 2010).

Field trials conducted at West Bengal condition to evaluate the harvest residue of tebuconazole 25.9 EC in paddy and groundnut revealed that the fungicide residues were below the detection limit of the instrument (< 0.01 ppm) irrespective of doses in paddy and groundnut and the use of tebuconazole may be advocated for the control of diseases in paddy and groundnut without any residual toxicity problem (Kundu *et al.,* 2011).

Studies on residual and dissipation dynamics of flusilazole in apple and soil after treatment with the fungicide at recommended and high dosage levels showed that half life of flusilazole in apple and soil were 4.23 - 7.77 days and 3.04 - 5.14 days respectively. Residues of flusilazole in apple at harvest time were below 0.05 mg/kg at both recommended and high dosage levels (Shuang *et al*., 2011).

Investigation of pesticide residues in vegetables and fruits grown in various regions of Hatay, Turkey indicated that residues of carbendazim, fenarimol, fludioxonil, metalaxyl, thiabendozole and triadimenol were found at levels between 0.003 and 0.759 mg/kg. However, the detected residue amounts were less than the MRLs declared in the Turkish Food Codex and EU MRLs (Sungur and Tunur, 2012).

Dikic *et al.* (2012) reported that prolonged intake of low doses of carbendazim through fruits and vegetables impend changes in metabolic patterns, hepatotoxicity and subsequent liver damage in human.

Material and methods

3. MATERIALS AND METHODS

The present study entitled "Integrated management of Fusarium wilt and anthracnose of vegetable cowpea (*Vigna unguiculata* subsp. s*esquipedalis* (L.) Verdcourt) using new generation fungicides" was carried out in the Department of Plant Pathology, College of Agriculture, Vellayani during the year 2011 - 2014. Details of materials used and the methods followed for the study are presented below.

3.1. SURVEY ON INCIDENCE, SEVERITY (DISEASE INDEX) AND EXTENT OF YIELD LOSS DUE TO MAJOR FUNGAL DISEASES IN COWPEA

Potential cowpea growing areas of Thiruvananthapuram district were periodically surveyed during 2011 - 2012 to assess the prevalence of major fungal diseases in cowpea *viz*; Fusarium wilt, anthracnose, web blight and collar rot, Cercospora leaf spot, powdery mildew and rust. Observations were recorded on disease incidence, index and extent of yield loss.

3.1.1. Disease Incidence

The incidence of disease under natural epiphytotic (Singh, 2002) condition was recorded as:

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

3.1.2. Disease Index

Based on the extent of damage caused by the disease, standard score charts with pictorial diagrams were developed for assessing the severity (disease index) due to major fungal diseases in cowpea (Singh, 2002).

3.1.2.1. Score Chart for Assessing the Severity of Fusarium wilt in Cowpea

Data on the severity of Fusarium wilt in cowpea was recorded following the 0 - 4 rating scale (Plate 1) (Senthil, 2003) where,

0- Healthy plants

1- Slight yellowing of leaves

2- Yellowing and necrosis of leaves, initiation of basal swelling

3- Basal swelling, yellowing and necrosis of leaves

4- Basal swelling, distortion, yellowing and necrosis of leaves. Total wilting

3.1.2.2. Score Chart for Assessing the Severity of Anthracnose in Cowpea

Data on the severity of anthracnose in cowpea was recorded following the 0 -9 rating scale (Plate 2) (Mayee and Dattar, 1986) where,

0- No symptoms

1- Small, circular to irregular, brown lesions covering 1 % or less of the area of leaves and spindle shaped lesions covering 1 % or less of the area of vines.

3- Lesions big covering 1 - 10 % of the area of the leaves and vines.

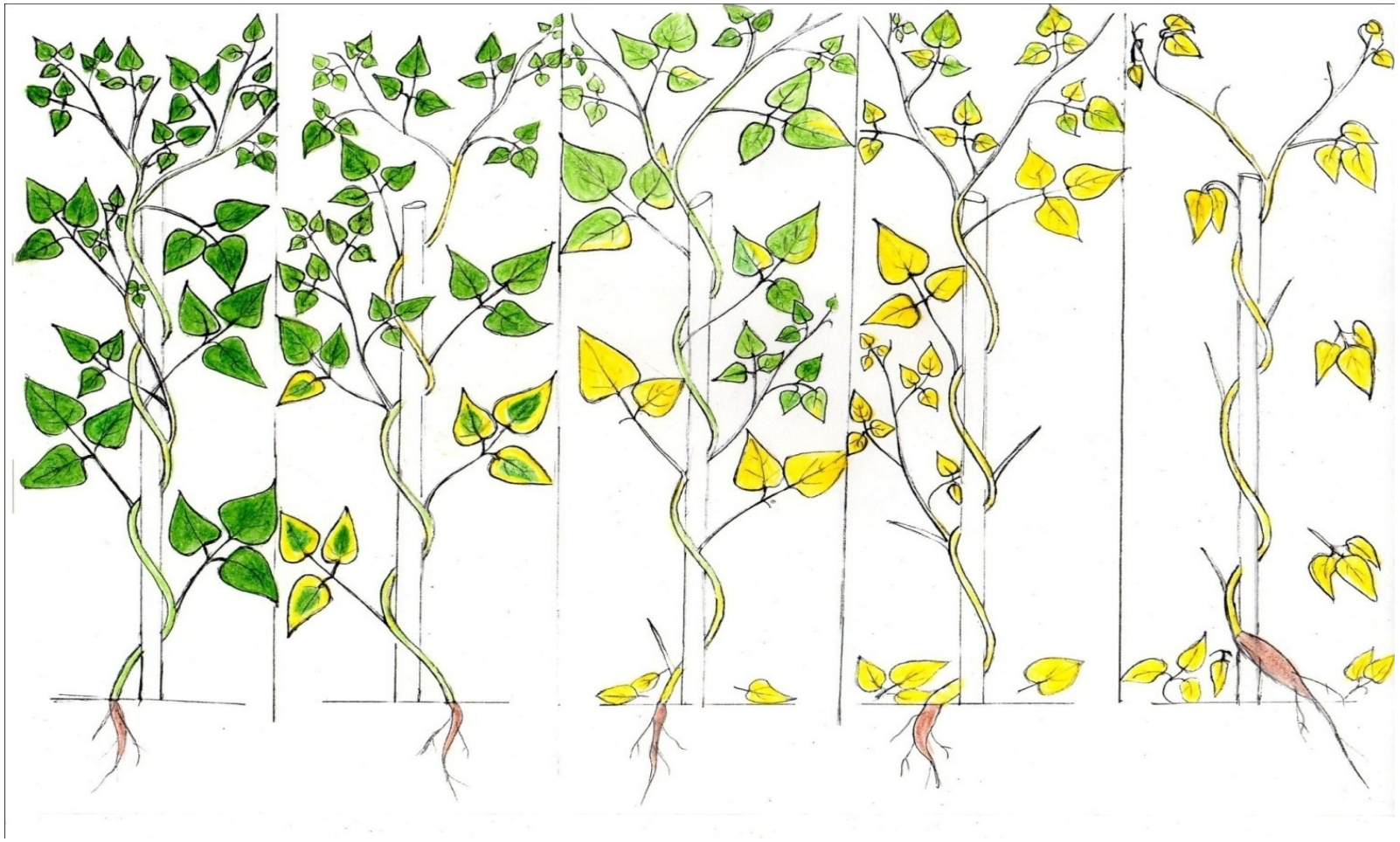
5- Lesions covering 11 - 25 % of the area of leaves and vines.

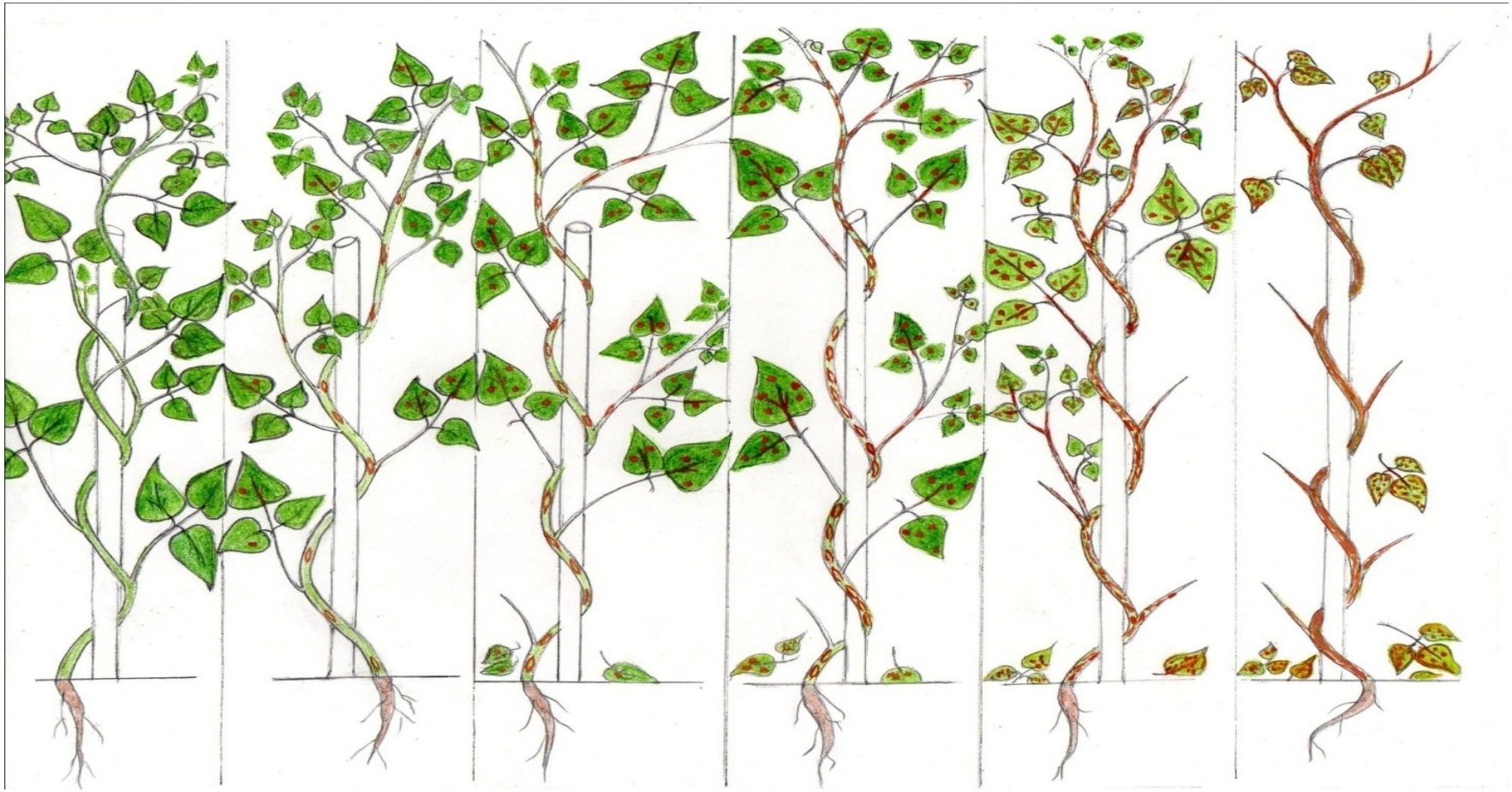
7- Lesions covering 26 - 50 % of the area of leaves and vines. Lesions on stem and pods.

9 - Lesions covering > 51 % of the area of leaves and vines. Deep lesions on stem and pods. Defoliation of leaves and blighting of plant.

0 **1** **2** **3** **4**

Plate 1. Standard area diagram for assessing the severity of Fusarium wilt in cowpea





0 **1** **3** **5** **7** **9**

Plate 2. Standard area diagram for assessing the severity of anthracnose of cowpea

3.1.2.3. Score Chart for Assessing the Severity of Web blight in Cowpea

Data on the severity of web blight in cowpea was recorded following the 0 - 9 rating scale (Plate 3) (Mayee and Dattar, 1986) where,

0- No symptoms

1- 1 - 10 % of leaf area infection

3- 11 - 25 % of leaf area infection

5- 26 - 50 % of leaf area infection

7- 51 - 75 % of leaf area infection

9- > 75 % of leaf area infection

3.1.2.4. Score Chart for Assessing the Severity of Cercospora leaf spot in Cowpea

Data on the severity of Cercospora leaf spot in cowpea was recorded following the 0 - 9 rating scale (Plate 4) (Mayee and Dattar, 1986) where,

0- No symptoms

1- Small reddish brown spots covering 1 % or less of the leaf area

3- Well defined spots, bound by veins covering 1 - 10 % of the leaf area

5- Vein bound, well defined spots with purple margin and gray centre covering 11 - 25 % of the leaf area

7- Spots enlarging, with purple margin and gray centre covering 26 - 50 % of the leaf area along with shot- hole symptoms

9- Spots covering 51 % or more of the leaf area along with shot- hole symptoms. Spots on pods

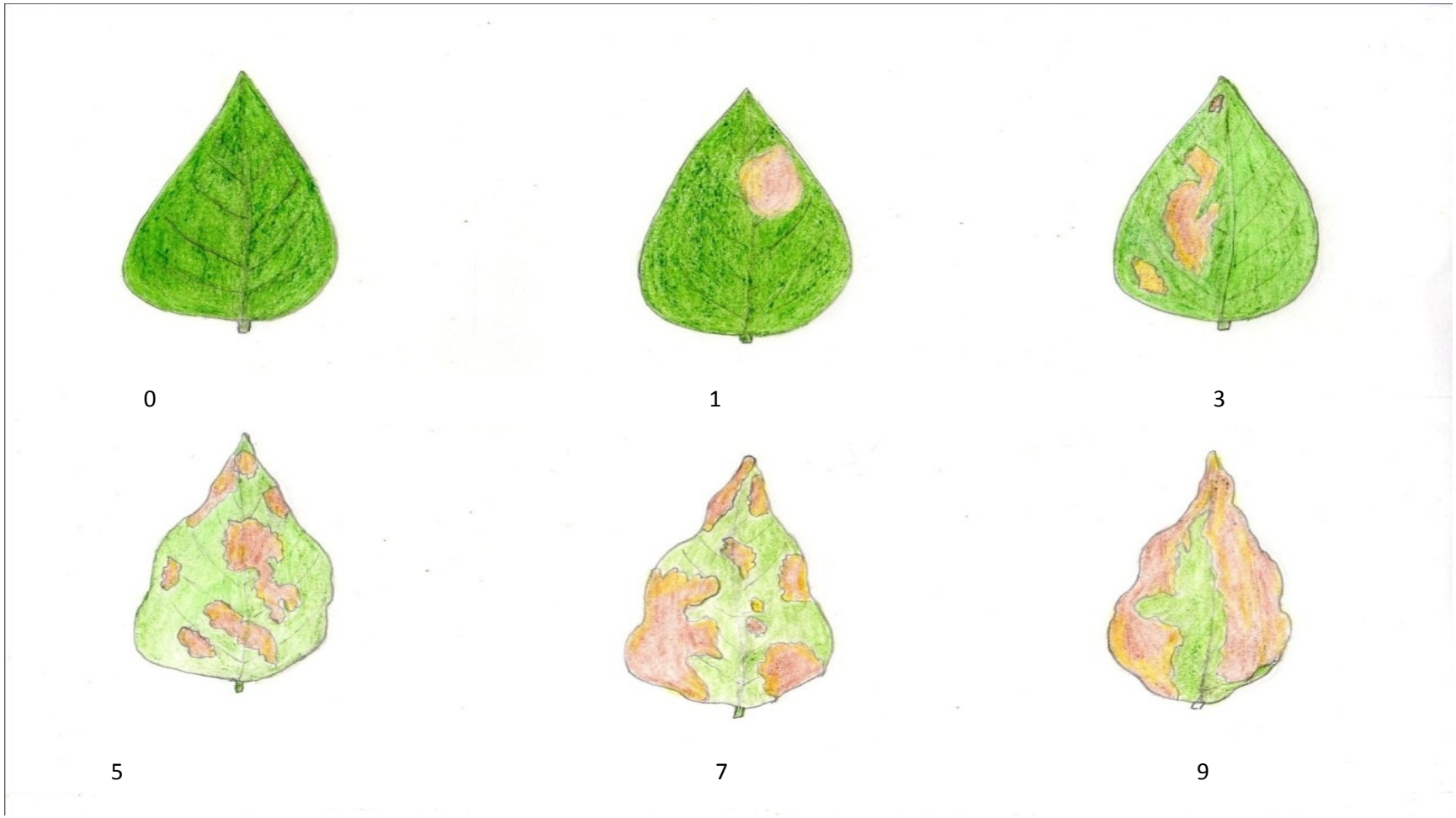


Plate 3. Standard area diagram for assessing the severity of web blight in cowpea

0 **1** **3** **5** **7** **9**

Plate 4. Standard area diagram for assessing the severity of Cercospora leaf spot in cowpea

3.1.2.5. Score Chart for Assessing the Severity of Powdery mildew in Cowpea

Data on the severity of powdery mildew in cowpea was recorded following the 0 - 9 rating scale (Plate 5) (Mayee and Dattar, 1986) where,

0- No symptoms on leaves

1- Small powdery flecks covering 1 % or less of the leaf area.

3- Small powdery lesions covering 1 – 10 % of the leaf area.

5- Powdery lesions enlarging, irregular covering 11 – 25 % of the leaf area

7- Powdery lesions coalescing forming irregular white to grey patches covering 26 - 50 % of the leaf area.

9- Powdery patches covering 51 % or more of the leaf area. Symptoms on pods and flowers.

3.1.2.5. Score Chart for Assessing the Severity of Rust in Cowpea

Data on the severity of rust in cowpea was recorded following the 0 - 9 rating scale (Plate 6) (Mayee and Dattar, 1986) where,

0- No symptoms on leaves

1- Small, round, powdery brown uredosori covering 1 % or less of the leaf area.

3- Typical uredosori covering 1 - 10 % of the leaf area.

5- Typical uredosori covering 11 - 25% of the leaf area

7- Typical uredosori covering 26 - 50 % of the leaf area

9- Uredosori cover 51 % or more of the leaf area. Withering of leaves.

Plate 5. Standard area diagram for assessing the severity of powdery mildew in cowpea

0 **1** **3** **5** **7** **9**

Plate 6. Standard area diagram for assessing the severity of rust in cowpea

Based on the scores assigned to each diseased plant/leaf, severity (disease index) was worked out using the formula described by McKinney (1923).

$$\text{Disease index} = \frac{\text{Sum of individual ratings}}{\text{Total number of plants/ leaves observed}} \times \frac{100}{\text{Maximum grade}}$$

3.1.3. Yield Loss

The loss in yield was calculated as described by Singh (2002).

$$\text{Yield loss} = \frac{Yp - Yx}{Yp} \times 100$$ where,

Yp : Potential yield

Yx : Actual yield with any measured degree of disease incidence or severity

During the course of periodic surveys, infected cowpea plants showing characteristic symptoms of Fusarium wilt and anthracnose on roots, stem and leaves were collected, microscopically examined and stored for further studies in a refrigerator.

3.2. SYMPTOMATOLOGY STUDIES

Detailed studies on the symptomatology of Fusarium wilt and anthracnose of cowpea were carried out including the stages of disease development. The natural incidence of Fusarium wilt and anthracnose in cowpea was observed from farmers' fields and the sequence of events leading to the final death of the plants were recorded.

3.3. ISOLATION OF PATHOGENS

3.3.1. Isolation of Pathogen Associated with Fusarium wilt of Cowpea

The roots and stem of infected cowpea plants were washed in running tap water to remove the adhering soil particles and were cut into small bits of size one to two mm with sterilized blade. These bits were then surface sterilized with

0.1 % aqueous solution of mercuric chloride (Hg Cl_2) for one min followed by washing with three changes of sterile water. The bits were transferred aseptically into sterile petridishes containing solidified Potato Dextrose Agar (PDA) (Appendix I) and incubated at room temperature. The fungal growth that appeared on plates was transferred to the PDA slants (Aneja, 2003).

The fungal isolates thus obtained were purified by single spore isolation technique. Serially diluted spore suspension of each isolate prepared from seven to eight days old culture was plated on sterilized plain agar (2 %) in petriplates under aseptic conditions. The plates were incubated at room temperature for 24 h. A single spore located under microscope was marked with a fine tip pen and transferred to PDA slants for further studies (Aneja, 2003).

3.3.2. Isolation of Pathogen Associated with Anthracnose of Cowpea

Standard tissue isolation procedure was followed to isolate the causal organism. The infected tissues of the leaves and twigs were cut into small bits of one to two mm size and surface sterilized in 0.1 % mercuric chloride (Hg Cl_2) solution for one min and washed thrice in sterile distilled water to remove the traces of mercuric chloride before transferring them into sterile petridishes containing solidified PDA under aseptic conditions. The plates were incubated at room temperature and observed for fungal growth. Further, the pure culture of the fungus was obtained by single spore isolation method as described in 3.3.1.

3.4. CHARACTERIZATION OF PATHOGENS

3.4.1. Morphological and Cultural Characterization of Pathogen Associated with Fusarium wilt of Cowpea

The morphological and cultural characters of different isolates of the pathogen were studied by growing them on PDA and compared with those mentioned by Booth (1971). Micro-morphological characters of conidia and conidiophores were studied by following slide culture technique as described by

Riddel (1974). The size and shape of conidia were measured using an image analyzer (Motic images plus 2.0 software). The mode of chlamydospore production *viz.,* solitary, pairs, chains and location were observed. Cultural characters such as the nature and growth of mycelium, color/pigmentation of the different isolates and sporulation were recorded after seven to eight days of inoculation. The radial growth of pathogen was recorded up to 10 days after inoculation.

3.4.1.1. Slide Culture

Sterile plain agar medium was poured in petridishes to a thickness of two mm. After solidification six mm square pieces were cut using a sterile needle. One square disc was placed at the centre of a sterile slide and each of the four sides of the agar block was inoculated with mycelial bits of the pathogen. A cover slip was placed on the top of the inoculated agar disc and the slides were placed in moist petridish chambers consisting of wet filter paper in the bottom in which two glass rods kept as support for the slides. The dish with the slide was then incubated at room temperature for two to three days. After this the cover slip was lifted off gently and mounted on another slide using lactophenol cotton blue stain (Appendix II). The agar block was removed from the culture slide and another mount was prepared on it without disturbing the fungal growth on it. The slides were examined under low power and high power objectives of a compound microscope and micro-morphological characters of conidia and conidiophores were studied.

3.4.2. Morphological and Cultural Characterization of Pathogen Associated with Anthracnose of Cowpea

The nature of growth, color, branching pattern and septation in the hyphae of the different isolates of the pathogen associated with anthracnose of cowpea were recorded after seven days of incubation on PDA. The occurrence of sectors, vegetative and reproductive structures and the characters of acervuli and setae were described. Colony diameter was recorded daily for a week and the growth

rate was calculated as the seven day average of mean daily growth (cm/day). The size and shape of conidia were measured using an image analyzer (Motic images plus 2.0 software). Color of the conidial mass and zonation were also recorded from the colonies grown on PDA plates at room temperature (Chowdhry and Varshney, 2000). The shape of appressoria was studied using a slide culture method modified from Hawksworth (1974).

3.4.3. Molecular Characterization of the Pathogen Isolates Associated with Fusarium wilt and Anthracnose of Cowpea by Partial Sequencing of Internal Transcribed Spacer (ITS) Region of rDNA

The isolates of the two pathogens obtained from different locations were characterized on molecular basis by comparison of the ITS sequences of the isolates. The procedure for molecular characterization was as follows:

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

About 100 mg of the tissue/mycelium was homogenized in 400 µl of buffer PL1. 10 µl of RNase A solution was added and inverted to mix. The homogenate was incubated at 65ºC for 10 min. The lysate was transferred to a Nucleospin filter and centrifuged at 11000 x g for two min. The flow through liquid was collected and the filter was discarded. 450 µl of buffer PC was added and mixed well. The solution was transferred to a Nucleospin Plant II column, centrifuged for one min and the flow through liquid was discarded. 400 µl buffer PW1 was added to the column, centrifuged at 11000 x g for one min and flow though liquid was discarded. Then 700 µl PW2 was added, centrifuged at 11000 x g and flow through liquid was discarded. Finally 200 µl of PW2 was added and centrifuged at 11000 x g for two min to dry the silica membrane. The column was transferred to a new 1.7 ml tube and 50 µl of buffer PE was added and incubated at 65ºC for five min. The column was then centrifuged at 11000 x g for one min to elute the DNA. The eluted DNA was stored at 4ºC.

3.4.3.2. Agarose Gel Electrophoresis for DNA Quality Check

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

3.4.3.3. PCR Analysis

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

Target	Primer name	Direction	Sequence (5' → 3')	Reference
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).

3.4.3.4. PCR Amplification Profile

98 ^{o}C - 30 sec

98 ºC	-	5 sec	40 cycles
60 ºC	-	10 sec	
72 ºC	-	15 sec	
72 ºC	-	60 sec	
4 ºC	-	∞	

3.4.3.5. Agarose Gel Electrophoresis of PCR Products

The PCR products were checked in 1.2 % agarose gels prepared in 0.5 X 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 0.5 X TBE as electrophoresis buffer for about 1 - 2 h, until the bromophenol 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 transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.4.3.6. ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications. Five µl of PCR product is mixed with two µl of ExoSAP-IT and incubated at 37ºC for 15 min followed by enzyme inactivation at 80ºC for 15 min.

3.4.3.7. Sequencing Using BigDye Terminator v3.1

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

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM (either F/R)
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.

3.4.3.8. Post Sequencing PCR Clean up

A master mix I of 10 µl milli Q and two µl 125mM EDTA per reaction was made. 12 µl of master mix I to each reaction containing 10 µl of reaction contents was added and were properly mixed. A master mix II of two µl of 3M sodium acetate pH 4.6 and 50 µl of ethanol per reaction was made. 52 µl of master mix II was added to each reaction. The contents were mixed by inverting and incubated at room temperature for 30 min. Further it was centrifuged at 14,000 rpm for 30 min. The supernatant was decanted and 100 µl of 70 % ethanol was added. It was spinned at 14,000 rpm for 20 min. The supernatant was decanted and 70 % ethanol wash was repeated. The supernatant was decanted and the pellet was air dried. The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.4.3.9. Sequence Analysis and Submission of Sequences in NCBI

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al.,* 2010). The identity of ITS- rDNA conserved region of the pathogen isolates associated with Fusarium wilt and anthracnose of cowpea was established by performing a similarity search using Basic Local Alignment Search Tool (BLAST) in the

National Centre for Biotechnology Information (NCBI) database and the sequences were matched with existing available database for species confirmation. Based on the sequence matching results, the rDNA sequences were bankitted in the NCBI database and accession numbers were obtained.

3.4.3.10. Phylogenetic Analysis

The data set based on the ITS- rDNA region of the pathogen isolates associated with Fusarium wilt and anthracnose of cowpea and other *Fusarium* and *Colletotrichum* reference sequences were retrieved from NCBI Genbank database (USA) and compared. Multiple sequence alignment was done using ClustalW2 and phylogenetic analysis through Phylogeny.fr software (Dereeper *et al.,* 2008). A phylogeny tree was constructed using neighbor-joining (NJ) method. All traits had equal weight and gaps were treated as 'missing' values. Transitions and transversions were included in the analysis. The branch support of the trees resulting from the neighbor- joining (NJ) analysis was assessed by bootstrapping with 1000 replicates using the heuristic search option and indicated at the nodes as percentage.

3.4.4. Identification of Pathogens

The identification of the pathogen isolates associated with Fusarium wilt and anthracnose of cowpea was done based on the morphological, cultural and molecular characters. The identity of the species was further confirmed through ITS- rDNA sequence analyses.

3.5. PATHOGENICITY TESTS

3.5.1. Fusarium wilt of Cowpea

The pathogenicity of different isolates of the pathogen was proved following Koch's postulates. Healthy cowpea seedlings were planted in paper cups filled with sterile soil, artificially infected by pouring 50 ml conidial suspension (1×10^7 cfu ml^{-1}) of each isolate of the pathogen associated with Fusarium wilt of cowpea

into each cup. The trial had three replicates. Sterilized soil watered with 50 ml of sterile water per cup was used as the control. The isolates capable of producing wilt and the number of days taken for the initiation of symptoms were recorded. On the basis of initiation of wilt after inoculation, virulence rating of the pathogen isolates was done and the most virulent isolate of the pathogen was selected for further studies (Jasnic *et al.,* 2005).

3.5.2. Anthracnose of Cowpea

Artificial inoculation of the fungus was carried out to prove the pathogenicity. The cowpea seedlings were raised in plastic cups and inoculated with a conidial suspension (1×10^6 cfu ml^{-1}) of each pathogen isolate or with sterile water by spraying the foliage and incubated in growth chamber to maintain high relative humidity (90 %). Three replications were maintained. The inoculated plants were monitored for expression of symptoms which appeared four to five days after incubation. The fungus was re-isolated from infected leaf of the plant and was compared with original culture for confirmation. On the basis of initiation of symptoms, virulence rating of the pathogen isolates was done and the most virulent isolate of the pathogen was selected for further studies (Ansari *et al.,* 2004).

3.6. *IN VITRO* EVALUATION OF FUNGICIDES AGAINST PATHOGENS ASSOCIATED WITH FUSARIUM WILT AND ANTHRACNOSE OF COWPEA

Twelve selected fungicides (Appendix III) *viz.,* propiconazole (0.1 %), chlorothalonil (0.2 %), thiophanate-methyl (0.1 %), flusilazole (0.1 %), azoxystrobin (0.15 %), tebuconazole (0.1 %), captan + hexaconazole (0.1 %), carboxin+thiram (0.4 %), copper hydroxide (0.25 %), mancozeb (0.25 %), carbendazim (0.1 %) and copper oxychloride (0.2 %) were evaluated *in vitro* against pathogens associated with Fusarium wilt and anthracnose of cowpea*,* by poisoned food technique (Nene and Thapliyal, 1993).

The required quantity of fungicide was thoroughly mixed with 50 ml of sterile water in a 250 ml conical flask. The fungicidal suspension was poured into another 250 ml conical flask containing 50 ml of double strength melted PDA and mixed thoroughly. The medium was poured into sterile petridishes. After solidification of the medium each plate was inoculated at the centre with a four mm agar disc of the test pathogen. Plates containing non poisoned media served as control. Three replications each were maintained for each treatment. The plates were incubated at room temperature and linear growth of the pathogen was recorded. Per cent inhibition of mycelial growth of the test pathogen over control was calculated using the formula described by Vincent (1927).

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

where,

I = Per cent inhibition

C = Growth of the pathogen in control plate (cm)

T = Growth of the pathogen in test plate (cm)

3.7. COMPATIBILITY STUDIES OF FUNGICIDES WITH BENEFICIAL MICROBES

The experiment was conducted to test the compatibility of 12 selected fungicides *viz.,* propiconazole (0.1 %), chlorothalonil (0.2 %), thiophanate-methyl (0.1 %), flusilazole (0.1 %), azoxystrobin (0.15 %), tebuconazole (0.1 %), captan + hexaconazole (0.1 %), carboxin + thiram (0.4 %), copper hydroxide (0.25 %), mancozeb (0.25 %), carbendazim (0.1 %) and copper oxychloride (0.2 %) with biocontrol agents (*Trichoderma viride* and *Pseudomonas fluorescens*) and root nodule bacterium (*Rhizobium* spp.).

3.7.1. Compatibility of Fungicides with *T. viride*

The compatibility of fungicides with *T. viride* was tested by poisoned food technique as described in 3.6. Observation on the per cent suppression of the mycelial growth of *T. viride* over control was noted.

3.7.2. Compatibility of Fungicides with *P. fluorescens*

The compatibility of fungicides with *P. fluorescens* was tested by the method of Bandopadhyaya *et al*. (1979). Sterile filter paper discs of 10 mm diameter were dipped in appropriate concentrations of fungicides as listed under 3.8. Kings'B medium (Appendix I) was melted and allowed to cool to 40^0C. This was seeded with 48 h old culture of *P. fluorescens*. Medium was poured into sterile petridishes and allowed to solidify. A filter paper disc dipped in appropriate fungicidal solution was placed at the centre of the dish. Plates having filter paper discs dipped in sterile water served as control. Three replications were maintained for each treatment. Observations on the zone of growth inhibition were noted after 48 h of incubation at room temperature.

3.7.3. Compatibility of Fungicides with *Rhizobium* spp.

The compatibility of fungicides with *Rhizobium* spp. was tested as described under 3.8.2 using Nutrient Agar medium (Appendix I).

3.8. *IN VIVO* EVALUATION OF FUNGICIDES AGAINST PATHOGENS ASSOCIATED WITH FUSARIUM WILT AND ANTHRACNOSE OF COWPEA

Two pot experiments were laid out to find the efficacy of 12 selected fungicides against pathogens associated with Fusarium wilt and anthracnose of cowpea. The details of experiments are as follows: Design – CRD, Treatments - 13, Replications – 5 and Variety – Malika. The treatment details for the experiments were as follows: T_1 – Propiconazole (0.1 %), T_2 – Chlorothalonil (0.1 %), T_3 – Thiophanate- methyl (0.1 %), T_4 – Flusilazole (0.1 %), T_5 –

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Azoxystrobin (0.15 %), T_6 – Tebuconazole (0.1 %), T_7 – Captan + Hexaconazole (500 g/ha), T_8 – Carboxin + Thiram (4 g/kg seed), T_9 – Copper hydroxide (0.25 %), T_{10} – Mancozeb (0.25 %), T_{11} – Carbendazim (0.1%), T_{12} – Copper oxychloride (0.2 %), T_{13} – Control.

The potting mixture consisting of sand: soil: cowdung @ 1:1:1 was prepared and fumigated with 5 % formaldehyde solution. Sterilized potting mixture was filled in earthen pots of size 12″ x 12″. Cowpea seeds of the variety Malika were sown in the pots. The plants were maintained as per the Package of Practice Recommendations: Crops (KAU, 2011) by giving timely application of fertilizers and adopting need based plant protection measures. Artificial inoculation of the plants was done using mass multiplied pathogen inoculum. Three rounds of fungicide application were done at 30, 45 and 60 days after seed emergence either as soil drenching or as foliar spray.

Onset of wilt and anthracnose were recorded. Disease incidence and index were worked out as described in 3.1.1 and 3.1.2. Biometric observations such as plant height, root length, fresh and dry weight of shoots and roots, number of pods, pod yield and number of root nodules were recorded at harvest.

3.8.1. Mass Multiplication and Inoculation of *Fusarium* spp.

Fusarium was mass multiplied on sand – maize mixture using the modified method of Lewis and Papavizas (1984). Maize was mixed with sand in the ratio 1:9. The mixture was moistened with sufficient water enough to promote fungal growth. This mixture was taken in 1000 ml conical flasks and autoclaved at 1.05 kg/cm^2 pressure for two hours. After sterilization, actively growing fungal disc of *Fusarium* spp. was aseptically transferred into the flasks and incubated at room temperature for 15 days to promote fungal growth. The mass multiplied pathogen inoculum (23 x 10^7 cfu g^{-1}) was applied at the root zone of the plant and thoroughly incorporated into the soil 15 days after seed emergence.

3.8.2. Mass Multiplication and Inoculation of *Colletotrichum* spp.

Colletotrichum spp. was mass multiplied on Potato Dextrose broth and the conidial suspension (1 x 10^6 cfu ml^{-1}) was sprayed on to the leaves 15 days after seed emergence.

3.9. INTEGRATED MANAGEMENT OF FUSARIUM WILT AND ANTHRACNOSE OF VEGETABLE COWPEA USING FUNGICIDES, BIO AGENTS AND ECO FRIENDLY METHODS

Based on the results of pot experiments three effective fungicides with least disease incidence and severity along with bio agents and eco friendly methods were selected to evaluate under field conditions to determine their efficacy in the management of Fusarium wilt and anthracnose of cowpea. The details of experiment are as follows: Design – RBD, Treatments – 9, Replications – 3, Variety – Malika, Location – Instructional Farm, College of Agriculture, Vellayani, Plot size – 6.75 m^2 and No. of observational plants/plot – 4.

The treatment details for the experiment were as follows: T_1 – Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + flusilazole (0.1 %), T_2 – Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + tebuconazole (0.1%), T_3 – Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + carbendazim (0.1%), T_4 – Soil solarization + *Trichoderma* enriched neem cake organic manure mixture, T_5 – Flusilazole (0.1 %), T_6 –Tebuconazole (0.1%), T_7 – Carbendazim (0.1%), T_8 – Copper oxychloride (0.2 %) (Chemical check), T_9 – Control. Seed treatment was done uniformly for all the treatments with carbendazim (@ 2 g/kg seed).

All standard and recommended Package of Practices (KAU, 2011) was followed for the cultivation of crop. Lime application was done for all the treatments at the time of field preparation. Fungicide application was done thrice at 30, 45 and 60 days after seed emergence either as soil drenching or as foliar spray. Biometric observations such as shoot length, root length, fresh and dry

weight of shoots and roots, number of pods, pod yield and number of root nodules were recorded at the time of harvest of the crop. Onset of wilt and anthracnose were also recorded. Disease incidence and idex were worked out as described in 3.1.1 and 3.1.2.

3.9.1. Soil Solarization

Soil solarization was carried out in four treatments arranged in a randomized block design with three replications, for a period of six weeks from mid August till the end of September, 2013. The experimental site was incorporated with required quantity of organic manure in the soil, irrigated, ploughed and levelled prior to imposing solarization treatment. Later, the plots were covered with transparent polythene sheets of 120 gauge thickness. The edges of the sheet were sealed with soil to keep it in position and also to maintain the temperature and moisture inside the polythene mulch. Adequate care was taken to keep the sheet in close contact with the surface of soil to prevent the formation of air pockets between the soil and the polythene sheet. Soil temperature during solarization was recorded at 1400 h at a depth of 10 cm using soil thermometer. After the period of solarization, the sheet was removed and sowing was done (Katan, 1981).

3.9.2. Preparation and Application of *Trichoderma* Enriched Neem cake Organic manure Mixture

Dry neem cake and cowdung was powdered and mixed in the ratio 1: 9 to get a coarse texture and was moistened by sprinkling water. The commercial preparation of *Trichoderma* spp. was added @ one kg/100 kg of neem cake cowdung mixture. After thorough mixing, it was covered with a perforated polythene sheet and kept in shade for 10 days for multiplication. Again it was mixed well and kept for five more days for further multiplication. Later the preparation was applied at the root zone of the plant @ one kg/pit and thoroughly incorporated into the soil (KAU, 2011).

3.10. PERSISTENCE AND DEGRADATION KINETICS OF FUNGICIDE RESIDUES

The studies on persistence and degradation of fungicide residues were done in the Pesticide Residue Research and Analytical Laboratory of the All India Network Project on Pesticide Residues, KAU Centre, College of Agriculture, Vellayani.

3.10.1. Glass wares and Reagents

The following glass wares, reagents and equipments were used for the study.

Laboratory glass wares	Chemical reagents	Equipments
Centrifuge tubes 15 ml and 50 ml	Acetonitrile (HPLC grade)	Analytical balance
Micropipette 100 µl, 1 ml and 5 ml	Magnesium sulphate (anhydrous) (GR grade)	Vortex shaker
Turbovap tubes 20 ml and 30 ml	Sodium chloride (GR grade)	Turbovap LV
Graduated test tubes 5 ml and 10 ml	n-Hexane (HPLC grade)	Laboratory centrifuge
Micro syringe 10 µl and 500 µl	Acetone (HPLC grade)	Mechanical shaker
Conical flasks 250 ml	Sodium sulphate (anhydrous) (GR grade)	Rotary vacuum flash evaporator
Beakers 100, 250, 500 ml	Primary Secondary Amine	Hot air oven

	(PSA)	
Standard flasks 10 ml, 25 ml, 50 ml, 100 ml	-	UPLC-MS/MS system: AB Sciex API 3200 mass spectrometer with Waters Acquity UPLC system

The glass wares were initially washed with clean tap water followed by washing with one per cent laboline and again with tap water and distilled water followed by rinsing with acetone and kept inverted at room temperature for drying. Fully dried glasswares were kept in hot air oven at 50^0C. Syringes were thoroughly rinsed with acetone followed by hexane. The solvents were distilled with glass distillation apparatus. Sodium sulphate was pre washed with acetone, dried at room temperature, activated in an oven at 110^0C for three hours and stored under moisture free condition.

3.10.2. Preparation of Standard Fungicide Mixtures

Certified reference materials of different fungicides *viz.,* flusilazole, tebuconazole and carbendazim were obtained from Bayer Crop Science Ltd., Mumbai. Stock solutions (1000 µg ml^{-1}) of the fungicides were prepared by dissolving a weighed quantity of the analytical grade material in HPLC grade methanol. The stock solutions were serially diluted to prepare an intermediate stock of 100 µg ml^{-1}. The intermediate stock solutions were further diluted with HPLC grade methanol to prepare working standard mixtures (10 µg ml^{-1}) of the fungicides to be analyzed by positive electro spray ionization. The working standard mixtures were then serially diluted to obtain 1.00, 0.50, 0.25, 0.10, 0.075, 0.05, 0.025, 0.01 and 0.005 µg ml^{-1} concentrations of analytical grade fungicides.

3.10.3. Determination of Limit of Detection (LOD)

Ten µl of the working standards of 1.00, 0.50, 0.25, 0.10, 0.075, 0.05, 0.025, 0.01 and 0.005 µg ml^{-1} concentrations were injected under set standard UPLC-MS/MS conditions. Each standard was injected thrice and the limit of detection of the instrument of each fungicide was calculated based on the lowest quantity of the pesticide standard that could be identified under standard UPLC-MS/MS conditions. The lowest concentration for which a signal to noise (S/N) ratio greater than three was considered as LOD of the particular compound.

3.10.4. Preparation of Calibration Curve

A calibration curve (linearity response line) was prepared by plotting concentration vs. peak area.

3.10.5. Dissipation of Fungicides

3.10.5.1. Sampling

Mature pods of cowpea were collected from each plot two hours, one, three, five, seven, fifteen and twenty one days after the third application of fungicides which was done at 60 days after seed emergence. Samples were brought to the laboratory in polythene bags and processed immediately for residue analysis.

3.10.5.2. Residue Extraction

The Multiresidue estimation procedure recommended for fruits and vegetables as per QuEChERS method (Anastassiades *et al.,* 2003) with suitable modifications was adopted for residue extraction and clean up in cowpea. The harvested fruits were macerated as such in a high-speed blender (BLIXER 6 vv Robot Coupe) for three min and a representative sample of 25 g of ground fruits was taken in a 50 ml centrifuge tube. HPLC grade acetonitrile (50 ml) was added to the samples and homogenized with a high speed tissue homogenizer (Heidolph Silent Crusher- M) at 14000 rpm for three min. This was followed by the addition of 10 g activated sodium chloride (NaCl) and shook for two min for separation of

the acetonitrile layer. The samples were then centrifuged at 2500 rpm for four min and 16 ml of the clear upper layer was transferred into 50 ml centrifuge tubes containing six grams pre activated sodium sulphate and vortexed for two min. The acetonitrile extracts were subjected to clean up dispersive solid phase extraction (DSPE). For this, 12 ml of the upper layer was transferred into centrifuge tubes (15 ml) containing 0.2 g PSA and 1.2 g anhydrous magnesium sulphate. The mixtures were then shaken in vortex for 30 sec and again centrifuged for three min at 2500 rpm. The supernatant liquids (five ml each) were transferred to turbovap tube and evaporated to dryness under a gentle stream of nitrogen using a Turbovap set at 45^0C and 7.5 psi nitrogen flow. The residues were reconstituted in two ml of methanol and filtered through a 0.2 micron filter prior to LC-MS/MS.

3.10.5.3. Residue Estimation

The chromatographic separation was achieved using Waters Acquity UPLC system equipped with a reversed phase Atlantis dC-18 (2.1 X100 mm, five micron particle size) column. A gradient system involving the following two elute components: A: 10 % methanol in water + 0.1 % formic acid + 50 mM ammonium acetate; B: 10 % water in methanol + 0.1 % formic acid + 50 mM ammonium acetate was used as mobile phase for the separation residues. The gradient elution was as follows; 0 min isocratic 20 % B, 0.0 - 4.0 min linear from 20 % to 90 % B, 4.0 - 5.0 min linear from 90 % to 95 % B and 5 - 9.0 min linear from 95% to 100 % B, with 9.0-10.00 min for initial conditions of 20 % B. The flow remained constant at 0.8 ml/min and injection volume was 10 µl. The column temperature was maintained at 40^0C. The effluent from the LC system was introduced into Triple quadrapole API 3200 MS/MS system equipped with an electrospray ionization interface (ESI), operating in the positive ion mode. The source parameters were temperature 550^0C; ion gas (GSI) 50 psi, ion gas (GS2) 50 psi, ion spray voltage 5,500 V, curtain gas 13 psi. Under these operating conditions, the retention time of carbendazim, flusilazole and tebuconazole was found to be 0.943, 3.79 and 3.92 min respectively.

3.10.5.4. Residue Quantification

Based on the peak area of the chromatogram obtained for various fungicides, the quantity of residue was determined as detailed below.

Residue (mg/kg) = Concentration obtained from chromatogram by using the calibration curve x Dilution factor

$$\text{Dilution factor} = \frac{\text{Volume of the solvent added}}{\text{Weight of sample (g)}} \times \frac{\text{Final volume of the extract}}{\text{Volume of extract taken for concentration}}$$

The persistence of fungicides was expressed in terms of half life (DT_{50}) i.e., time for the disappearance of fungicide to 50 % of its initial concentration. The half life as well as the time required to reach below tolerance level (T_{tol}) were calculated using Hoskin's formula (Hoskin, 1961).

3.11. BENEFIT: COST RATIO

The weight of fruits harvested were recorded and expressed as kg/plant and converted to t/ha. The parameters *viz.,* increase in yield over control, additional monetary returns over control and additional cost of plant protection and integrated disease management measures were calculated to compute the benefit cost ratio. The benefit cost ratio for each treatment was obtained by dividing additional monetary benefits by additional cost incurred on each treatment.

3.12. STATISTICAL ANALYSIS

Analysis of variance was done on the data collected using the statistical package MSTAT (Freed, 1986). Multiple comparisons among the treatment means were done using DMRT.

Results

4. RESULTS

The experimental results obtained from the study entitled "Integrated management of Fusarium wilt and anthracnose of vegetable cowpea (*Vigna unguiculata* subsp. s*esquipedalis* (L.) Verdcourt) using new generation fungicides" are presented below.

4.1. SURVEY ON INCIDENCE, SEVERITY (DISEASE INDEX) AND EXTENT OF YIELD LOSS DUE TO MAJOR FUNGAL DISEASES IN COWPEA

A survey was conducted in the predominant cowpea growing areas of Thiruvananthapuram district during 2011 - 2012 for assessing the incidence, severity (disease index) and extent of yield loss due to major fungal diseases in cowpea (Figure 3, 4 & 5).

The results revealed that the incidence and index of Fusarium wilt varied from 0 – 20 % and 0 - 20 respectively in the different areas surveyed. The highest incidence (20.00 %) and index (20.00) of the disease was recorded at Palapoor and Pappanchani areas whereas, the incidence of Fusarium wilt was absent in Vizhinjam and Kottukal.

The incidence and index of anthracnose ranged from 0 – 55 % and 0 - 33.33 respectively. Maximum incidence (55.00 %) was observed at Kalliyoor followed by Kattakada (45.00 %) whereas, the incidence of anthracnose was absent in Muttacaud, Venniyoor, Vizhinjam and Kottukal. Disease index was maximum (33.33) at Pothencode followed by Kalliyoor (31.70) and least (8.30) at Palapoor and Pappanchani.

The incidence of web blight and collar rot was noticed at Vizhinjam (10.00 %), Balaramapuram (10.00 %) and Palapoor (8.00 %), Kottukal (5.00 %) and Pappanchani (5.00 %). The index of web blight ranged between 0 - 10.33 in the areas surveyed.

The incidence and index of Cercospora leaf spot in cowpea varied from 0 - 37 % and 0 - 14.16 respectively. The maximum incidence was recorded at Palapoor, Pappanchani and Kalliyoor areas whereas incidence was absent in Vizhinjam, Muttacaud, Kottukal, Kazhakkuttom and Venniyoor. Disease index was highest (14.16) at Kalliyoor and least (5.33) at Balaramapuram.

Negligible incidence of powdery mildew and rust was observed at Balaramapuram and Palapoor respectively during the course of survey.

Among the six diseases, considerable mean yield losses were noticed due to Fusarium wilt (7.05 %) and anthracnose (3.92 %) of cowpea in the different areas surveyed.

4.2. SYMPTOMATOLOGY STUDIES

4.2.1. Fusarium wilt of Cowpea

The affected plants showed yellowing, withering and drooping of leaves followed by defoliation. The vines showed blackening and drying. The diseased plants became stunted and weak. The base of the stem became swollen and resembled a small tuber which later disintegrated resulting in shredding. The taproot and lateral roots were also affected. When the main stem, primary branches and roots were split open, internal browning of conducting tissues and white mycelial growth of the pathogen became visible. Occasionally, abnormal flattening of the stem along the growing tip was noticed. The flowers showed fasciation and sterility resulting in severe yield reduction (Plate 7).

4.2.2. Anthracnose of Cowpea

The initial symptoms appeared as minute, circular to irregular spots on the leaves which later increased in size and turned light brown to dark brown in color. In the advanced stages of disease development, they coalesced together to form large, necrotic spots on the leaves with shot holes (Plate 8). On the stem and vines, symptom appeared as spindle shaped lesions with light grey centre and

a. Foliar yellowing

b. Defoliation

c. Drying up of vines

d. Basal stem swelling

e. Shredding

f. Damage of tap roots and lateral roots

g. Damage of conducting tissues

Plate 7. Symptoms of Fusarium wilt of cowpea

a. Leaf spots

b. Lesions on midrib

c. Lesions on pods

d. Spindle shaped lesions on vines

e. Drying up of vines

Plate 8. Symptoms of anthracnose of cowpea

reddish brown margin which enlarged up to 10 - 12 mm in length. In the later stages, small irregular deep seated reddish brown spots appeared on the pods also.

4.3. ISOLATION OF PATHOGENS

4.3.1. Isolation of Pathogen Associated with Fusarium wilt of Cowpea

The pathogen causing Fusarium wilt in cowpea was isolated from infected root and stem showing typical symptoms and purified by single spore isolation technique. A total of 12 isolates of *Fusarium* spp. were obtained from infected plants collected from major cowpea growing areas of Thiruvananthapuram and were designated as F1 to F12. The isolates were subcultured periodically on PDA slants and stored in refrigerator at 4°C. The list of *Fusarium* isolates obtained and their respective locations are presented in Table 1.

4.3.2. Isolation of Pathogen Associated with Anthracnose of Cowpea

The isolation of pathogen associated with anthracnose of cowpea was made from infected leaves and twigs, sub cultured periodically and kept in a low temperature for further studies. Eight isolates of *Colletotrichum* spp. were obtained from various locations which were serially numbered from C1 to C8 (Table 2).

4.4. CHARACTERIZATION OF PATHOGENS

4.4.1. Morphological and Cultural Characterization of *Fusarium* spp.

The 12 isolates obtained were cultured on PDA for their morphological and cultural characterization (Plate 9) based on standard keys (Booth, 1971) and the results are presented in Table 3.

The isolates F1, F2, F3, F4, F6, F8 and F9 had medium growth rate of 0.90 – 1.42 cm/day. Colonies were appressed to floccose in texture, white on the upper surface, reddish brown or faint pink on the lower side of the petridish. Conidiophores consisted of single phialides, arising laterally on the hyphae or

Table 1. *Fusarium* isolates obtained and their respective locations

Sl. No.	Isolate	Location
1	F1	Vellayani
2	F2	Palapoor
3	F3	Pappanchani
4	F4	Pappanchani
5	F5	Venniyoor
6	F6	Balaramapuram
7	F7	Muttacaud
8	F8	Thazhava
9	F9	Pothencode
10	F10	Kattakada
11	F11	Kazhakkuttom
12	F12	Kalliyoor

Table 2. *Colletotrichum* isolates obtained and their respective locations

Sl. No.	Isolate	Location
1	C1	Vellayani
2	C2	Pappanchani
3	C3	Kalliyoor
4	C4	Balaramapuram
5	C5	Pothencode
6	C6	Azhur
7	C7	Kattakada
8	C8	Kulathoor

short branched conidiophores. Single, sub cylindrical to slightly obclavate phialides arose from primary or secondary conidiophores. Macroconidia were 3 -5 septate, 4.6 - 7.4 x 1.4 - 2.7 µm in size, thin walled, fusoid, pointed ends, occasionally falcate with terminal cell, hooked and pedicellate basal cell. Microconidia were abundant, oval to ellipsoidal, cylindrical, straight or curved. Chlamydospores when present were terminal in position.

The isolates F7, F11 and F12 had medium growth rate of 0.86 - 1.05 cm/day. Colonies appeared floccose in texture, dull white on the upper surface and yellowish to dark brown on the lower side of the petridish. Conidiophores consisted of single lateral phialides in aerial mycelium. Phialides were short, compact and obclavate. Macroconidia were mostly 3 - 5 septate, 14.8 - 17.2 x 2.2 - 3µm in size, thick walled, typically falcate, tapering towards both ends, bent at central part with elongated apical cell and pedicellate basal cell. Microconidia were oval to ellipsoidal in shape. Chlamydospores when present were intercalary, solitary or in chains.

The isolates F5 and F10 had a fast growth rate of 1.3 and 1.04 cm/day respectively. Colonies appeared sparse with creamy white upper surface and dirty white on the lower side of the petridish. The primary conidiophores arose laterally from hyphae on aerial mycelium which was unbranched or sparsely branched. Short, subcylindrical, obclavate monophialides produced slender macroconidia and long microconidia. Macroconidia were 3 - 5 septate, thick walled, subcylindrical, slightly curved, short and bent apically, indistinctly pedicellate basal cell and measured 7.9 - 10.2 x 1.8 - 2.6 µm in size. Microconidia were ovoid and straight or rarely ellipsoidal to curved. Chlamydospores were abundant, terminal or intercalary in position formed in single, pairs or chains.

Table 3. Morphological and cultural characters of *Fusarium* isolates inciting wilt of cowpea

Isolate No.	No. of days taken to cover 9 cm petridish	Average growth rate (cm/day)	Colony texture	Colony color		Conidiophore arrangement			Phialides	
				Upper	Lower	Single/ group	Loose/ compact	Branching	Number	Shape
F1	10	0.9	Appressed	Dull white	Dark brown	Single	Loose	Lateral	Mono/poly	Cylindrical
F2	8	1.16	Appressed	White	Faint pink	Single/group	Loose	Lateral	Mono	Sub cylindrical
F3	7	1.39	Appressed	White	Faint pink	Single	Loose	Lateral	Mono	Sub cylindrical
F4	6	1.42	Appressed	White	Reddish brown	Single	Loose	Lateral	Mono	Sub cylindrical
F5	7	1.3	Sparse	Creamy white	Dirty white	Single	Loose	Unbranched	Mono	Sub cylindrical
F6	7	1.33	Floccose	White	Brown	Single	Loose	Lateral	Mono	Obclavate
F7	9	1.05	Floccose	Dull white	Yellowish brown	Single	Compact	Lateral	Mono	Obclavate
F8	7	1.37	Floccose	White	Reddish brown	Single	Loose	Lateral	Mono	Sub cylindrical
F9	9	0.98	Floccose	White	Reddish brown	Single	Loose	Lateral	Mono	Sub cylindrical
F10	9	1.04	Sparse	Creamy white	Dirty white	Single	Loose	Unbranched	Mono	Sub cylindrical
F11	11	0.86	Floccose	Dull white	Yellowish brown	Single	Compact	Lateral	Mono	Obclavate
F12	9	1.02	Floccose	Dull white	Yellowish brown	Single	Compact	Lateral	Mono	Obclavate

Table 3. Continued..

Isolate No.	Macroconidia					Microconidia shape	Chlamydospores	
	Septation	Average length (µm)	Average breadth(µm)	Basal cell type	Shape		Position	Formation (single/chain/cluster)
F1	1-5	5.0	1.1	Not pedicellate	Sub cylindrical	Pear shaped	Not formed	-
F2	3	5.6	2.3	Pedicellate	Falcate	Oval	Not formed	-
F3	3	4.6	1.4	Pedicellate	Falcate	Oval	Not formed	-
F4	3-5	7.1	1.5	Pedicellate	Falcate	Ellipsoidal	Not formed	-
F5	3-5	10.2	2.6	Not pedicellate	Sub cylindrical	Oval	Terminal or intercalary	Single, pair or chains
F6	3	5.4	2.6	Pedicellate	Falcate	Oval	Not formed	-
F7	3-5	17.2	2.7	Pedicellate	Falcate	Oval/ ellipsoidal	Not formed	-
F8	3	5.5	1.9	Pedicellate	Falcate	Ellipsoidal	Not formed	-
F9	3	6.4	2.7	Pedicellate	Falcate	Ellipsoidal	Terminal	Single
F10	3-5	7.9	1.8	Not pedicellate	Sub cylindrical	Oval	Terminal or intercalary	Single, pair or chains
F11	3-5	15.4	3	Pedicellate	Falcate	Oval/ ellipsoidal	Not formed	-
F12	3-5	14.8	2.2	Pedicellate	Falcate	Oval/ ellipsoidal	Not formed	-

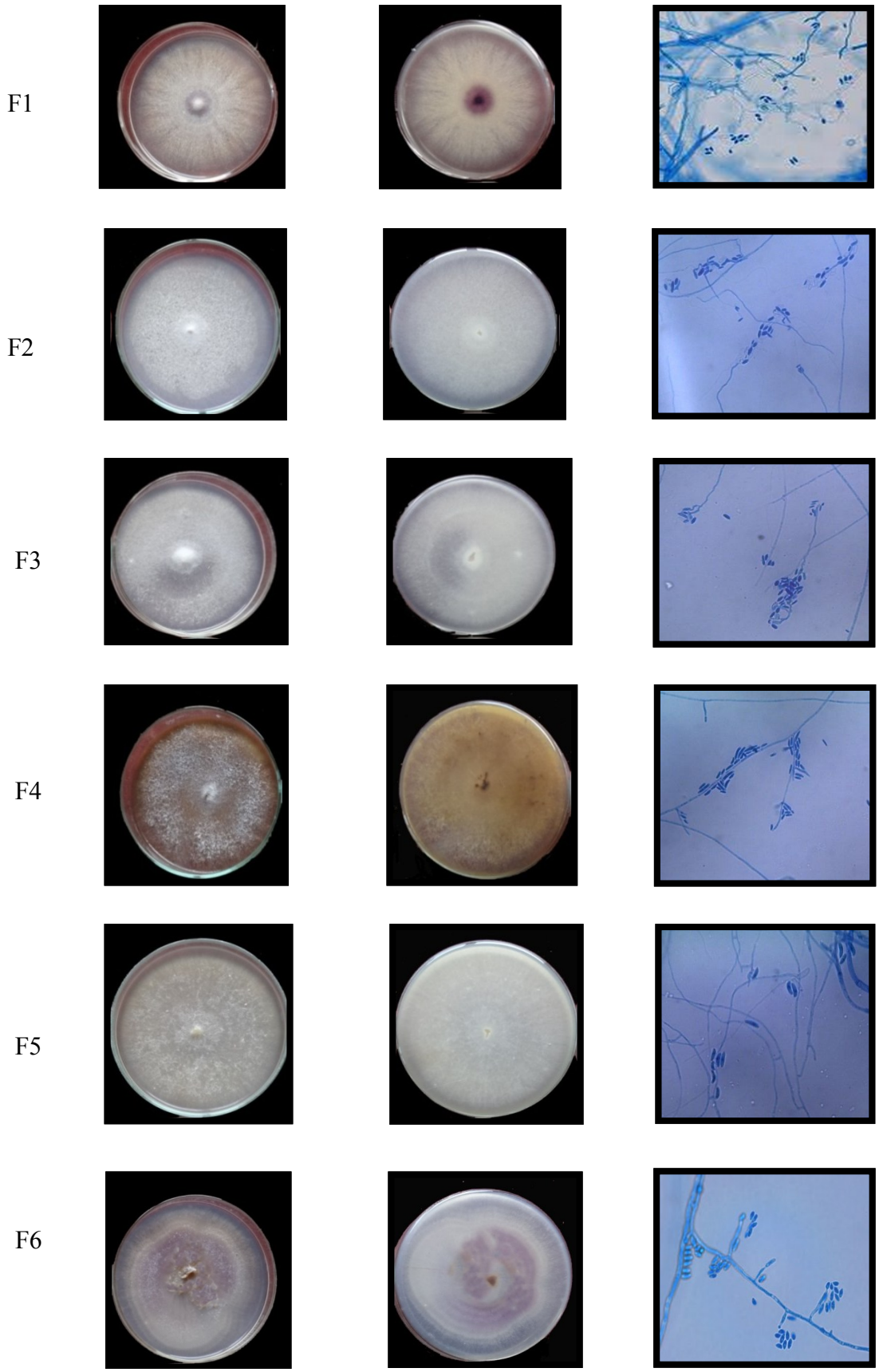
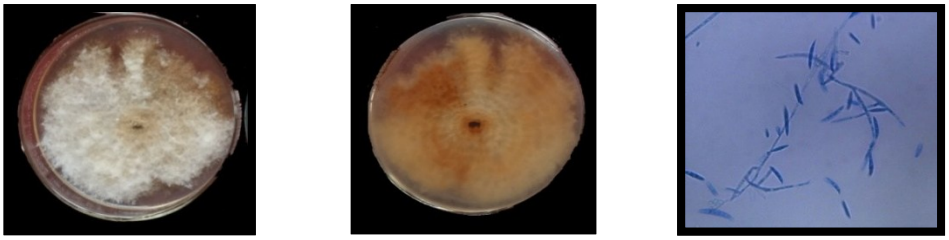


Plate 9. Morphological and cultural characters of *Fusarium* isolates a) upper and b) reverse of cultures on PDA c) conidia

F7



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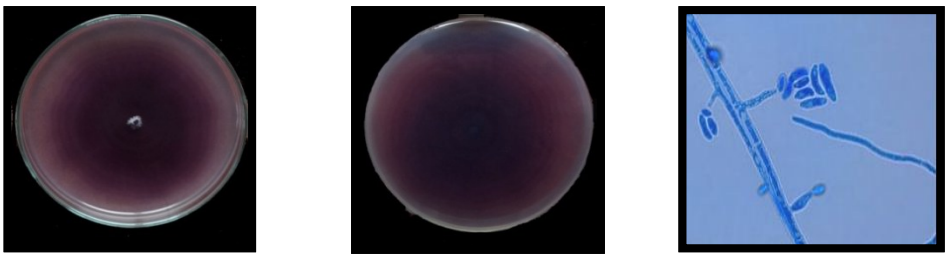

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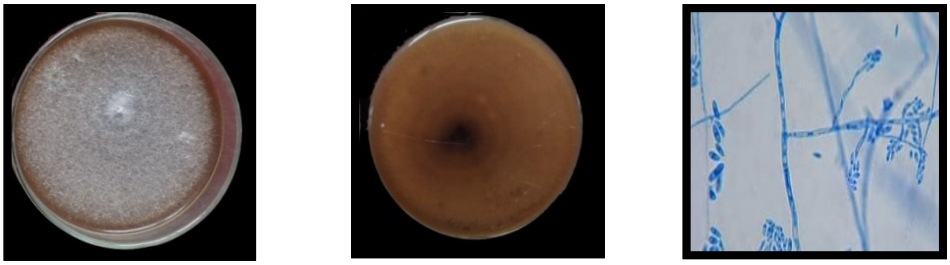
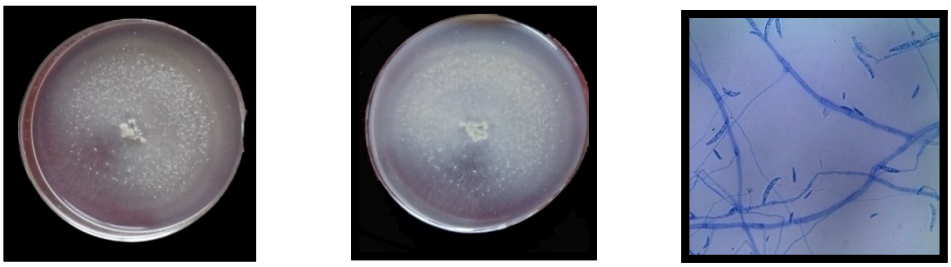


F10



F11

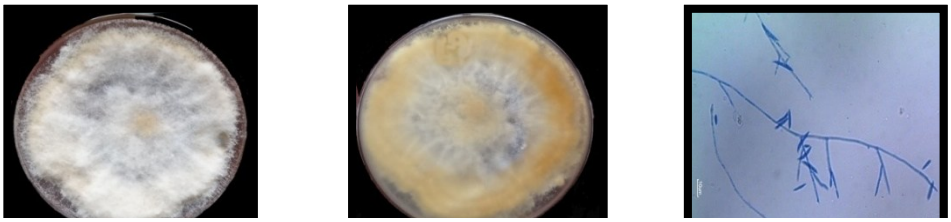
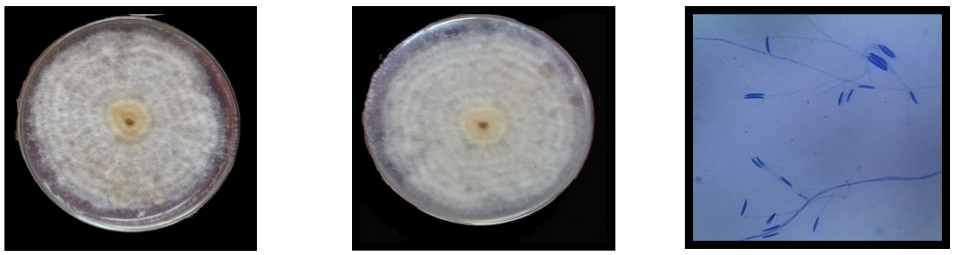


Plate 9. Morphological and cultural characters of *Fusarium* isolates a) upper

F12



4.4.2. Morphological and Cultural Characterization of *Colletotrichum* spp.

The morphological and cultural characters of the eight isolates of *Colletotrichum* spp. obtained (Plate 10) were studied based on standard keys (Chowdhry and Varshney, 2000) and the results are presented in Table.4. The isolates C1, C2, C3 and C5 had a fast growth rate of 1.21 - 1.39 cm/day. The colonies appeared sparse, initially white becoming grey, as the cultures aged on PDA. Colony reverse was dark greyish to black. Light orange slimy spore mass were produced outward from the centre of the colony. Black acervuli and setae were also observed. Dark structures, which resembled perithecia were often observed. The mycelia were branched, septate and hyaline. Conidia were hyaline, cylindrical with round ends, slightly flattened and 9.0 - 11.3 x 3.7 - 4.3 µm in size. Appressoria were observed on the underside of sterile cover slips arising from vegetative hyphae. They were irregular in shape and varied from light to dark brown in color.

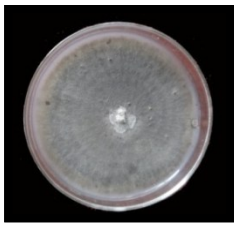
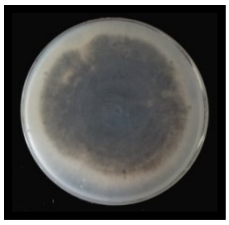
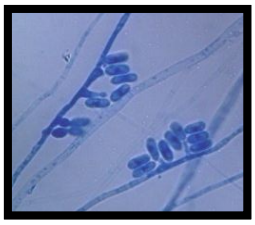
The isolates C7 and C8 had a fast growth rate of 1.35 and 1.30 cm/day respectively. The colonies appeared dense, white turning to grey, while the reverse side of the colonies was of dark grey, with regular margins. Bright orange colored slimy conidial mass were produced near the inoculation point. Black acervuli and abundant setae were observed. Perithecia appeared scattered and globose. Mycelium appeared hyaline, septate and branched. The conidia were cylindrical with both apices rounded or with one apex rounded and the other end pointed. The conidial size varied from 9.5 - 9.6 x 3.5 - 3.8 µm in size. Most of the appressoria formed in slide cultures were irregularly shaped and only a few were ovoid.

The isolate C4 had a mean growth rate of 1.04 cm/day. The colonies showed dense, fluffy, off-white lacking acervuli and setae. The reverse side of the colony was greyish. Sparse sporulation was noticed. The sexual fruiting bodies or perithecia were not noticed. The mycelia were hyaline, branched and septate.

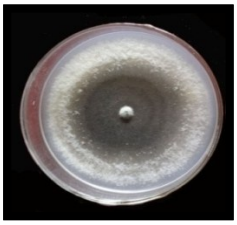
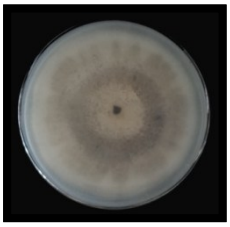
Table 4. Morphological and cultural characters of Colletotrichum isolates inciting anthracnose of cowpea

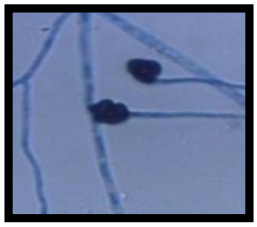
Isolate No.	No. of days taken to cover 9 cm petridish	Average growth rate (cm/day)	Colony texture	Colony color		Acervulate (Yes/No)	Setae	Perithecia	Conidia			Shape of appressoria
				Upper	Lower				Shape	Average length (µm)	Average breadth(µm)	
C1	6	1.38	Sparse	Initially white turning to grey	Dark greyish to black	Yes	Present	Scattered and globose	Cylindrical with round apices	9.8	4.3	Irregular
C2	7	1.21	Sparse	Initially white turning to grey	Dark greyish to black	Yes	Present	Not formed	Cylindrical with round apices	10.3	3.8	Irregular
C3	6	1.38	Sparse	Initially white turning to grey	Dark greyish to black	Yes	Present	Scattered and globose	Cylindrical with round apices	9.0	4.2	Irregular
C4	9	1.04	Dense	Off-white	Grey	No	Absent	Not formed	Straight obtuse apex	8.6	3.6	Clavate/Irregular
C5	6	1.39	Sparse	Initially white turning to grey	Dark greyish to black	Yes	Present	Scattered and globose	Cylindrical with round apices	11.3	3.7	Irregular
C6	10	0.80	Dense	Off-white	Grey	No	Absent	Not formed	Cylindrical with a pointed end	8.8	3.7	Clavate
C7	7	1.35	Dense	White turning to grey	Dark grey	Yes	Present	Scattered and globose	Cylindrical	9.5	3.5	Irregular/ovoid
C8	7	1.30	Dense	White turning to grey	Dark grey	Yes	Present	Not formed	Cylindrical	9.6	3.8	Irregular/ovoid

C1

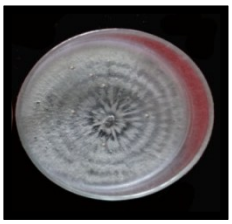
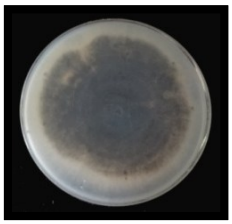
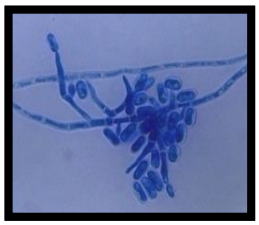
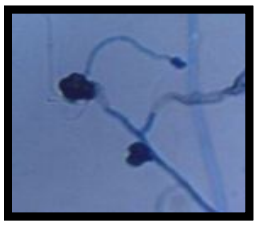





C2

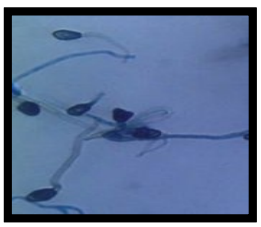


C3

C4



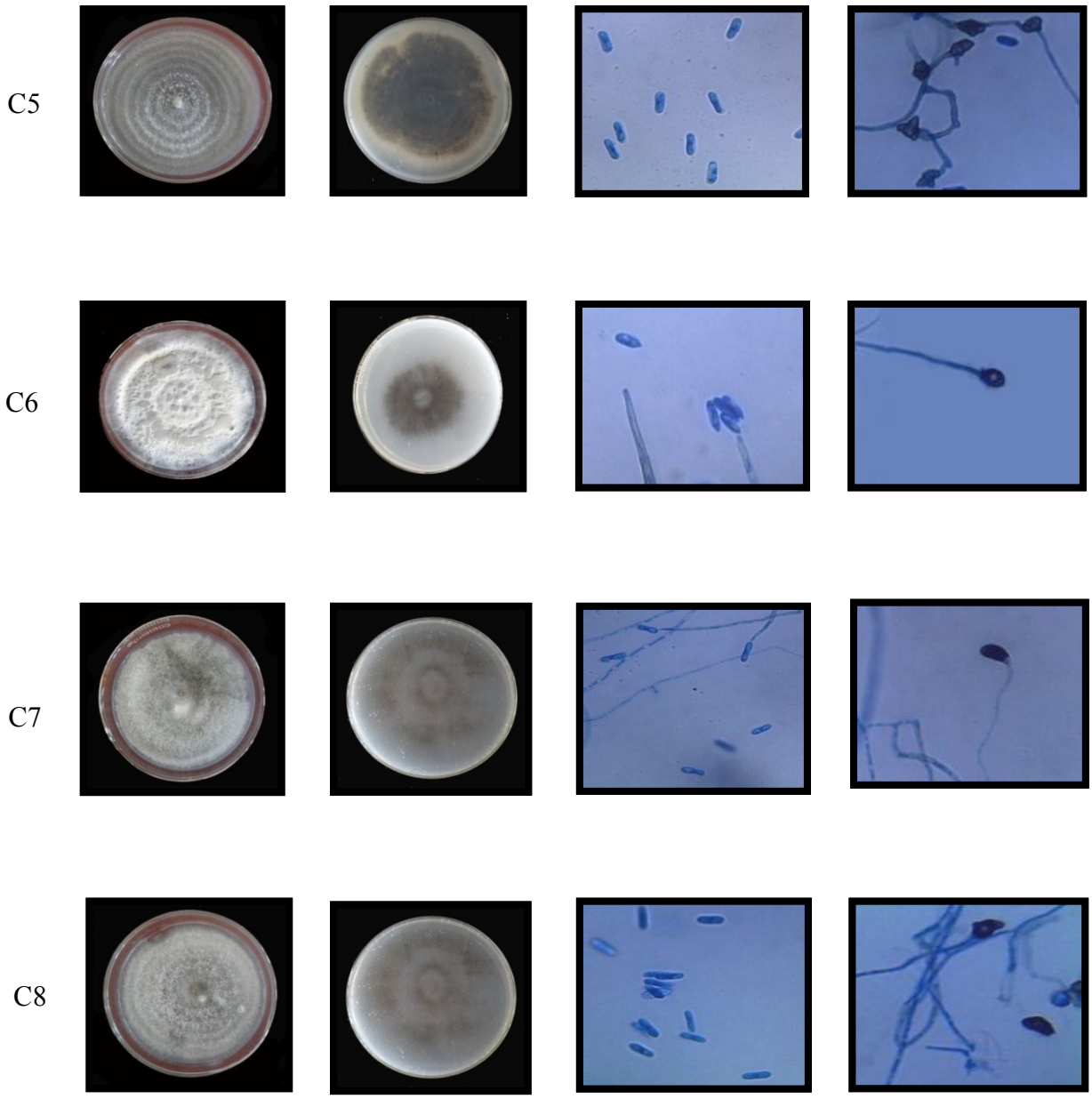


Plate 10. Morphological and cultural characters of *Colletotrichum* isolates a) upper and b) reverse of cultures on PDA c) conidia d) appressoria

Conidia produced were straight, obtuse at the apex and 8.6 x 3.6 µm in size. The flattened pressing organs or appressoria appeared clavate or irregular in shape.

The isolate C6 had a slow growth rate of 0.8 cm/day. The colonies appeared dense, fluffy and off-white, while the reverse side was greyish. Sporulation was sparse and acervuli and setae were lacking. Perithecia were not formed. Hyaline, septate and branched mycelia were observed. The conidia were straight, cylindrical with the apex rounded and the other end pointed and 8.8 x 3.7 µm in size. Most of the appressoria formed were clavate and melanized.

4.4.3. Molecular Characterization of the Pathogen Isolates by Partial Sequencing of Internal Transcribed Spacer (ITS) Region of rDNA

4.4.3.1. ITS-rDNA Sequencing of Isolates of Fusarium spp.

Amplification of the ITS- rDNA region of the 12 different isolates of *Fusarium* spp. using universal primers ITS 1 and ITS 4 yielded a PCR product of 500-530 bp long. Sequences of the isolates of *Fusarium* spp. (Appendix 1Va) were deposited in GenBank and used to search for similar sequences in NCBI database using BLAST program. An identity of 99 – 100 % was observed between the *Fusarium* isolates obtained in this study and other sequences of *Fusarium* spp. available in NCBI database. A comparison between the sequences available in GenBank and the representative isolates used in the study indicated that sequence similarity was not related to the geographical origin of the isolates. The PCR amplification profile of the ITS region of ribosomal DNA of *Fusarium* isolates is given in Plate 11. Multiple sequence alignment of the ITS- rDNA region of *Fusarium* spp. is given in Appendix Va.

A phylogenetic tree constructed from ITS- rDNA region sequences using neighbor-joining (NJ) method illustrated that *F. oxysporum*, *F. solani* and *F. equiseti* clearly formed four distinct clusters and this result was supported by a high bootstrap value. The tree was rooted with *Aspergillus oryzae* (EU680476.1) as an outgroup strain. Cluster I was pertaining to *F. oxysporum* isolates *viz.,* F2,

F3, F4, F6, F8 and F9 and four reference *F. oxysporum* isolates from GenBank; JN400710.1, HM756257.1, AY667489.1 and JQ954892.1 with 99 % bootstrap support. All the *F. equiseti* isolates *viz.,* F7, F11 and F12 together formed cluster II along with the reference isolate JQ690081.1 with high bootstrap support (100%). Cluster III included the *F. oxysporum* (F1) isolate along with the reference isolates from GenBank; KF534747.1 (*F. oxysporum*), KC859450.1 (*F. udum*) and JX177431.1 (*F. acuminatum*) with 93 % bootstrap support. While cluster IV included isolates F5 and F10 together with two reference *F. solani* isolates; JQ625562.1 and JX517202.1 with a high bootstrap support (100 %) (Figure 1).

4.4.3.2. ITS- rDNA Sequencing of Isolates of Colletotrichum spp.

The ITS- rDNA region of eight different isolates of *Colletotrichum* spp. was sequenced for the molecular characterization and identification of the pathogen. Amplification using primers ITS 1 and ITS 4 resulted in an amplicon of approximately 540 bp long (Plate. 12). Sequences of the eight isolates of *Colletotrichum* spp. (Appendix 1Va) were deposited in GenBank and used to search for similar sequences in NCBI database using BLAST program. Alignment of the sequences of representative isolates collected from different locations in this study with other known sequences of *Colletotrichum* spp. obtained from GenBank revealed that an identity of 99 – 100 % exist among the sequences. Multiple sequence alignment of the ITS- rDNA region of *Colletotrichum* spp. is given in Appendix Vb.

Phylogenetic analysis grouped the *Colletotrichum* isolates into three clusters with *Pyricularia grisea* (FN555114.1) as an outgroup strain. All *Colletotrichum* isolates grouped in cluster I included *C. gloeosporioides* isolates in this study *viz.,* C1, C2, C3 and C5 along with three reference isolates from Genbank; KJ002768.1 (*C. lindemuthianum*), JX669450.1 (*C. gloeosporioides*) and FJ172225.1 (*C. gloeosporioides*) with 50 % bootstrap support. The top of cluster II had three *C.gloeosporioides* isolates; C4, C7 and C8 grouped with reference isolates;

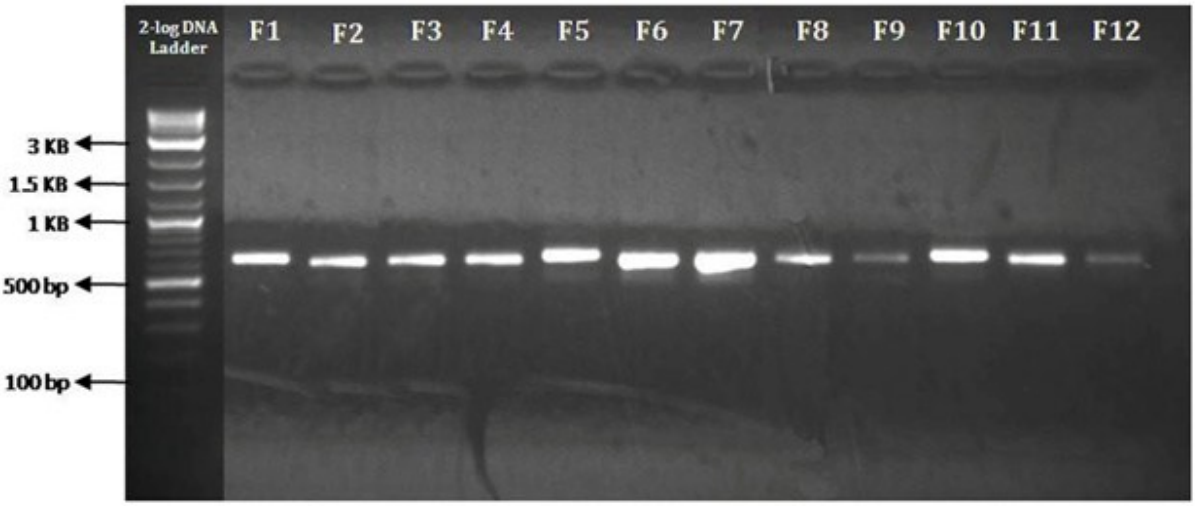


Plate 11. PCR amplification profile of the ITS- rDNA region of *Fusarium* isolates

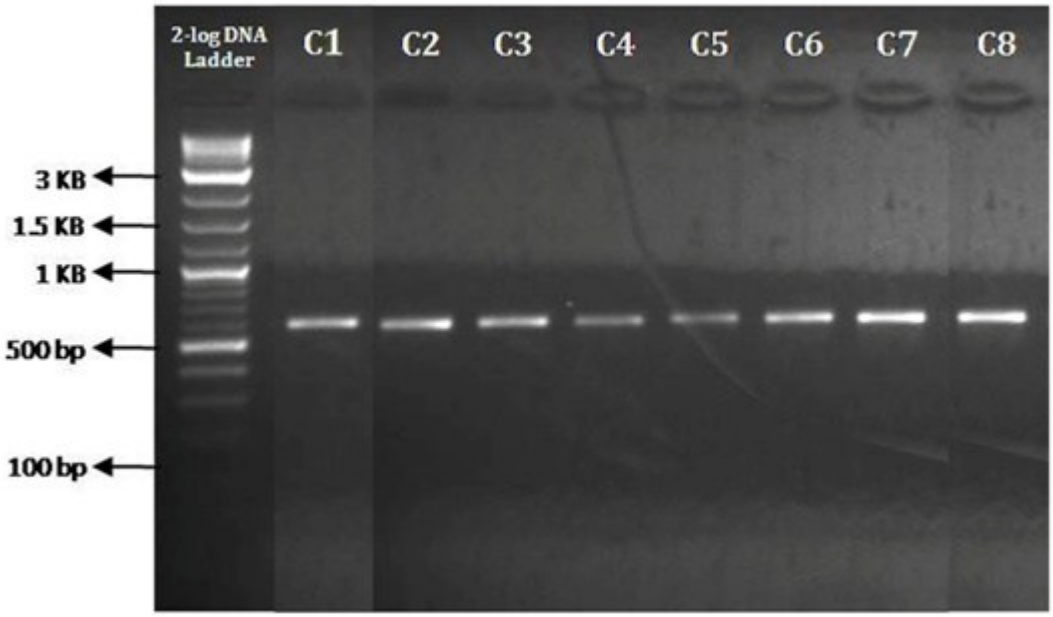


Plate 12. PCR amplification profile of the ITS- rDNA region of *Colletotrichum* isolates

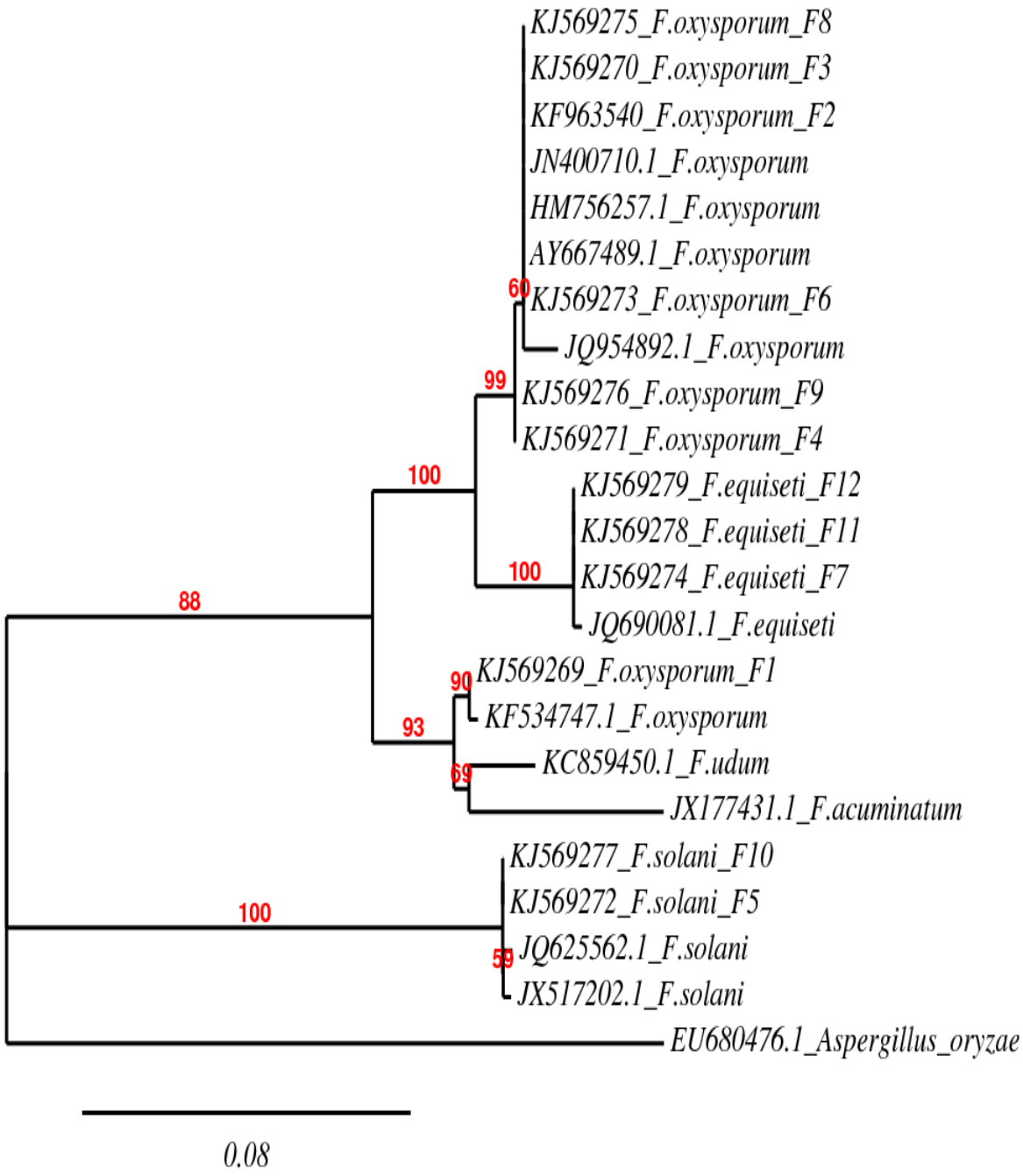


Figure 1. Phylogenetic tree generated from ITS- rDNA sequences of *Fusarium* spp. by Neighbour Joining (NJ) analysis (Scale bar = 0.08 substitutions per site)

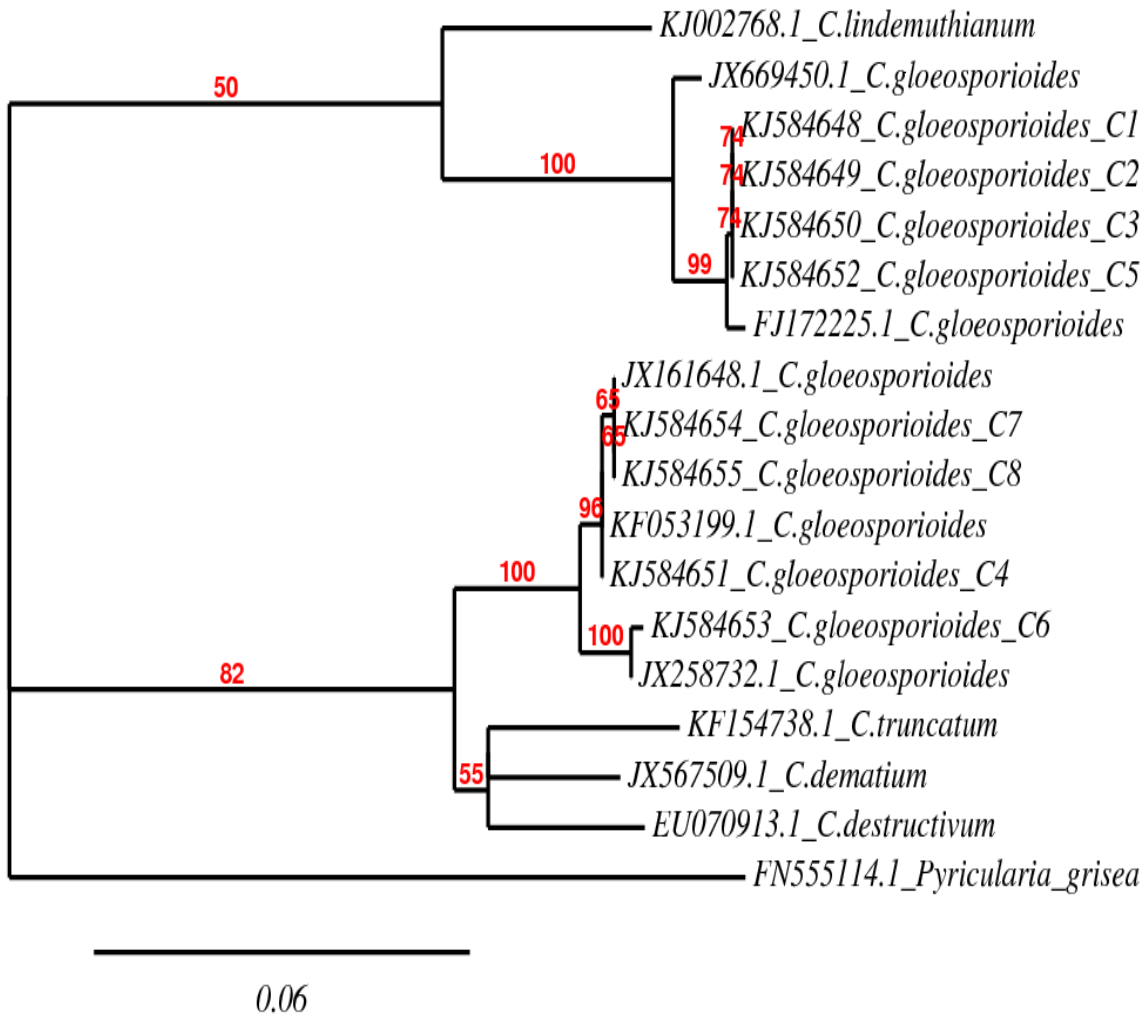


Figure 2. Phylogenetic tree generated from ITS- rDNA sequences of *Colletotrichum* spp. by Neighbour Joining (NJ) analysis (Scale bar = 0.06 substitutions per site)

JX161648.1 and KF053199.1 with 95 % bootstrap support and the other branch of this cluster comprised of isolate C6 and the reference isolate JX258732.1 with 100 % bootstrap support. Cluster III included other principal *Colletotrichum* reference isolates; KF154738.1 (*C. truncatum*), JX567509.1 (*C. dematium*) and EU070913.1 (*C. destructivum*) infecting grain legumes, with 55 % bootstrap support (Figure 2).

4.4.4. Identification of the Pathogens

4.4.4.1. Identification of the Pathogen Associated with Fusarium wilt of Cowpea

Based on the morphological and cultural characters, the isolates of the pathogen associated with Fusarium wilt of cowpea were tentatively identified as, *F. oxysporum* Schlecht, *F. equiseti* (Corda) Sacc. and *F. solani* (Mart.) Sacc. Among the 12 isolates, *F. oxysporum* was the prevalent species and widely distributed in Trivandrum, as it was found to be present in six locations examined. On the contrary, *F. equiseti* and *F. solani* were noted as less frequent species with three and two isolates each (Table 1). The identity of the species was further confirmed through ITS-rDNA sequence analyses. The sequences were deposited in GenBank and the accession numbers obtained are given in Table 5.

4.4.4.2. Identification of the Pathogen Associated with Anthracnose of Cowpea

The isolates of the pathogen associated with anthracnose of cowpea were tentatively identified as, *C. gloeosporioides* Penz. based on the morphological and cultural characters. The identity of the species was further confirmed through ITS- rDNA sequence analyses, the sequences were deposited in GenBank and the accession numbers obtained are given in Table 6.

4.5. PATHOGENICITY TESTS

4.5.1. Fusarium wilt of Cowpea

The pathogenicity and comparative virulence of 12 different isolates of *Fusarium* in causing wilt was assessed by planting healthy cowpea seedlings in

Table 5. *Fusarium* isolates and their identity as revealed by molecular characterization using ITS-rDNA sequence analysis

Isolate	Species identification	GenBank Accession No.	Matching organism in NCBI GenBank with accession number	Identity (%)
F1	*F. oxysporum*	KJ569269	*F. oxysporum* KF534747.1	99
F2	*F. oxysporum*	KF963540	*F. oxysporum* JN400710.1	100
F3	*F. oxysporum*	KJ569270	*F. oxysporum* JN400710.1	100
F4	*F.oxysporum*	KJ569271	*F. oxysporum* JN400697.1	100
F5	*F. solani*	KJ569272	*F. solani* JQ625562.1	100
F6	*F. oxysporum*	KJ569273	*F. oxysporum* JN400697.1	100
F7	*F. equiseti*	KJ569374	*F. equiseti* HQ332532.1	100
F8	*F. oxysporum*	KJ569275	*F. oxysporum* JN400697.1	100
F9	*F. oxysporum*	KJ569276	*F. oxysporum* JN400697.1	100
F10	*F. solani*	KJ569277	*F. solani* JQ625562.1	100
F11	*F. equiseti*	KJ569278	*F. equiseti* HQ332532.1	100
F12	*F. equiseti*	KJ569279	*F. equiseti* HQ332532.1	100

Table 6. *Colletotrichum* isolates and their identity as revealed by molecular characterization using ITS- rDNA sequence analysis

Isolate	Species identification	GenBankAccession No.	Matching organism in NCBI GenBank with accession number	Identity (%)
C1	*C. gloeosporioides*	KJ584648	*C. gloeosporioides* FJ172225.1	99
C2	*C. gloeosporioides*	KJ584649	*C. gloeosporioides* FJ172225.1	99
C3	*C. gloeosporioides*	KJ584650	*C. gloeosporioides* FJ172225.1	99
C4	*C. gloeosporioides*	KJ584651	*C. gloeosporioides* KF053199.1	100
C5	*C. gloeosporioides*	KJ584652	*C. gloeosporioides* FJ172225.1	100
C6	*C. gloeosporioides*	KJ584653	*C. gloeosporioides* JX258732.1	99
C7	*C. gloeosporioides*	KJ584654	*C. gloeosporioides* JX161648.1	100
C8	*C. gloeosporioides*	KJ584655	*C. gloeosporioides* JX161648.1	100

artificially inoculated soil. Of the 12 isolates tested, eight induced wilt with vascular discoloration and foliar yellowing (Plate 13), whereas the isolates F7, F8, F10 and F11 were found to be non – pathogenic and did not produce any symptoms. The isolate F2 from Palapoor took only seven days for the development of symptoms and was found to be highly pathogenic followed by the isolates F3 and F5, whereas the isolate F9 took maximum time for disease development. The results of pathogenicity and comparative virulence of *Fusarium* isolates are presented in Table 7. Based on the virulence rating, the isolate F2 identified as *F. oxysporum* was selected as the test pathogen for further studies.

4.5.2. Anthracnose of Cowpea

The pathogenicity and comparative virulence of eight isolates of the pathogen associated with anthracnose was assessed by inoculating cowpea seedlings with the conidial suspension of the fungus. Anthracnose appeared as irregular brown spots of 2 - 3 mm on the leaves (Plate 14). Among the eight isolates tested, the isolate C7 from Kattakada took only four days for the initiation of symptoms and was found to be highly pathogenic. The isolates C1 and C3 took the maximum time (eight days) for symptom development, whereas, the isolates C2, C4 and C6 did not produce any symptoms. The results of pathogenicity and comparative virulence of *Colletotrichum* isolates are presented in Table 8. Based on the virulence rating, the isolate C7 identified as *C. gloeosporioides* was selected as the test pathogen for further studies.

4.6. *IN VITRO* EVALUATION OF FUNGICIDES AGAINST PATHOGENS ASSOCIATED WITH FUSARIUM WILT AND ANTHRACNOSE OF COWPEA

The *in vitro* evaluation of 12 fungicides against the most virulent isolates *viz.,* F2 (*F. oxysporum*) and C7 (*C. gloeosporioides*) associated with Fusarium wilt and anthracnose of cowpea was done by poisoned food technique and the results are presented in Tables 9 and 10.

Table 7. Pathogenicity and comparative virulence of *Fusarium* isolates on cowpea

Isolate	Pathogenicity	Time taken for symptom development (days)	Virulence rating
F1	Wilt producing type	9	++
F2	Wilt producing type	7	+++
F3	Wilt producing type	8	++
F4	Wilt producing type	9	++
F5	Wilt producing type	8	++
F6	Wilt producing type	9	++
F7	Non -pathogenic	-	-
F8	Non -pathogenic	-	-
F9	Wilt producing type	10	++
F10	Non -pathogenic	-	-
F11	Non -pathogenic	-	-
F12	Wilt producing type	9	++

Table 8. Pathogenicity and comparative virulence of *Colletotrichum* isolates on cowpea

Isolate	Pathogenicity	Time taken for symptom development (days)	Virulence rating
C1	Pathogenic	8	+
C2	Non-pathogenic	-	-
C3	Pathogenic	8	+
C4	Non- pathogenic	-	-
C5	Pathogenic	7	+
C6	Non- pathogenic	-	-
C7	Pathogenic	4	+++
C8	Pathogenic	6	++

+ pathogenic ++ virulent +++ highly virulent – non pathogenic

a. Healthy

b. Inoculated

Plate 13. Pathogenicity testing for Fusarium wilt of cowpea

a. Healthy

b. Inoculated

Plate 14. Pathogenicity testing for anthracnose of cowpea

4.6.1. *In vitro* Evaluation of Fungicides against *F. oxysporum*

The results (Table 9) revealed that all the fungicides tested *in vitro* against *F. oxysporum* significantly inhibited the mycelial growth of the test pathogen at the recommended concentrations over the untreated control. Of the 12 fungicides, tebuconazole (0.1 %), carboxin + thiram (0.4 %), mancozeb (0.25 %) and carbendazim (0.1 %) gave 100 % inhibition of the mycelial growth of the test pathogen and differed significantly from all other treatments. It was followed by captan + hexaconazole (0.1 %) which recorded a minimum mean colony diameter of 0.60 cm and 95.48 % inhibition of the mycelial growth of the pathogen which was on par with propiconazole (0.1 %) and thiophanate- methyl (0.1 %). The maximum mean colony diameter (5.27 cm) and the least per cent inhibition (41.50 %) of the mycelial growth of the pathogen were recorded by the fungicide, azoxystrobin (0.15 %) (Plate 15).

4.6.2. *In vitro* Evaluation of Fungicides against *C. gloeosporioides*

The *in vitro* evaluation of fungicides against *C. gloeosporioides* revealed that all the fungicides tested significantly inhibited the mycelial growth of the test pathogen at the recommended concentrations as compared to control (Table 10). Among the 12 fungicides, propiconazole (0.1 %), flusilazole (0.1 %), tebuconazole(0.1 %), carboxin + thiram (0.4 %) and mancozeb (0.25 %) were the best in inhibiting (100 %) the growth of *C. gloeosporioides* and differed significantly from all other treatments. It was followed by thiophanate-methyl (0.1 %) and carbenbendazim (0.1 %) which gave 92.58 % and 91.30 % inhibition of the mycelial growth of the pathogen. The maximum mean colony diameter (6.72 cm) and least per cent inhibition (25.37 %) of the mycelial growth of the pathogen were observed in azoxystrobin (0.15 %) (Plate 16).

Table 9. Efficacy of fungicides on the *in vitro* suppression of *F. oxysporum*

Sl. No.	Fungicide	Dose (%)	Colony(dia)	Per cent growth inhibition**
1	Propiconazole	0.1	0.67^{b}	92.58^{bc} (74.16)
2	Chlorothalonil	0.1	1.83^{c}	79.64^{d} (63.15)
3	Thiophanate- methyl	0.1	0.63^{b}	92.94^{bc} (74.56)
4	Flusilazole	0.1	0.83^{b}	90.74^{c} (72.25)
5	Azoxystrobin	0.15	5.27^{f}	41.50^{f} (40.09)
6	Tebuconazole	0.1	0.00^{a}	100.00^{a} (90.00)
7	Captan+Hexaconazole	0.1	0.60^{b}	95.48^{b} (77.69)
8	Carboxin+Thiram	0.4	0.00^{a}	100.00^{a} (90.00)
9	Copper hydroxide	0.25	2.95^{d}	67.27^{e} (55.08)
10	Mancozeb	0.25	0.00^{a}	100.00^{a} (90.00)
11	Carbendazim	0.1	0.00^{a}	100.00^{a} (90.00)
12	Copper oxychloride	0.2	4.83^{e}	45.97^{f} (42.67)
13	Control	-	9.00^{g}	0.00^{g} (0.00)
	SE	-	0.10	1.76
	CD (0.05)	-	0.296	5.118

*Mean of five replications

** Values in parenthesis are arc-sine transformed

Treatment means with similar alphabets in superscript, do not differ significantly

Table 10. Efficacy of fungicides on the *in vitro* suppression of *C.gloeosporioides*

Sl. No.	Fungicide	Dose (%)	Colony dia	Per cent growth inhibition**
1	Propiconazole	0.1	0.00^{f}	100.00^{a} (90.00)
2	Chlorothalonil	0.1	2.87^{c}	68.44^{e} (55.80)
3	Thiophanate- methyl	0.1	0.67^{e}	92.58^{b} (74.16)
4	Flusilazole	0.1	0.00^{f}	100.00^{a} (90.00)
5	Azoxystrobin	0.15	6.72^{b}	25.37^{f} (30.23)
6	Tebuconazole	0.1	0.00^{f}	100.00^{a} (90.00)
7	Captan+Hexaconazole	0.1	1.12^{de}	87.60^{cd} (69.36)
8	Carboxin+Thiram	0.4	0.00^{f}	100.00^{a} (90.00)
9	Copper hydroxide	0.25	1.43^{d}	84.13^{d} (66.50)
10	Mancozeb	0.25	0.00^{f}	100.00^{a} (90.00)
11	Carbendazim	0.1	0.78^{e}	91.30^{bc} (72.82)
12	Copper oxychloride	0.2	6.28^{b}	30.09^{f} (33.26)
13	Control	-	9.00^{a}	0.00^{g} (0.00)
	SE	-	0.18	0.53
	CD (0.05)	-	0.531	3.693

*Mean of five replications

** Values in parenthesis are arc-sine transformed

Treatment means with similar alphabets in superscript, do not differ significantly

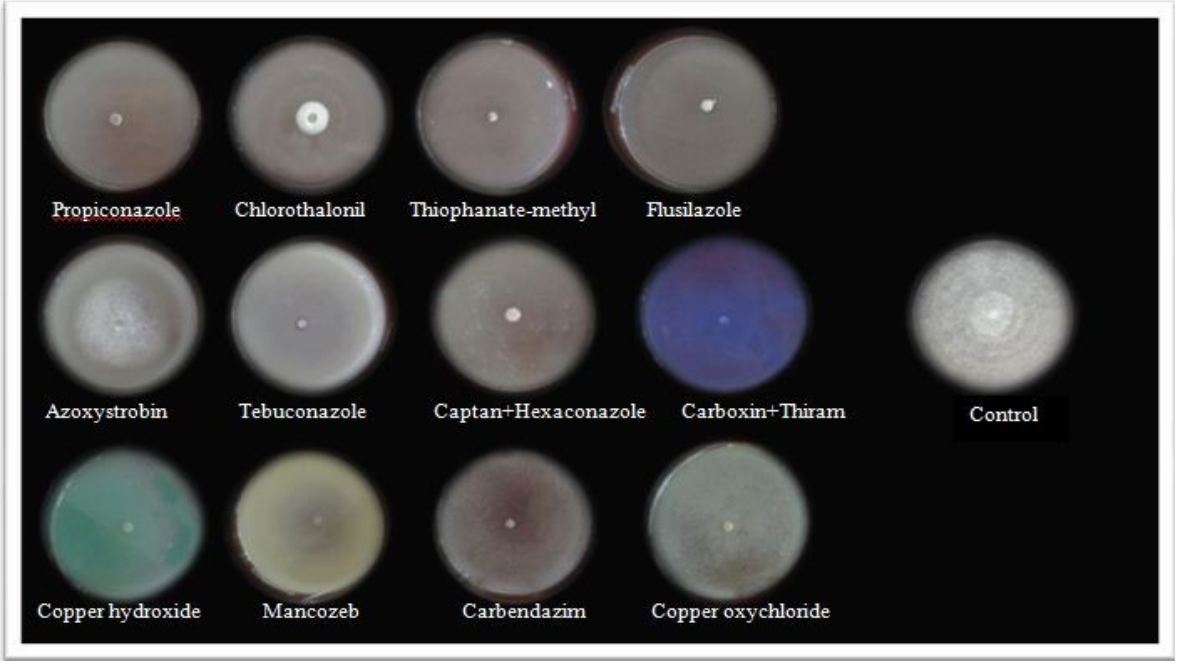


Plate 15. Effect of fungicides on the *in vitro* suppression of *F. oxysporum*

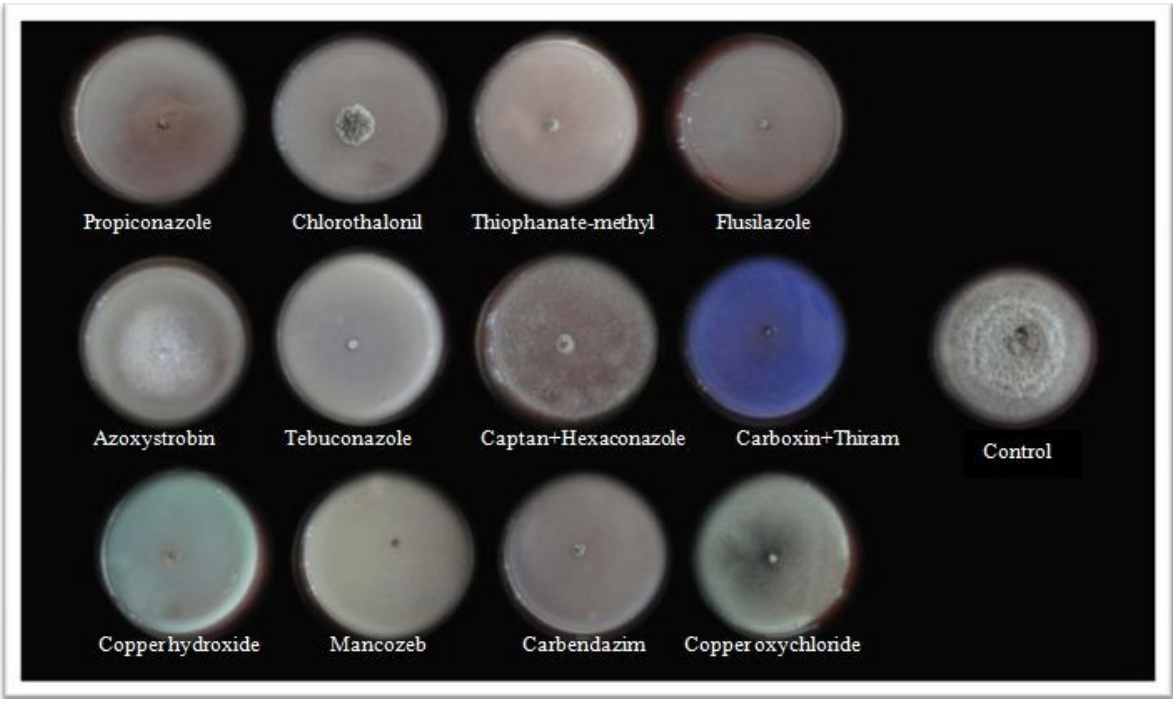


Plate 16. Effect of fungicides on the *in vitro* suppression of *C.gloeosporioides*

4.7. COMPATIBILITY STUDIES OF FUNGICIDES WITH BENEFICIAL MICROBES

The compatibility of 12 selected fungicides with biocontrol agents (*T. viride* and *P. fluorescens*) and root nodule bacterium (*Rhizobium* spp.) was tested based on *in vitro* sensitivity and the results are presented in Table 11.

4.7.1. Compatibility of Fungicides with *T.viride*

Fungicidal compatibility studies with *T. viride* revealed that, propiconazole (0.1 %), flusilazole (0.1 %), tebuconazole (0.1 %) and carbendazim (0.1 %) were incompatible with *T. viride*, showing 100 % inhibition of the mycelial growth at their recommended dosage and differed significantly from all other treatments. While, chlorothalonil (0.1 %), thiophanate-methyl (0.1 %), captan + hexaconazole (0.1 %), carboxin + thiram (0.4 %) and copper hydroxide (0.25 %) were found to be least compatible showing more than 85 % inhibition of the mycelial growth. Mancozeb (0.25 %) and copper oxychloride (0.2 %) were moderately compatible with mycelial growth inhibition in the range of 20 – 50 %. Only azoxystrobin (0.15 %) was found to be highly compatible without the inhibition (0 %) of mycelial growth of *T. viride* (Plate 17).

4.7.2. Compatibility of Fungicides with *P. fluorescens*

The results revealed that none of the fungicides tested inhibited the growth of *P. fluorescens* at their recommended concentration and was found to be highly compatible.

4.7.3. Compatibility of Fungicides with *Rhizobium* spp.

The studies on compatibility of fungicides with *Rhizobium* spp. indicated that, all the fungicides tested were highly compatible with *Rhizobium* spp., while, captan + hexaconazole (0.1 %), carboxin + thiram (0.4 %), copper hydroxide (0.25 %) and copper oxychloride (0.2 %) were found only moderately compatible with bacterial growth inhibition in the range of 25 – 45 % (Plate 18).

Table 11. Effect of fungicides on the growth of beneficial microbes

Sl. No.	Fungicide	Dose (%)	Per cent growth suppression*		
			*T.viride***	*P.fluorescens*	*Rhizobium* spp.**
1	Propiconazole	0.1	100.00[a] (90.00)	0.00	0.00[f] (0.00)
2	Chlorothalonil	0.1	91.70[c] (73.22)	0.00	16.70[e] (24.12)
3	Thiophanate- methyl	0.1	90.93[cd] (72.45)	0.00	0.00[f] (0.00)
4	Flusilazole	0.1	100.00[a] (90.00)	0.00	0.00[f] (0.00)
5	Azoxystrobin	0.15	0.00[g] (0.00)	0.00	0.00[f] (0.00)
6	Tebuconazole	0.1	100.00[a] (90.00)	0.00	0.00[f] (0.00)
7	Captan+Hexaconazole	0.1	89.98[cd] (71.52)	0.00	22.97[d] (28.60)
8	Carboxin+Thiram	0.4	96.26[b] (78.82)	0.00	31.07[c] (33.82)
9	Copper hydroxide	0.25	85.28[d] (67.41)	0.00	44.07[a] (41.59)
10	Mancozeb	0.25	22.94[f] (28.61)	0.00	0.00[f] (0.00)
11	Carbendazim	0.1	100.00[a] (90.00)	0.00	0.00[f] (0.00)
12	Copper oxychloride	0.2	49.63[e] (44.71)	0.00	36.50[b] (37.12)
13	Control	-	0.00[g] (0.00)	0.00	0.00
	SE		1.80	-	-
	CD (0.05)	-	5.233	NS	2.378

*Mean of five replications

** Values in parenthesis are arc-sine transformed

Treatment means with similar alphabets in superscript, do not differ significantly

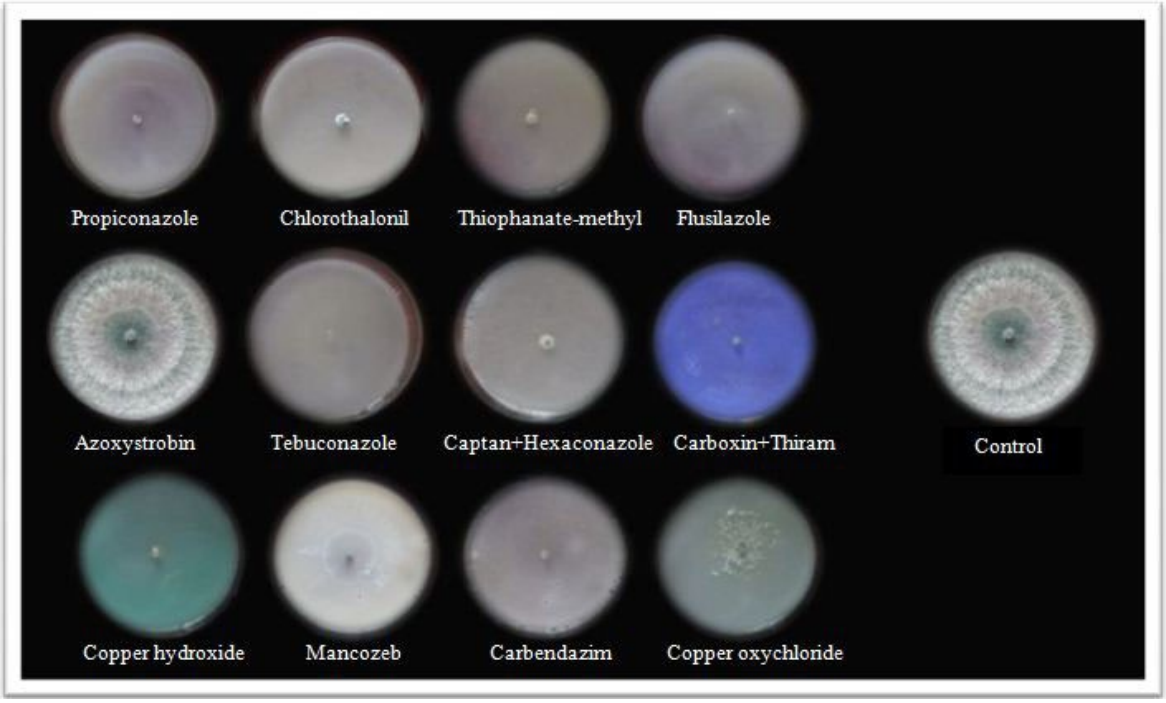


Plate 17. Effect of fungicides on the mycelial growth of *T. viride*

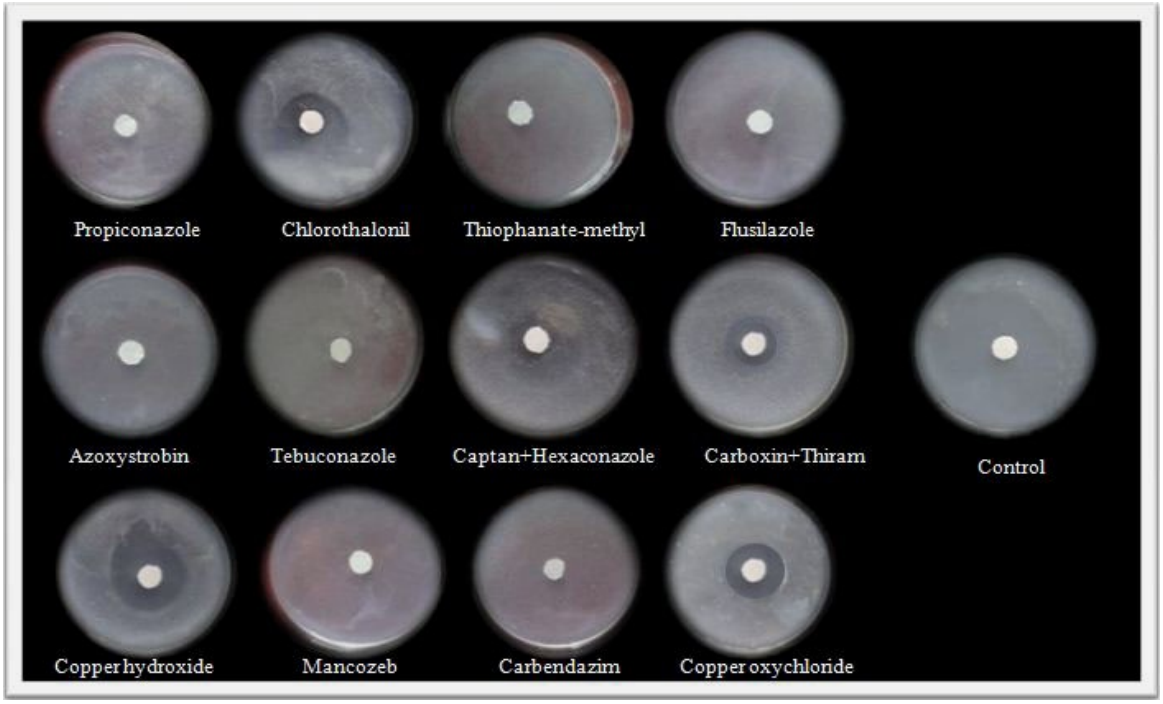


Plate 18. Effect of fungicides on the growth of *Rhizobium* spp.

4.8. *IN VIVO* EVALUATION OF FUNGICIDES AGAINST PATHOGENS ASSOCIATED WITH FUSARIUM WILT AND ANTHRACNOSE OF COWPEA

Two pot experiments were conducted in CRD with five replications to assess the efficacy of 12 selected fungicides against Fusarium wilt and anthracnose of cowpea. Observations on disease incidence, index and biometric characters such as plant height, root length, fresh and dry weight of shoots and roots, number of pods, pod yield and number of root nodules were recorded.

4.8.1. *In vivo* Evaluation of Fungicides against Fusarium wilt of Cowpea

4.8.1.1. Disease Incidence

The results revealed that all the treatments caused significant reduction in wilt incidence over untreated control. However, soil drenching of flusilazole (0.1 %), tebuconazole (0.1 %) or carbendazim (0.1 %) were highly effective in the total suppression of wilt incidence and differed significantly from other treatments. Among the fungicides tested, treatment with captan + hexaconazole (0.1 %) and carboxin + thiram (0.4 %) recorded a higher incidence of 66.70 % and were on par with each other. The pathogen inoculated control plants showed basal swelling and yellowing of leaves, 45 days after seed emergence followed by defoliation and complete death of the plants, recording 100 % wilt incidence and differed significantly from all other treatments (Table 12).

4.8.1.2. Disease Index

The plants treated with flusilazole (0.1 %), tebuconazole (0.1 %) or carbendazim (0.1 %) recorded the lowest disease index (0.00) and differed significantly from all other treatments (Table 12). It was followed by treatment with propiconazole (0.1 %), thiophanate-methyl (0.1 %) or azoxystrobin (0.15 %) with an index of 16.70 and were on par with each other. Soil drenching with contact fungicides *viz.,* chlorothalonil (0.2 %), copper hydroxide (0.25 %), mancozeb (0.25 %) and copper oxychloride (0.2 %) registered an index of 25.00

Table 12. Effect of fungicides on the incidence and index of Fusarium wilt of cowpea under *in vivo* conditions

Treatments	Disease incidence (%)*	Disease index*
T_1	33.30[b] (35.23)	16.70[b] (24.11)
T_2	33.30[b] (35.23)	25.00[c] (29.99)
T_3	33.30[b] (35.23)	16.70[b] (24.11)
T_4	0.00[a] (0.00)	0.00[a] (0.00)
T_5	33.30[b] (35.23)	16.70[b] (24.11)
T_6	0.00[a] (0.00)	0.00[a] (0.00)
T_7	66.70[c] (54.73)	66.70[c] (54.73)
T_8	66.70[c] (54.73)	50.00[d] (44.98)
T_9	33.30[b] (35.23)	25.00[c] (29.99)
T_{10}	33.30[b] (35.23)	25.00[c] (29.99)
T_{11}	0.00[a] (0.00)	0.00[a] (0.00)
T_{12}	33.30[b] (35.23)	25.00[c] (29.99)
T_{13}	100.00[d] (90.00)	100.00[f] (90.00)
SE	0.01	0.007
CD (0.05)	0.029	0.021

* Values in parenthesis are arc-sine transformed

Treatment means with similar alphabets in superscript, do not differ significantly

T_1–Propiconazole (0.1%) T_2 –Chlorothalonil (0.1%) T_3 –Thiophanate- methyl (0.1%) T_4 – Flusilazole (0.1 %) T_5 – Azoxystrobin (0.15%) T_6 – Tebuconazole (0.1%) T_7 – Captan + Hexaconazole (500 g/ha) T_8 – Carboxin + Thiram (4 g/kg seed) T_9 – Copperhydroxide (0.25%) T_{10} – Mancozeb (0.25%) T_{11}–Carbendazim (0.1%) T_{12}– Copper oxychloride (0.2 %) T_{13} – Control

and were statistically on par with each other. The highest disease index (100.00) was observed with the control plants and differed significantly from all other treatments.

4.8.1.3. Plant Height

The plants treated with flusilazole (0.1 %) registered the maximum height (395.83 cm) followed by the treatment with tebuconazole (0.1 %) (382.50 cm). The minimum plant height (292.50 cm) was shown by the plants treated with carboxin + thiram (4 g/kg seed) (Table 13).

4.8.1.4. Root Length

Significantly longer roots (44.27 cm) were produced in plants treated with carbendazim (0.1 %) followed by treatment with tebuconazole (0.1 %) (42.33 cm) and flusilazole (0.1 %) (41.00 cm) and were on par with each other. Root length was minimum (14.33 cm) for untreated control plants (Table 13).

4.8.1.5. Fresh Weight – Shoot

The maximum fresh weight (361.67 g) was observed with carbendazim (0.1 %) treated plants followed by treatment with flusilazole (0.1 %) (333.33 g) and azoxystrobin (0.15 %) (328.33 g) and were on par with each other. The minimum fresh weight (36.67 g) was noticed with untreated control plants and differed significantly from all other treatments (Table 13).

4.8.1.6. Dry Weight – Shoot

The highest dry weight (56.67 g) of shoot was recorded in treatment with either carbendazim (0.1 %) or flusilazole (0.1 %) and was on par with plants treated with azoxystrobin (0.15 %) (55.00 g). The lowest dry weight of shoot (25.00 g) was recorded with untreated control plants (Table 13).

4.8.1.7. Fresh Weight – Root

There was no significant difference among the treatments with respect to fresh weight of root. Plants drenched with chlorothalonil (0.1 %) showed the highest fresh root weight (46.67 g) whereas the lowest fresh weight of root (26.67 g) was observed in plants treated with mancozeb (0.25 %) and the untreated control plants (Table 13).

4.8.1.8. Dry Weight – Root

Plants drenched with chlorothalonil (0.1 %) showed the highest dry root weight (10.30 g), whereas, the lowest dry weight of root (5.53 g) was observed in plants treated with mancozeb (0.25 %) (Table 13).

4.8.1.9. Number of Pods

The plants treated with carbendazim (0.1 %) recorded the highest number (59.00) of pods followed by treatment with azoxystrobin (0.15 %) (47.67) and were on par with each other. The lowest number of pods (27.00) was noticed in plants treated with carboxin + thiram (4 g/kg seed) (Table 13).

4.8.1.10. Pod Yield

With regard to pod yield, plants drenched with carbendazim (0.1 %) registered the maximum pod yield (1186.33 g/plant) and differed significantly from all other treatments, followed by treatment with azoxystrobin (0.15 %) (950.67 g/plant). However, those plants treated with carboxin + thiram (4 g/kg seed) recorded the minimum yield (541.00 g/plant) which was on par with the treatment with captan + hexaconazole (500 g/ha) (544.00 g/plant). (Table 13).

4.8.1.11. Number of Root Nodules

Significant difference was noticed among the treatments with regard to number of root nodules (Table 13). The highest number (71.00) of root nodules was observed in plants treated with propiconazole (0.1 %) which was on par with

Table 13. In vivo efficacy of different fungicides on the growth and yield parameters of cowpea plants inoculated with F. oxysporum

Treatments	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)	No. of pods	Pod yield (g plant)	No. of root nodules
T_1	307.50^{abc}	31.33^{abc}	193.33^{bc}	36.67^{bcd}	40.00^{a}	6.70^{a}	31.33^{b}	621.33^{h}	71.00^{a}
T_2	361.67^{ab}	35.00^{ab}	193.33^{bc}	33.33^{cd}	46.67^{a}	10.30^{a}	42.00^{ab}	842.33^{d}	47.00^{abc}
T_3	352.10^{abc}	32.33^{abc}	263.33^{abc}	41.67^{abcd}	33.33^{a}	6.80^{a}	37.33^{b}	743.33^{f}	53.67^{ab}
T_4	395.83^{a}	41.00^{a}	333.33^{ab}	56.67^{a}	36.67^{a}	8.30^{a}	44.67^{ab}	893.33^{c}	33.00^{abc}
T_5	298.33^{abc}	34.00^{abc}	328.33^{ab}	55.00^{a}	41.67^{a}	8.37^{a}	47.67^{ab}	950.67^{b}	43.67^{abc}
T_6	382.50^{ab}	42.33^{a}	326.67^{abc}	53.33^{ab}	33.33^{a}	7.10^{a}	34.00^{b}	682.33^{g}	29.67^{abc}
T_7	355.83^{abc}	23.00^{bcd}	226.67^{abc}	46.67^{abc}	38.33^{a}	7.53^{a}	27.33^{b}	544.00^{j}	10.00^{bc}
T_8	262.50^{c}	20.33^{cd}	206.67^{bc}	41.67^{abcd}	35.00^{a}	7.63^{a}	27.00^{b}	541.00^{j}	19.33^{bc}
T_9	327.50^{abc}	30.00^{abc}	278.33^{abc}	41.67^{abcd}	38.33^{a}	8.50^{a}	33.67^{b}	674.67^{g}	18.33^{bc}
T_{10}	340.00^{abc}	31.33^{abc}	278.33^{abc}	45.00^{abc}	26.67^{a}	5.53^{a}	40.33^{ab}	805.33^{e}	27.00^{abc}
T_{11}	331.67^{abc}	44.27^{a}	361.61^{a}	56.67^{a}	38.33^{a}	6.77^{a}	59.00^{a}	1186.33^{a}	53.33^{ab}
T_{12}	300.00^{abc}	32.33^{abc}	286.67^{abc}	40.00^{abcd}	41.67^{a}	7.80^{a}	36.67^{b}	741.00^{f}	50.33^{ab}
T_{13}	292.50^{bc}	14.33^{d}	36.67^{d}	25.00^{d}	26.67^{a}	6.00^{a}	29.00^{b}	581.33^{i}	1.00^{c}
SE	33.67	5.00	47.36	6.15	9.79	2.47	7.43	37.03	17.09
CD (0.05)	97.908	14.545	137.711	17.881	NS	NS	21.617	8.924	49.699

Treatment means with similar alphabets in superscript, do not differ significantly

T_1–Propiconazole (0.1%) T_2–Chlorothalonil (0.1%) T_3–Thiophanate- methyl (0.1%) T_4 – Flusilazole (0.1 %) T_5 – Azoxystrobin (0.15%)

T_6 – Tebuconazole (0.1%) T_7 – Captan + Hexaconazole (500 g/ha) T_8 –Carboxin + Thiram (4 g/kg seed) T_9 – Copperhydroxide (0.25%)

T_{10} – Mancozeb (0.25%) T_{11}–Carbendazim (0.1%) T_{12}– Copper oxychloride (0.2 %) T_{13} – Control

the treatment with thiophanate-methyl (0.1 %) (53.67). The lowest number of root nodules was noticed in untreated control plants.

4.8.2. *In Vivo* Evaluation of Fungicides against Anthracnose of Cowpea

4.8.2.1. Disease Incidence

Among the fungicides, foliar application of flusilazole (0.1 %) recorded the least disease incidence (50.00 %) followed by treatments with propiconazole (0.1 %), tebuconazole (0.1 %) or carbendazim (0.1 %) (75.00 %) and were on par with each other. Plants treated with contact fungicides *viz.,* chlorothalonil (0.2 %), copper hydroxide (0.25 %), mancozeb (0.25 %) and copper oxychloride (0.2 %) registered an incidence of 96.99 %. However, the untreated control plants recorded cent per cent disease incidence (Table 14).

4.8.2.2. Disease Index

The data on disease index revealed that all the treatments were significantly superior to the untreated control. The lowest disease index (5.60) was recorded by the plants treated with flusilazole (0.1 %) which was on par with the treatment with tebuconazole (0.1 %) (8.30). This was followed by treatment with either propiconazole (0.1 %), carbendazim (0.1%) or thiophanate–methyl (0.1 %) with an index of 13.90 and 15.74 respectively and were statistically on par with each other. Among the contact fungicides tested, treatment with copper oxychloride (0.2 %) registered the lowest disease index of 20.30. The untreated control plants recorded the highest disease index (83.30) and differed significantly from all other treatments (Table 14).

4.8.2.3. Plant Height

Maximum plant height (354.17 cm) was recorded in case of plants treated with thiophanate-methyl (0.1 %) which was on par with carbendazim (0.1 %) (349.17 cm) and tebuconazole (0.1 %) (347.50 cm). The untreated control plants recorded the minimum height (264.17 cm) (Table 15).

Table 14. Effect of fungicides on the incidence and index of anthracnose of cowpea under *in vivo* conditions

Treatments	Disease incidence (%)*	Disease index*
T_1	75.00^{ab} (59.98)	13.90^{bc} (21.88)
T_2	96.99^{abc} (79.99)	40.70^{f} (39.63)
T_3	93.32^{bc} (74.99)	15.74^{c} (23.30)
T_4	50.00^{a} (44.98)	5.60^{a} (13.68)
T_5	93.32^{bc} (74.99)	40.70^{f} (39.63)
T_6	75.00^{ab} (59.98)	8.30^{ab} (16.77)
T_7	82.16^{abc} (64.99)	33.00^{ef} (35.05)
T_8	75.02^{ab} (59.99)	25.74^{de} (30.47)
T_9	96.99^{bc} (79.99)	55.61^{g} (48.20)
T_{10}	96.99^{bc} (79.99)	40.70^{f} (39.63)
T_{11}	75.00^{ab} (59.98)	13.90^{bc} (21.88)
T_{12}	96.99^{bc} (79.99)	20.30^{cd} (26.77)
T_{13}	100.00^{c} (90.00)	83.30^{h} (65.85)
SE	9.81	1.93
CD (0.05)	28.527	5.610

* Values in parenthesis are arc-sine transformed

Treatment means with similar alphabets in superscript, do not differ significantly

T_1–Propiconazole (0.1%) T_2–Chlorothalonil (0.1%) T_3–Thiophanate- methyl (0.1%) T_4 – Flusilazole (0.1 %) T_5 – Azoxystrobin (0.15%) T_6 – Tebuconazole (0.1%) T_7 – Captan + Hexaconazole (500 g/ha) T_8 – Carboxin + Thiram (4 g/kg seed) T_9 – Copperhydroxide (0.25%) T_{10} – Mancozeb (0.25%) T_{11}–Carbendazim (0.1%) T_{12}– Copper oxychloride (0.2 %) T_{13} – Control

4.8.2.4. Root Length

The plants treated with thiophanate- methyl (0.1 %) produced longer roots (36.17 cm) and differed significantly from those treated with mancozeb (0.25 %) which produced the smaller roots (17.50 cm) (Table 15).

4.8.2.5. Fresh Weight – Shoot

Significant difference was noticed among the treatments with respect to fresh weight of shoots. Maximum fresh weight (298.33 g) of shoot was noticed in plants treated with thiophanate-methyl (0.1 %) followed by treatment with flusilazole (0.1 %) (261.67 g) and was on par with each other. The untreated control plants showed the minimum fresh weight (88.33 g) and differed significantly from other treatments (Table 15).

4.8.2.6. Dry Weight – Shoot

Analysis of data on shoot dry weight indicated that the plants treated with either chlorothalonil (0.1 %) or thiophanate- methyl (0.1 %) registered the maximum dry shoot weight (40.00 g) and was on par with each other whereas, the untreated control plants recorded the minimum dry shoot weight (21.67 g) (Table 15).

4.8.2.7. Fresh Weight – Root

The plants treated with flusilazole (0.1 %) recorded the maximum (55.00 g) fresh root weight which was on par with the treatment with propiconazole (0.1%) (51.67 g). The untreated control plants recorded the minimum (16.67 g) fresh root weight (Table 15).

4.8.2.8. Dry Weight – Root

The data regarding dry root weight indicated that the plants treated with propiconazole (0.1 %) registered the maximum (12.47 g) dry root weight followed by treatment with flusilazole (0.1 %) (11.23 g) and was on par with each other.

The untreated control plants recorded the minimum (3.67 g) dry root weight (Table 15).

4.8.2.9. Number of Pods

The plants treated with flusilazole (0.1 %) recorded the highest (59.67) number of fruits followed by treatment with azoxystrobin (0.15 %) (51.00) and carbendazim (0.1 %) (45.00). The lowest number of pods was noticed in untreated control plants (Table 15).

4.8.2.10. Pod Yield

With respect to pod yield, the plants treated with flusilazole (0.1 %) recorded the maximum (1192.33 g/plant) yield and differed significantly from all other treatments, followed by treatment with azoxystrobin (0.15 %) (1021.33 g/plant) and carbendazim (0.1%) (900.33 g/plant) and differed significantly from each other. The minimum yield (603.67 g/plant) was noticed in untreated control plants (Table 15).

4.8.2.11. Number of Root Nodules

With regard to the number of root nodules, the plants treated with flusilazole (0.1 %) registered the maximum (51.67) number of root nodules which was on par with the treatment with carboxin+thiram (4 g/kg seed) (46.00), whereas the plants treated with mancozeb (0.25 %) recorded the minimum number of root nodules (18.33) (Table 15).

4.9. INTEGRATED MANAGEMENT OF FUSARIUM WILT AND ANTHRACNOSE OF VEGETABLE COWPEA USING FUNGICIDES, BIO AGENTS AND ECOFRIENDLY METHODS

Based on the results of pot experiments, three effective fungicides *viz.,* flusilazole (0.1 %), tebuconazole (0.1 %) and carbendazim (0.1 %) which showed the least incidence and index of both the diseases along with seed treatment

Table 15. *In vivo* efficacy of different fungicides on the growth and yield parameters of cowpea plants inoculated with *C. gloeosporioides*

T_1	335.00^{ab}	22.17^{abc}	193.33^{bcd}	36.67^{a}	51.67^{ab}	12.47^{a}	42.33^{a}	845.67^{de}	31.67^{abc}
T_2	324.17^{ab}	33.67^{ab}	241.67^{abc}	40.00^{a}	40.00^{abcd}	8.97^{abc}	42.67^{a}	853.67^{d}	35.00^{abc}
T_3	354.17^{a}	36.17^{a}	298.33^{a}	40.00^{a}	38.33^{abcd}	8.23^{abcd}	42.33^{a}	844.67^{de}	41.00^{abc}
T_4	304.17^{ab}	25.17^{abc}	261.67^{ab}	38.33^{a}	55.00^{a}	11.23^{ab}	59.67^{a}	1192.33^{a}	51.67^{a}
T_5	265.00^{b}	28.63^{abc}	211.67^{bc}	31.67^{abc}	33.33^{bcde}	7.63^{abcd}	51.00^{a}	1021.33^{b}	40.00^{abc}
T_6	347.50^{a}	21.33^{abc}	188.33^{bcd}	31.67^{abc}	31.67^{bcde}	5.20^{cd}	34.33^{a}	686.67^{h}	25.67^{bc}
T_7	320.00^{ab}	25.00^{abc}	125.00^{de}	25.00^{bc}	31.67^{bcde}	6.67^{bcd}	35.33^{a}	710.33^{g}	27.00^{bc}
T_8	323.33^{ab}	25.67^{abc}	220.00^{bc}	31.67^{abc}	41.67^{abc}	9.83^{abc}	33.67^{a}	668.33^{h}	46.00^{ab}
T_9	309.17^{ab}	20.63^{abc}	181.67^{cd}	36.67^{a}	45.00^{abc}	10.50^{ab}	33.67^{a}	673.00^{h}	37.33^{abc}
T_{10}	307.50^{ab}	17.50^{c}	203.33^{bc}	33.33^{ab}	30.00^{cde}	6.17^{bcd}	41.67^{a}	833.67^{e}	18.33^{c}
T_{11}	333.33^{ab}	21.33^{abc}	218.33^{bc}	36.67^{a}	20.00^{de}	4.70^{cd}	45.00^{a}	900.33^{c}	33.33^{abc}
T_{12}	349.17^{a}	20.00^{bc}	190.00^{bcd}	33.33^{ab}	38.33^{abcd}	9.40^{abc}	36.67^{a}	742.33^{f}	35.00^{abc}
T_{13}	264.17^{b}	21.00^{abc}	88.33^{e}	21.67^{c}	16.67^{e}	3.67^{d}	30.33^{a}	603.67^{i}	20.67^{c}
SE	28.20	5.37	26.70	3.61	7.10	1.79	7.02	38.01	8.03

Treatment means with similar alphabets in superscript, do not differ significantly

T_1–Propiconazole (0.1%) T_2–Chlorothalonil (0.1%) T_3–Thiophanate- methyl (0.1%) T_4 – Flusilazole (0.1 %) T_5 – Azoxystrobin (0.15%)

T_6 – Tebuconazole (0.1%) T_7 – Captan + Hexaconazole (500 g/ha) T_8 –Carboxin + Thiram (4 g/kg seed) T_9 – Copperhydroxide (0.25%)

T_{10} – Mancozeb (0.25%) T_{11}–Carbendazim (0.1%) T_{12}– Copper oxychloride (0.2 %) T_{13} – Control

(carbendazim @ 2g/kg seed), soil solarization (Plate 19) and *Trichoderma* enriched neem cake organic manure mixture were evaluated in the field to determine their efficacy in the management of Fusarium wilt and anthracnose of cowpea (Plate 20). Observations on disease incidence, index and growth parameters such as plant height, root length, fresh and dry weight of shoots and roots, number of pods, pod yield and number of root nodules were recorded.

4.9.1. Incidence of Fusarium wilt

All the treatments were effective in the suppression of Fusarium wilt of cowpea on field evaluation. However, treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and soil drenching of either tebuconazole (0.1 %) or carbendazim (0.1 %) was highly effective for the management of Fusarium wilt with total suppression of wilt incidence. This was followed by soil drenching of tebuconazole (0.1 %), carbendazim (0.1 %) or copper oxychloride (0.2 %) alone which registered wilt incidence of 16.67 % while, treatment with flusilazole (0.1 %) alone recorded a higher incidence of 33.33 %. The untreated control plants showed symptoms like basal swelling and foliar yellowing followed by complete death of the plants and recorded the highest disease incidence (41.67 %) (Table 16).

4.9.2. Severity (Disease Index) of Fusarium wilt

The plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and soil drenching of either tebuconazole (0.1 %) or carbendazim (0.1 %) recorded the lowest (0.00) disease index which was followed by treatment with copper oxychloride (0.2 %) (8.33). The highest disease index (41.67) was observed among the untreated control plants (Table 16).

4.9.3. Incidence of Anthracnose

Statistical analysis of the data on anthracnose incidence revealed that foliar application of either flusilazole (0.1 %) or tebuconazole (0.1 %) registered the

Plate 19. Solarization of beds

Plate 20. General view of the experimental plot

Table 16. Effect of soil solarization, *Trichoderma* enriched neem cake organic manure mixture and chemicals on the incidence and index of Fusarium wilt of cowpea under field conditions

Treatments	Disease incidence (%)*	Disease index*
T_1	16.67[a] (14.99)	16.67[a] (14.99)
T_2	0.00[a] (0.00)	0.00[a] (0.00)
T_3	0.00[a] (0.00)	0.00[a] (0.00)
T_4	25.00[a] (19.99)	25.00[a] (19.99)
T_5	33.33[a] (30.00)	29.17[a] (23.09)
T_6	16.67[a] (14.99)	16.67[a] (14.99)
T_7	16.67[a] (14.99)	16.67[a] (14.99)
T_8	16.67[a] (14.99)	8.33[a] (10.00)
T_9	41.67[a] (39.98)	41.67[a] (39.98)
SE	14.54	12.56
CD (0.05)	NS	NS

* Values in parenthesis are arc-sine transformed

Treatment means with similar alphabets in superscript, do not differ significantly

T_1–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + flusilazole (0.1 %) T_2–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + tebuconazole (0.1%) T_3–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + carbendazim (0.1%) T_4–Soil solarization +*Trichoderma* enriched neem cake organic manure mixture T5 – Flusilazole (0.1 %) T_6 – Tebuconazole (0.1%) T_7– Carbendazim (0.1%) T_8 – Copper oxychloride (0.2 %) (Chemical Check) T_9 – Control

lowest (8.33 %) anthracnose incidence followed by treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) (25.00 %) and were on par with each other. Plants treated with copper oxychloride (0.2%) recorded 83.33 % anthracnose incidence. Treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) and foliar spray of carbendazim (0.1 %) alone registered an incidence of 91.67 %. However, all the observational plants in the untreated control plants and also in the treatment with soil solarization and *Trichoderma* enriched neem cake organic manure mixture alone without fungicide spray showed incidence of anthracnose (Table 17).

4.9.4. Severity (Disease Index) of Anthracnose

Significant difference was noted among the treatments with respect to disease index of anthracnose. The foliar application of either flusilazole (0.1 %) or tebuconazole (0.1 %) recorded the lowest (0.93) disease index which was on par with the treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) (4.63). This was followed by treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and flusilazole (0.1 %) and registered an index of 9.26. Foliar application of the contact fungicide, copper oxychloride (0.2%) recorded an index of 18.52. Plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) and foliar spray of carbendazim (0.1 %) alone registered an index of 32.41 and 56.48 respectively and differed significantly. The untreated control plants and also the plants treated with soil solarization and *Trichoderma* enriched neem cake organic manure mixture alone registered the highest disease index (75.9) and differed significantly from all other treatments (Table 17).

4.9.5. Plant Height

Significantly higher plants (593.33 cm) were produced in plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and

Table 17. Effect of soil solarization, *Trichoderma* enriched neem cake organic manure mixture and chemicals on the incidence and index of anthracnose of cowpea under field conditions

Treatments	Disease incidence (%)*	Disease index*
T_1	33.33^{b} (34.99)	9.26^{de} (16.82)
T_2	25.00^{b} (24.99)	4.63^{ef} (9.69)
T_3	91.67^{a} (79.99)	32.41^{c} (34.40)
T_4	100.00^{a} (90.00)	75.93^{a} (60.62)
T_5	8.33^{b} (10.00)	0.93^{f} (3.20)
T_6	8.33^{b} (10.00)	0.93^{f} (3.20)
T_7	91.67^{a} (79.99)	56.48^{b} (48.84)
T_8	83.33^{a} (69.98)	18.52^{cd} (25.38)
T_9	100.00^{a} (90.00)	75.92^{a} (60.74)
SE	9.34	3.76
CD (0.05)	27.992	11.270

* Values in parenthesis are arc-sine transformed

Treatment means with similar alphabets in superscript, do not differ significantly

T_1–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + flusilazole (0.1 %) T_2–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + tebuconazole (0.1%) T_3–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + carbendazim (0.1%) T_4–Soil solarization +*Trichoderma* enriched neem cake organic manure mixture T5 – Flusilazole (0.1 %) T_6 – Tebuconazole (0.1%) T_7– Carbendazim (0.1%) T_8 – Copper oxychloride (0.2 %) (Chemical Check) T_9 – Control

tebuconazole (0.1 %) followed by treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) (592.00 cm) and were on par with each other. The plant height was minimum (455.00 cm) for untreated control plants (Table 18).

4.9.6. Root Length

The plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) registered the maximum root length (55.33 cm) followed by treatment with either soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) (52.00 cm) or treatment with soil solarization and *Trichoderma* enriched neem cake organic manure mixture (43.67 cm) and were on par with each other. The minimum root length (17.00 cm) was observed among the untreated control plants which was on par with the treatment with either carbendazim (0.1 %) (29.00 cm) or copper oxychloride (0.2 %) (32.00 cm) (Table 18).

4.9.7. Fresh Weight – Shoot

The maximum fresh weight (434.33 g) was noticed among the plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) which was on par with the treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) (425.33 g) or tebuconazole (0.1 %) alone (365.00 g). The minimum fresh weight (227.00 g) was observed among the untreated control plants (Table 18).

4.9.8. Dry Weight – Shoot

The highest dry weight of shoot (117.00 g) was registered with the plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) followed by treatment with either soil solarization, *Trichoderma* enriched neem cake organic manure mixture and

tebuconazole (0.1 %) (112.33 g) or soil solarization, *Trichoderma* enriched neem cake organic manure mixture and flusilazole (0.1 %) (91.00 g) and were on par with each other. The lowest dry weight of shoot (41.67 g) was noticed among the untreated control plants which was on par with the treatment with flusilazole (0.1 %) (65.00 g), carbendazim (0.1 %) (62.33 g) or copper oxychloride (0.2 %) (56.67g) (Table 18).

4.9.9. Fresh Weight – Root

The maximum fresh root weight (57.67 g) was observed among the plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and either tebuconazole (0.1 %) or carbendazim (0.1 %) which was on par with the treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and flusilazole (0.1 %) (46.33 g). The untreated control plants recorded the minimum fresh root weight (19.67 g) which was on par with the treatment with carbendazim (0.1 %) (39.00 g), copper oxychloride (0.2 %) (29.67 g), or flusilazole (0.1 %) (27.00 g) (Table 18).

4.9.10. Dry Weight – Root

The statistical analysis on dry root weight indicated that the plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) registered the maximum dry root weight (9.75 g) followed by treatment with either soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) (9.30 g) or treatment with soil solarization and *Trichoderma* enriched neem cake organic manure mixture (7.81 g) and were on par with each other. The dry root weight was minimum (4.33 g) for untreated control plants (Table 18).

4.9.11. Number of Pods

The plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) recorded the highest number of

pods (56.00) which was on par with the treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) (55.00) whereas, the untreated control plants registered the lowest number (32.33) of pods and differed significantly from all other treatments (Table 18).

4.9.12. Pod Yield

The plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) recorded the maximum yield (1.14 kg/plant) which was on par with the treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) (1.13 kg/plant), followed by treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and flusilazole (0.1 %) (1.10 kg/plant). The untreated control plants registered the minimum yield (0.80 kg/plant) and differed significantly from all other treatments (Table 18).

4.9.13. Number of Root Nodules

With respect to the number of root nodules, the plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) recorded the maximum number of root nodules (58.00) which was on par with the treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) (53.00) or tebuconazole (0.1 %) alone (47.00). The number of root nodules was found minimum (28.67) for untreated control plants (Table 18).

4.10. PERSISTENCE AND DEGRADATION KINETICS OF FUNGICIDE RESIDUES

4.10.1. LOQ and LOD

The LOQ of flusilazole, tebuconazole and carbendazim was found to be 0.01 mg kg^{-1} and LOD being 0.005 mg kg^{-1}.

Table 18. Effect of soil solarization, *Trichoderma* enriched neem cake organic manure mixture and chemicals on the growth and yield parameters of cowpea plants under field conditions

T_1	559.00^{a}	39.00^{abc}	328.67^{abc}	91.00^{abc}	46.33^{abc}	7.09^{a}	54.67^{ab}	1.10^{a}	43.33^{ab}
T_2	593.33^{a}	55.33^{a}	434.33^{ab}	112.33^{ab}	57.67^{ab}	9.75^{a}	55.50^{ab}	1.13^{a}	58.00^{a}
T_3	592.00^{a}	52.00^{ab}	425.33^{ab}	117.00^{a}	57.67^{a}	9.30^{ab}	56.00^{a}	1.14^{a}	53.00^{a}
T_4	564.00^{a}	43.67^{abc}	352.67^{abc}	88.33^{abcd}	44.33^{ab}	7.81^{ab}	54.00^{b}	1.08^{a}	46.67^{ab}
T_5	464.00^{b}	25.67^{cd}	268.00^{abc}	65.00^{cde}	27.00^{bc}	5.77^{ab}	39.27^{d}	1.02^{a}	29.00^{b}
T_6	472.00^{b}	37.67^{abc}	365.00^{abc}	82.00^{bcd}	42.00^{ab}	7.50^{b}	42.87^{c}	1.08^{a}	47.00^{ab}
T_7	479.00^{b}	29.00^{cd}	262.33^{abc}	62.33^{cde}	39.00^{abc}	5.27^{b}	44.00^{c}	1.05^{a}	31.67^{ab}
T_8	469.00^{b}	32.00^{bcd}	247.00^{bc}	56.67^{de}	29.67^{bc}	5.83^{b}	42.70^{c}	1.06^{a}	38.00^{ab}
T_9	455.00^{b}	17.00^{d}	227.00^{c}	41.67^{e}	19.67^{c}	4.33^{b}	32.33^{e}	0.80^{b}	28.67^{b}
SE	13.35	6.86	61.21	11.39	7.04	1.20	26.35	0.22	7.48

Treatment means with similar alphabets in superscript, do not differ significantly

T_1–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + flusilazole (0.1 %) T_2–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + tebuconazole (0.1%) T_3–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + carbendazim (0.1%) T_4–Soil solarization +*Trichoderma* enriched neem cake organic manure mixture T5 – Flusilazole (0.1 %) T_6 – Tebuconazole (0.1%) T_7– Carbendazim (0.1%) T_8 – Copper oxychloride (0.2 %) (Chemical Check) T_9 – Control

4.10.2. Dissipation of Fungicides

4.10.2.1. Flusilazole

Flusilazole (0.1 %) sprayed on cowpea resulted in an initial deposit of 0.61 mg kg^{-1} on fruits two hours after spraying (Table 19). On the next day, the residue became 0.53 mg kg^{-1}, indicating 13.11 % dissipation of the residues. On the third day, the dissipation increased to 37.70 % and the concentration of residue registered a lower value of 0.38 mg kg^{-1}. The residues degraded by 77.05 % on the fifth day, recording a concentration of 0.14 mg kg^{-1}. From the initial deposit, 96.72 % of the residues got dissociated on the seventh day, whereby the concentration was recorded to be 0.02 mg kg^{-1}. No residues were detected from the tenth day of spraying.

4.10.2.2. Tebuconazole

An initial deposit of 0.56 mg kg^{-1} residue of tebuconazole (0.1 %) was recorded two hours after spraying and the residue got reduced to 0.54 mg kg^{-1} with a dissipation percentage of 3.57 after one day (Table 20). On the third day, 21.43 % reduction in the initial deposit of the residues was noted, the concentration of residues being 0.44 mg kg^{-1}. Fifth day recorded an average residue deposit of 0.22 mg kg^{-1} and the dissipation percentage increased to 60.71 %. The residue level was 0.02 mg kg^{-1} on the seventh day, the dissipation percentage being 96.43 which reached below detectable level on the tenth day of spraying.

4.10.2.3. Carbendazim

The mean residue level of carbendazim (0.1 %) detected on cowpea fruits at different intervals (Table 21) showed an initial deposit of 1.65 mg kg^{-1}, dissipated to 1.35 mg kg^{-1}, with a reduction of 18.18 % one day after spraying. On the third day, the residue was reduced to 1.05 mg kg^{-1} and the dissipation percentage increased to 36.36. The residue degraded further by 73.94 % on the fifth day, the residue level recorded being 0.43 mg kg^{-1}. On the seventh day, the fruit sample

Table 19. Residue and dissipation of flusilazole in cowpea pods from treated plots

DAS	Residue mg kg^{-1}			Mean ± SD	Dissipation %
	R1	R2	R3		
0 (2 hr after spraying)	0.63	0.56	0.63	0.61±0.040	-
1	0.66	0.56	0.51	0.53±0.025	4.92
3	0.40	0.42	0.32	0.38±0.053	37.70
5	0.12	0.16	0.13	0.14±0.021	77.05
7	0.02	0.02	0.03	0.02±0.006	96.72
10	BDL	BDL	BDL	-	-

BDL – Below Detectable Level

Table 20. Residue and dissipation of tebuconazole in cowpea pods from treated plots

DAS	Residue mg kg^{-1}			Mean ± SD	Dissipation %
	R1	R2	R3		
0 (2 hr after spraying)	0.61	0.61	0.47	0.56±0.081	-
1	0.53	0.53	0.57	0.54±0.023	3.57
3	0.38	0.49	0.46	0.44±0.057	21.43
5	0.19	0.22	0.25	0.22±0.024	60.71
7	0.02	0.02	0.02	0.02±0.002	96.43
10	BDL	BDL	BDL	-	-

BDL – Below Detectable Level

Table 21. Residue and dissipation of carbendazim in cowpea pods from treated plots

DAS	Residue mg kg^{-1}			Mean ± SD	Dissipation %
	R1	R2	R3		
0 (2 hr after spraying)	1.49	1.50	1.95	1.65±0.263	-
1	1.42	1.04	1.58	1.35±0.277	18.18
3	1.02	1.01	1.13	1.05±0.067	36.36
5	0.38	0.50	0.42	0.43±0.061	73.94
7	0.31	0.33	0.23	0.29±0.053	82.42
10	0.12	0.12	0.15	0.13±0.017	92.12
15	0.03	0.03	0.04	0.04±0.004	97.58
21	BDL	BDL	BDL	-	-

BDL – Below Detectable Level DAS – Days After Spraying

recorded a residue level of 0.29 mg kg^{-1} and the degradation percentage increased to 82.42 %. The residue level reached 0.13 mg kg^{-1} with a dissipation percentage of 92.12 on the tenth day. On the 15^{th} day, the residue became 0.04 mg kg^{-1}, where 97.58 % of the residues got dissipated. The residue was below detectable level from the 21^{st} day.

4.10.3. Half life and Waiting period

The half life and waiting period of the different fungicides calculated based on the MRL values prescribed by the European Union indicated that flusilazole (0.1 %) when sprayed on cowpea fruits had a half life of 1.5 days and waiting period of 8.15 days. Tebuconazole (0.1 %) degraded to half of its initial deposit in 3.5 days and the waiting period estimated was zero days. The half life recorded for carbendazim (0.1 %) was 2.7 days and the waiting period worked out was 8.53 days (Table 22).

4.11. BENEFIT: COST RATIO

The data on benefit-cost ratio (Table 23) of integrated management of Fusarium wilt and anthracnose of cowpea using new generation fungicides revealed that the returns from treatment with carbendazim (0.1%), copper oxychloride (0.2 %) and tebuconazole (0.1 %) were high being Rs. 5.9, 4.3 and 2.8, respectively for every rupee spent. However, the lowest returns were obtained from treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and flusilazole (0.1 %).

Table 22. Half life and waiting period of different fungicides in cowpea

Fungicide	MRL(EU) mg kg^{-1}	r	R^2	Linear equation	$T_{½}$ (days)	T_{tol} /WP (days)
Flusilazole	0.02	-0.94	0.8	Y=0.20x+1.96	1.5	8.15
Tebuconazole	2	-0.89	0.7	Y=0.19x+1.97	3.5	0
Carbendazim	0.2	-0.99	0.9	Y=0.11x+2.25	2.7	8.53

r - Correlation coefficient

R^2 - Coefficient of determination

$T_{1/2}$ - Half life

T_{Tol} / WP - Waiting period

MRL - Maximum Residue Limit

EU - European Union

Table 23. Economic analysis of the experiment entitled "Integrated management of Fusarium wilt and anthracnose of vegetable cowpea using new generation fungicides"

Treatments	Yield (t/ha)	Increase in yield over control (t/ha)	Additional monetary benefits (Rs. /ha)	Additional expenses (Rs. /ha)	B : C ratio
T_1	8.25	2.25	90000	129800	-
T_2	8.48	2.48	99200	62150	1.6
T_3	8.55	2.55	102000	45200	2.3
T_4	8.10	2.10	84000	32600	2.6
T_5	7.65	1.65	66000	97200	-
T_6	8.10	2.10	84000	29550	2.8
T_7	7.88	1.88	75200	12600	5.9
T_8	7.95	1.95	78000	18000	4.3
T_9	6.00	-	-	-	-

T_1–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + flusilazole (0.1 %) T_2–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + tebuconazole (0.1%) T_3–Soil solarization + *Trichoderma* enriched neem cake organic manure mixture + carbendazim (0.1%) T_4–Soil solarization +*Trichoderma* enriched neem cake organic manure mixture T5 – Flusilazole (0.1 %) T_6 – Tebuconazole (0.1%) T_7– Carbendazim (0.1%) T_8 – Copper oxychloride (0.2 %) (Chemical Check) T_9 – Control

Discussion

5. DISCUSSION

The cultivation of cowpea, an important vegetable crop grown in Kerala for meeting dietary protein requirements, suffer from serious setback due to its susceptibility to a large number of pests and pathogens, among which diseases caused by fungi play a major role. Among the various fungal diseases, Fusarium wilt and anthracnose are widely distributed at present in all cowpea growing regions of the State leading to significant yield losses. Taking into account the diversity and pliable nature of the pathogens, management strategies solely depending on any single method of control may not be adequate for effective suppression of the diseases. In this context, the present study was undertaken to investigate the efficacy of new generation fungicides for the management of Fusarium wilt and anthracnose of vegetable cowpea and to develop an integrated management strategy using the effective fungicides compatible with ecofriendly tactics.

A systematic survey was conducted in the main vegetable growing areas of Thiruvananthapuram district during 2011-2012 to understand the disease scenario in cowpea. The incidence and severity (disease index) of various diseases were attempted which indicated that among the six major fungal diseases, anthracnose was found to be the most predominant one (0 – 55 %), followed by Cercospora leaf spot (0 – 37 %), Fusarium wilt (0 – 20 %) and web blight and collar rot (0 – 10%). Negligible incidence of powdery mildew and rust was observed at Balaramapuram and Palapoor respectively during the course of survey (Figure 3). With regard to disease index, anthracnose index ranged between 0 - 33.3, followed by Fusarium wilt (0 - 20), Cercospora leaf spot (0 - 14.16) and web blight (0 - 10.33) (Figure 4). The results of survey conducted by Banyal *et al.* (2012) implied that anthracnose index in cowpea ranged from 19.7 - 53.5 in Himachal Pradesh. The disease index was observed maximum, when the plants were raised from diseased seeds and when grown in sick soil having abundant diseased plant debris. Prasanna (1985) also reviewed that seed infection due to

C. lindemuthianum was a primary source of anthracnose spread. However, among the major fungal diseases, considerable yield losses were found attributable due to Fusarium wilt and anthracnose of cowpea.

This study provides the first documentation regarding the occurrence, distribution, yield loss and relative predominance of diseases of cowpea in Thiruvananthapuram district. Incidence of Fusarium wilt varied from 0 - 20 % with the maximum incidence (20.00 %) and index (20.00) recorded at Palapoor and Pappanchani areas whereas the incidence was absent in Vizhinjam and Kottukal regions. Reghunath *et al.* (1995) reported that Fusarium wilt has been widely distributed all through the cowpea growing fields in Kerala since 1995-96. According to Senthil (2003), incidence of cowpea wilt was observed from the farmers' fields in Ookodu, Kalliyoor, Sasthavattam, Pallichal, Sasthankoil and Peringamala areas of Thiruvananthapuram district.

With regard to Cercospora leaf spot, incidence and index ranged between 0 – 37 % and 0 - 14.16 respectively with the maximum incidence recorded at Palapoor, Pappanchani and Kalliyoor areas whereas, incidence was absent in Vizhinjam, Muttacaud, Kottukal, Kazhakkuttom and Venniyoor. The incidence of web blight and collar rot was noticed at Vizhinjam (10.00 %), Balaramapuram (10.00 %) and Palapoor (8.00 %), Kottukal (5.00 %) and Pappanchani (5.00 %). Negligible incidence of powdery mildew and rust was observed at Balaramapuram and Palapoor respectively during the conduct of survey.

Data regarding extent of yield loss (Figure 5) revealed that, among the major fungal diseases, considerable yield losses were noticed due to Fusarium wilt and anthracnose (7.05 % and 3.92 %, respectively) of cowpea in the different areas surveyed. Literature scan revealed that losses in seed yield due to Fusarium wilt in cowpea ranged from 8.30 to 86.51% (Assuncao *et al.,* 2003) while, Enyiukwu and Awurum (2013) reported that cowpea anthracnose resulted in 50 % grain yield reduction in severely affected crop.

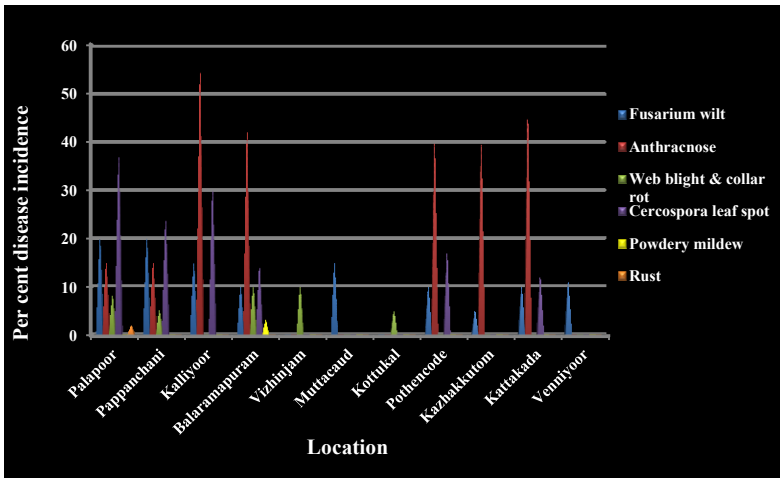


Figure 3. Incidence of major fungal diseases in cowpea during 2011-12

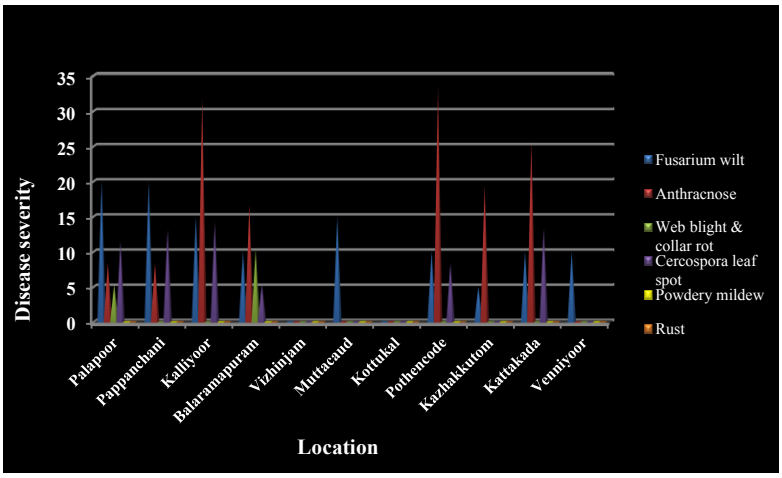


Figure 4. Severity (disease index) of major fungal diseases in cowpea during 2011-12

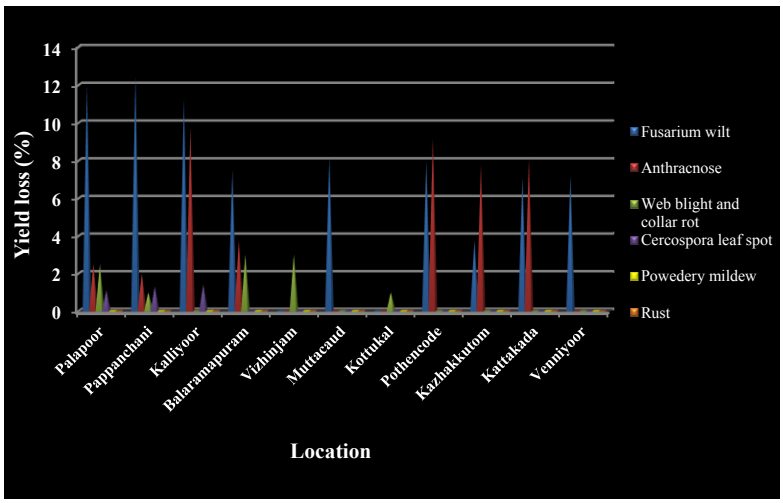


Figure 5. Yield loss due to major fungal diseases in cowpea during 2011-12

The characteristic symptoms of Fusarium wilt of cowpea appeared as yellowing, withering and drooping of leaves followed by defoliation and drying up of vines. The base of the stem became swollen and resembled a small tuber which later disintegrated resulting in shredding. The taproot and lateral roots were also affected. Occasionally, abnormal flattening of the stem along the growing tip, fasciation and sterility of flowers were also noticed. The symptoms observed were similar to the depictions given by Senthil (2003). Gokulapalan *et al.* (2006) had confirmed the association of *F. pallidoroseum* with fasciation of vegetable cowpea in Kerala.

Anthracnose of cowpea appeared as circular to irregular brown necrotic spots on leaves, spindle shaped lesions with light grey centre and reddish brown margin on the stem and vines and irregular deep seated reddish brown spots on the pods. Onesirosan and Barker (1971) observed brown to tannish pink, sunken stem lesions with dark margins which later girdled stem, branches and petioles in anthracnose affected cowpea plants. Brown sunken lesions appeared on pods also (Williams, 1975).

In the present study, isolates of *Fusarium* and *Colletotrichum* spp. obtained from major cowpea growing areas of Thiruvananthapum district showed much variability in their cultural, morphological and pathogenic attributes. The colonies of isolates F1, F2, F3, F4, F6, F8 and F9 of *Fusarium* spp. were appressed to floccose in texture, white on the upper surface, reddish brown or faint pink on the lower side of the petridish. Phialides were sub cylindrical to slightly obclavate. Macroconidia were 3 - 5 septate, 4.6 - 7.4 x 1.4 -2.7 µm in size, thin walled, fusoid, pointed ends, occasionally falcate with terminal cell hooked and pedicellated basal cell. Microconidia were abundant, oval to ellipsoidal, cylindrical, straight or curved. Chlamydospores when present were terminal in position. Honnareddy and Dubey (2007) also reported that isolates of *F. oxysporum* f. sp. *ciceris* collected showed variable pigmentation of medium from normal white to violet, brown, reddish violet, greenish violet, yellowish pink and dark green. The shape and size of macro conidia and micro conidia were in

accordance with the isolates of *F. oxysporum* f. sp. *udum* and *F. oxysporum* f. sp. *ricini* (Madhukeshwara, 2000 and Desai *et al.*, 2003).

The colonies of the isolates F7, F11 and F12 appeared floccose in texture, dull white on the upper surface and yellowish to dark brown on the lower side of the petridish. Phialides were short, compact and obclavate. Macroconidia were 3 - 5 septate, 14.8 - 17.2 x 2.2 – 3 µm in size, thick walled, typically falcate, tapering towards both ends, bent at central part with elongated apical cell and pedicellate basal cell. Microconidia were oval to ellipsoidal in shape. Chlamydospores when present were intercalary, solitary or in chains. Motlagh (2010) described *F. equiseti* associated with *Echinochloa* spp. as producing abundant mycelium that was initially white, turning brown on ageing with concomitant pale to dark brown pigmentation. Macroconidia were long and slender, had a dorsiventral curvature with tapered and elongated or whip-like apical cell and foot shaped basal cell.

Colonies of F5 and F10 appeared sparse with creamy white upper surface and dirty white on the lower side of the petridish. Short, sub cylindrical, obclavate monophialides produced slender macroconidia and long microconidia. Macroconidia were 3 - 5 septate, thick walled, subcylindrical, slightly curved, short and bent apically, indistinctly pedicellate basal cell and measured 7.9 - 10.2 x 1.8 - 2.6 µm in size. Microconidia were ovoid and straight or rarely ellipsoidal to curved. Chlamydospores were abundant, terminal or intercalary in position formed in single, pairs or chains. The results were in agreement with the depiction of morphological and cultural variability among the isolates of *F.solani* by Chandran and Kumar (2012).

Hence, based on the morphological and cultural characterization as well as the comparison with the standard keys described by Booth (1971), the isolates of *Fusarium* spp. were tentatively identified as *F. oxysporum* Schlecht, *F.equiseti* (Corda) Sacc*., F.equiseti* (Corda) Sacc. and *F.solani* (Mart.) Sacc. The species diversity in relation to geographical locations in the district has been scientifically

depicted for the first time with respect to Fusarium wilt. The study revealed variability did exist in the cultural and morphological characters as well as pathogenicity of the isolates of *Fusarium* spp. of cowpea from different locations. Among the 12 isolates, *F. oxysporum* was the prevalent species and widely distributed as it was found to be present in six locations examined. *F. equiseti* and *F. solani* were noted as less frequent species and were obtained from three and two locations, respectively.

Senthil (2003) also reported the association of *F. oxysporum* and *F. solani* with wilt of cowpea in Kerala. Barhate *et al.* (2006) and Mandhare *et al.* (2007) had also observed variability in morphological and cultural characters as well as pathogenicity among *F. udum* isolates involved in causing wilt of chickpea. Root infection in cowpea due to *F.equiseti* had been reported earlier by Ramachandran *et al.* (1982). The involvement of several isolates of *Fusarium* with wilt of cowpea points out the complexity of the disease. Hence, these observations foretell the chances of development of more severe and virulent strains through interspecific hybridization, mutation etc. which can lead to severe epiphytotics.

With regard to *Colletotrichum* spp., the colonies appeared sparse, dense or fluffy with whitish aerial mycelium becoming grey. Colony reverse was light-dark greyish to black. Light orange colored slimy spore mass were produced either outward from the centre of the colony or near the inoculation point. Black acervuli and abundant setae were observed. While, sporulation was sparse and acervuli and setae were lacking in C4 and C6. The mycelia appeared hyaline, septate and branched. Conidia were hyaline, straight, cylindrical with both apices rounded or with one apex rounded and the other end pointed. Size of conidia varied from 8.6 - 11.3 x 3.5 - 4.3 µm. Appressoria formed were irregular, clavate or ovoid and light to dark brown in color.

The nature of growth, color, branching pattern, septation in hyphae and the characters of acervuli and setae of different isolates of *Colletotrichum* spp. obtained in this study were in accordance with the characters of

C. gloeosporioides (Chowdappa *et al.,* 2012). The conidial measurements of isolates examined in this study also fit within the measurements of spore size of *C. gloeosporioides* reported previously (Jayasinghe *et al.,* 1997 and Chowdappa *et al.,* 2012). Thus, based on the morphological and cultural characterization as well as the comparison with the standard keys described by Chowdhry and Varshney (2000), the isolates of the pathogen associated with anthracnose of cowpea were tentatively identified as, *C.gloeosporioides* Penz. Earlier works conducted by Fassoni *et al.* (2012) and Mahmodi *et al.* (2012) had also reported the association of *C. gloeosporioides* with legumes.

The identity of the species of both the pathogens was further confirmed through ITS- rDNA sequence analyses. The internal transcribed spacer region of rDNA is the most used target sequence in the molecular detection of fungi and is also the most employed marker used to infer lower level taxonomy in fungi (Bruns, 2001). The ITS and 5.8S regions of rDNA are useful for *Fusarium* species identification and are used in analyses of phylogenetic relationships on the species level and below (O'Donnell and Cigelnik, 1997).

Amplification of the ITS- rDNA region of the 12 different isolates of *Fusarium* spp. using universal primers ITS 1 and ITS 4 yielded an amplicon of 500 - 530 bp long. The results were in accordance with findings that the amplification of ITS- rDNA region of *Fusarium* spp. belonging to sections Elegans, Gibbosum and Martiella which include *F. oxysporum*, *F. equiseti* and *F. solani* respectively, was approximately 550 bp (Lee *et al.,* 2000). An identity of 99 - 100% was observed between the *Fusarium* isolates obtained in this study and other sequences of *Fusarium* spp. available in NCBI database. The uniformity of ITS fragment size across several fungal groups makes nucleotide sequencing of ITS fragments necessary to reveal interspecific and intraspecific variations (Batista *et al.,* 2008).

According to the phylogenetic tree constructed from ITS- rDNA region, sequences of the 12 isolates of *Fusarium* spp. were divided into four clusters.

Cluster I and III included strains belonging to section Elegans with 99 % and 93 % bootstrap support respectively. All the strains belonging to section Gibbosum was included in cluster II with a high bootstrap support (100 %). While cluster IV included strains belonging to section Martiella with a high bootstrap support (100 %). Sequence analysis of ITS region to differentiate *Fusarium* at the species level and to determine the phylogenetic relationships had been attempted earlier by other workers as well (Lee *et al.,* 2000 and Shahnazi *et al*., 2012).

With respect to *Colletotrichum* spp., the amplification of the ITS- rDNA region of eight isolates using universal primers ITS 1 and ITS 4 yielded an amplicon of approximately 540 bp long which was in accordance with the findings of Chowdappa *et al.* (2012). The BLAST similarity search confirmed the results obtained as the ITS sequences from *C. gloeosporioides* in this study shared 99 - 100% sequence similarity with the sequences of *C. gloeosporioides* at NCBI.

A phylogenetic tree constructed from ITS- rDNA sequences using neighbor-joining (NJ) method grouped the isolates of *C. gloeosporioides* into two clusters indicating the complexity of the species while, other principal *Colletotrichum* spp. infecting grain legumes were grouped into a third cluster. The results were in accordance with Adhipathi *et al.* (2014) who used the amplification of ITS region and sequencing of *C. gloeosporioides* causing anthracnose in turmeric. Similar attempts had been performed by other workers as well (Photita *et al.*, 2005; Forseille *et al*., 2011; Arzanlou and Torbati, 2012 and Raj *et al*., 2013b).

Studies on pathogenicity and comparative virulence of the isolates of *Fusarium* spp. and *Colletotrichum* spp. was carried out following Koch's postulates. *Fusarium* spp. showed wide variability with respect to their pathogenesis related attributes. Of the 12 isolates tested, eight induced wilt with vascular discoloration and foliar yellowing, whereas the isolates F7, F8, F10 and F11 were found to be non – pathogenic and did not produce any symptoms. The isolate F2 (*F. oxysporum*) from Palapoor took only seven days for the

development of symptoms and was found to be highly pathogenic. Casas and Diaz (1985) also reported that isolates of *F. oxysporum* induced foliar yellowing with or without vascular discoloration along with collar and root necrosis on a chickpea cultivar 15 days after artificial inoculation. Similarly, Jasnic *et al.,* (2005) reviewed that *F. oxysporum* exhibited a significant amount of pathogenicity to artificially infected soybeans among other species *viz.,* *F. avenaceum, F. equiseti* and *F. poae*. isolated from diseased soybean plants. On the contrary, Senthil (2003) observed that some of the *F. oxysporum* isolates were non-pathogenic to artificially inoculated cowpea seedlings and it may be due to the endophytic nature of these isolates. Rodrigues and Menezes (2006) also identified the involvement endophytic *F. oxysporum* with cowpea seeds.

Among the eight isolates of *Colletotrichum* spp. tested, the isolate C7 (*C. gloeosporioides*) from Kattakada took only four days for the initiation of symptoms and was found to be highly pathogenic while, the isolates C2, C4 and C6 did not produce any symptoms. As observed by Singh and Singh (2006), cowpea plants sprayed with spore and hyphal suspension of *C. lindemuthianum* produced brown colored spots of 3 - 5 mm on the leaves after 48 h of incubation.

The above mentioned observations foresee the chances of development of more virulent pathogenic strains that could pave way for severe epiphytotics in future. For this reason, timely detection and management of Fusarium wilt and anthracnose of cowpea is necessary to avoid heavy crop loss due to these diseases. Among the management options, use of new generation fungicides that are ecologically safe with lower toxicological profiles sounds better. Hence, selected new generation fungicides *viz.,* propiconazole (0.1 %), chlorothalonil (0.2 %), thiophanate-methyl (0.1 %), flusilazole (0.1 %), azoxystrobin (0.15 %), tebuconazole (0.1 %), captan + hexaconazole (0.1 %), carboxin + thiram (0.4 %), copper hydroxide (0.25 %), mancozeb (0.25 %), carbendazim (0.1 %) and copper oxychloride (0.2 %) were evaluated *in vitro* and *in vivo*, so as to determine their efficacy against *F. oxysporum* and *C. gloeosporioides*.

The results of *in vitro* evaluation of fungicides against *F. oxysporum* (Figure 6) revealed that tebuconazole (0.1 %), carboxin + thiram (0.4 %), mancozeb (0.25 %) and carbendazim (0.1 %) gave 100 % inhibition of the mycelial growth of the pathogen. Total suppression of the mycelial growth of *F. oxysporum* by carbendazim had been observed earlier by other workers as well (Sharma *et al.,* 2002; Tripathi *et al.* 2007 and Singh *et al.*, 2010). Araujo *et al*. (2008) observed that carboxin + thiram @ 0.05 %, 0.1 %, 0.2 % and 0.3 % had the best effect on reducing the population of *F. oxysporum* f. sp. *cubense*. Kanwal *et al.* (2012) reported that tebuconazole (@ 0.007 %, 0.014 % and 0.033 %) completely arrested the mycelial growth of *F. oxysporum* f. sp. *lycopersici* at all concentrations tested. Among the fungicides, the least per cent inhibition (41.50 %) of the mycelial growth of the pathogen was recorded by azoxystrobin (0.15 %). On the contrary, Araujo *et al*. (2008) reported that Azoxystrobin (@ 0.01, 0.1, 1, 2, 3 and 4 ppm) was highly effective in reducing the population of *F. oxysporum* f. sp. *cubense*.

The results of *in vitro* evaluation of fungicides against *C. gloeosporioides* (Figure 6) revealed that propiconazole (0.1 %), flusilazole (0.1 %), tebuconazole (0.1 %), and carboxin + thiram (0.4 %) were the best in inhibiting (100 %) mycelial growth of the pathogen followed by thiophanate-methyl (0.1 %) and carbendazim (0.1 %) which gave 92.58 % and 91.30 % inhibition, respectively. The least per cent inhibition (25.37) of the mycelial growth was observed in azoxystrobin (0.15 %). Efficacy of propiconazole (@ 0.04 %) in the total suppression of mycelial growth of *Colletotrichum* spp. associated with legume anthracnose has been reported earlier (Shovan *et al*., 2008 and Rajesha *et al.*, 2010). Manjunath *et al*. (2013) noted 100 % inhibition of the mycelial growth of *C. lindemuthianum* by carbendazim (@ 0.01 %, 0.025 %, 0.05 % and 0.075 %).

Among the contact fungicides, mancozeb (0.25 %) completely inhibited the mycelial growth of *C. gloeosporioides* whereas, copper oxychloride (0.02 %) recorded the least (30.09 %) inhibition. Similarly, Manjunath *et al*. (2013) also found that mancozeb (0.2 %) was effective in inhibiting the mycelial growth

C. lindemuthianum up to 100 % while, the least inhibition (0.00 %) was observed with copper oxychloride.

Knowledge on the compatibility of fungicides with biocontrol agents and beneficial microbes is imperative for their successful adoption in an integrated disease management package. A laboratory experiment was carried out to assess the compatibility of selected new generation fungicides with the biocontrol agents (*T. viride* and *P. fluorescens*) and the root nodule bacterium (*Rhizobium* spp.) (Figure 7).

Studies on fungicide compatibility revealed that *T. viride* was incompatible with most of the common fungicides tested. Propiconazole (0.1 %), flusilazole (0.1 %), tebuconazole (0.1 %) and carbendazim (0.1 %) exerted 100 % inhibition of *T. viride* whereas; chlorothalonil (0.1 %), thiophanate-methyl (0.1 %), captan + hexaconazole (0.1 %), carboxin + thiram (0.4 %) and copper hydroxide (0.25 %) recorded more than 85 % inhibition of the mycelial growth of *T. viride* at their recommended dosage. Incompatibility of *T.viride* with propiconazole, tebuconazole, carbendazim and chlorothalonil had been reported earlier by other workers (Bagwan, 2010; Madhusudhan *et al.*, 2010 and Ranganathaswamy *et al*., 2012).

The contact fungicides, mancozeb (0.25 %) and copper oxychloride (0.2 %) were moderately compatible with mycelial growth inhibition in the range of 20 -50 %. The result was in accordance with the findings of Bagwan (2010). In the present study, azoxystrobin (0.15 %) was found highly compatible without the inhibition (0.00 %) of mycelial growth of *T. viride.* However, Ranganathaswamy *et al*. (2012) noted only a moderate compatibility of *T. viride* with azoxystrobin (0.1 %) with growth inhibition in the range of 20 – 45 %.

None of the fungicides tested inhibited the growth of *P. fluorescens* at their recommended concentration and was found to be highly compatible. Previous reports also support the compatibility of *P. fluorescens* with different fungicides

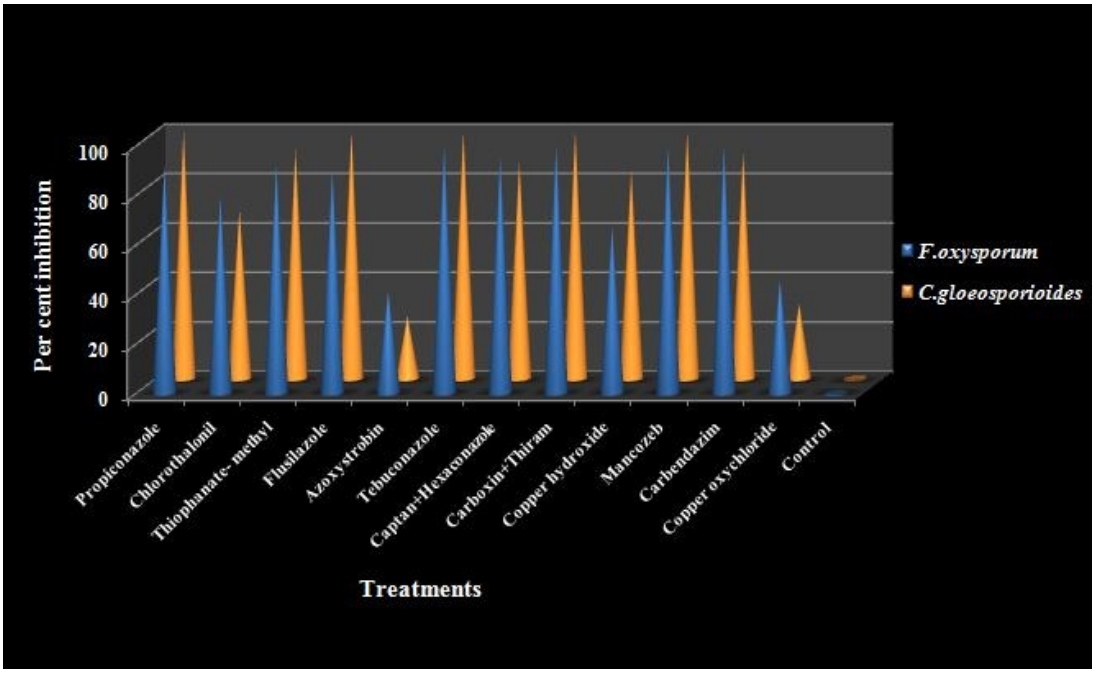


Figure 6. Efficacy of fungicides on the *in vitro* suppression of *F.oxysporum* and *C.gloeosporioides*

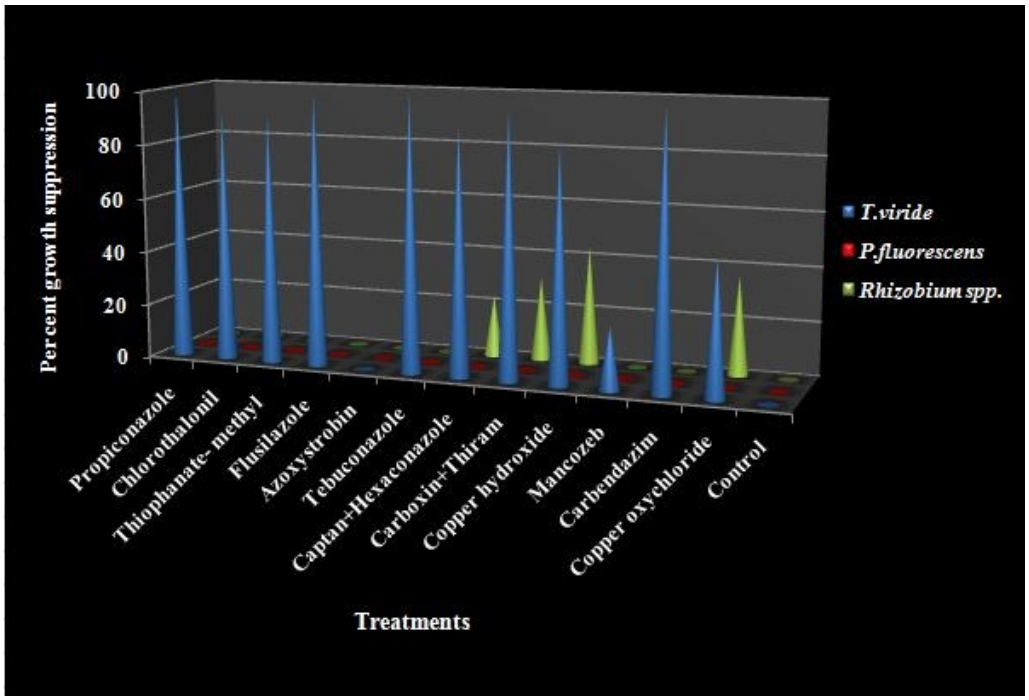


Figure 7. Effect of fungicides on the growth of beneficial microbes

(Vrinda, 2002; Khan and Gangopadhyay, 2008; Anand *et al.*, 2009 and Raj *et al.*, 2013).

Compatibility of fungicides with *Rhizobium* spp. indicated that, all the fungicides tested were highly compatible with *Rhizobium* spp. while, captan + hexaconazole (0.1 %), carboxin + thiram (0.4 %), copper hydroxide (0.25 %) and copper oxychloride (0.2 %) were found only moderately compatible. Singh and Mehta (2005) observed that carbendazim (0.1 %) and thiophanate-methyl (0.1 %) were fully compatible with *Rhizobium* spp. while, mancozeb showed inhibition at 0.2 %.

Pot culture experiments were also conducted to draw a more specific conclusion on the relative efficacy of the selected new generation fungicides against Fusarium wilt and anthracnose of cowpea. Observations on disease incidence, index and biometric characters such as plant height, root length, fresh and dry weight of shoots and roots, number of pods, pod yield and number of root nodules were recorded.

In vivo evaluation of new generation fungicides against Fusarium wilt of cowpea indicated that, soil drenching of flusilazole (0.1 %), tebuconazole (0.1 %) or carbendazim (0.1 %) @ 30, 45 and 60 days after seed emergence were highly effective in total suppression of wilt incidence (Figure 8) with the lowest disease index (0.00). Soil drenching with contact fungicides *viz.,* chlorothalonil (0.2 %), copper hydroxide (0.25 %), mancozeb (0.25 %) and copper oxychloride (0.2 %) registered an index of 25.00 (Figure 9). The findings were in agreement with Madhavi and Bhattiprolu (2011) who reported that soil drenching of carbendazim (0.1 %) and tebuconazole (0.3 %) recorded 100 % inhibition of *F. solani* at 10 and 15 cm inoculum depths. Senthil (2003) also observed that carbendazim was superior in the *in vivo* suppression of Fusarium wilt of cowpea.

In vivo evaluation of new generation fungicides against anthracnose of cowpea revealed that three rounds of foliar application of flusilazole (0.1 %) @ 30, 45 and 60 days after seed emergence recorded the least disease incidence

(50.00 %) followed by treatments with propiconazole (0.1 %), tebuconazole (0.1 %) or carbendazim (0.1 %) (75.00 %) (Figure 8). The data on anthracnose index revealed that the plants treated with flusilazole (0.1 %) recorded the lowest disease index (5.60) followed by treatment with tebuconazole (0.1%) (8.30). This was followed by treatment with propiconazole (0.1%), carbendazim (0.1 %) or thiophanate–methyl (0.1 %) with an index of 13.90 and 15.74 respectively. Among the contact fungicides tested, treatment with copper oxychloride (0.2 %) registered the lowest disease index of 20.30 (Figure 9).

Mafacioli *et al.* (2006) reported that tebuconazole (0.2 %) when sprayed seven times, at 15 days intervals significantly reduced anthracnose index on peach plants, providing control up to 68 – 78 %. Efficiency of carbendazim on reducing anthracnose index had been reported earlier by other workers as well (Purushothaman *et al.,* 2007; Gawade *et al.,* 2009 and Chauhan and Bhatia, 2012). On the contrary, Thomas *et al.* (2008) noted that tebuconazole and carbendazim were less effective against anthracnose incidence on lupin pods. Among the contact fungicides, the efficiency of copper oxychloride against anthracnose had been reported by Chauhan and Bhatia (2012) and Goswami *et al.*(2013).

The application of chemical fungicides also helped in improving the growth of the plants (Figure 10 & 11). The biometric parameters were increased to varying extent by fungicide application which was in accordance with previous findings (Senthil, 2003 and Singh *et al.,* 2010).

Even though, fungicide application remains the easiest and reliable approach for the maintenance of healthy crops in a commercial cultivation, sole use of these chemicals is associated with problems of resistant strain development, ecological risks and health hazards which had given way to the concept of integrated disease management (IDM) for crop protection and production improvement in a sustainable manner. Hence, based on the results of pot experiments, three effective fungicides *viz.,* flusilazole (0.1 %), tebuconazole (0.1 %) and carbendazim (0.1 %) which showed the least incidence and index of both the

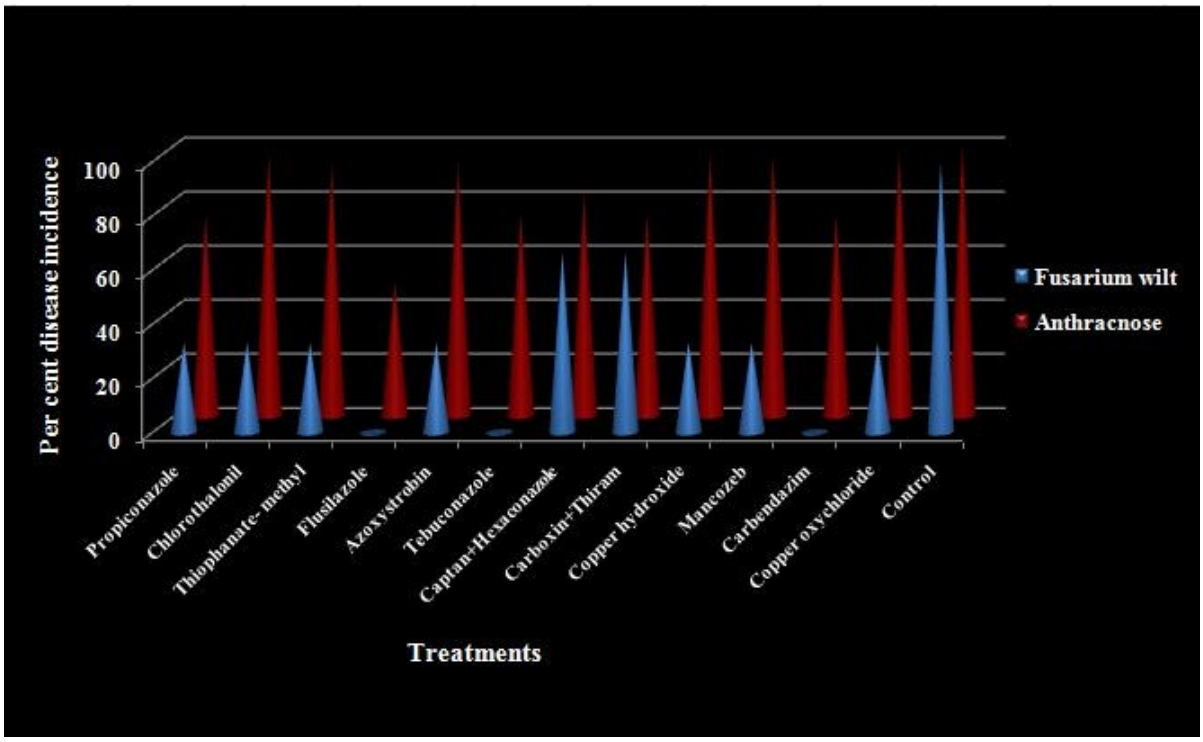


Figure 8. Effect of fungicides on the incidence of Fusarium wilt and anthracnose of cowpea under *in vivo* conditions

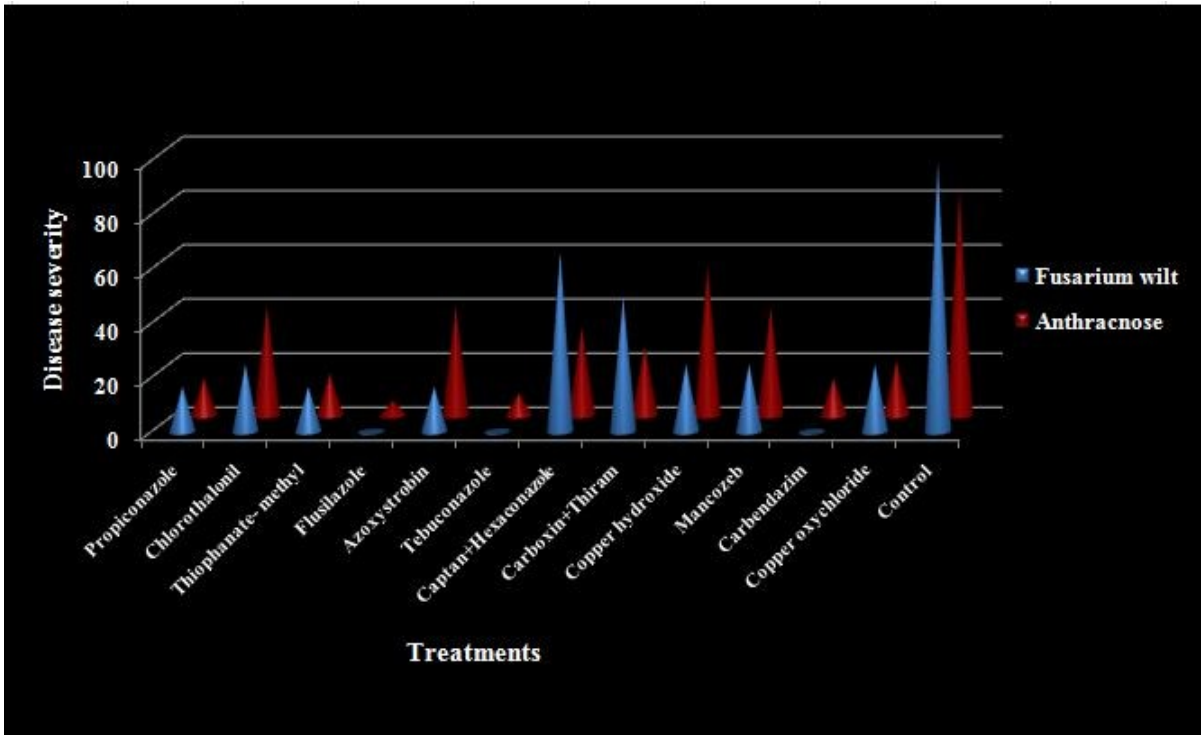


Figure 9. Effect of fungicides on the severity (disease index) of Fusarium wilt and anthracnose of cowpea under *in vivo* conditions

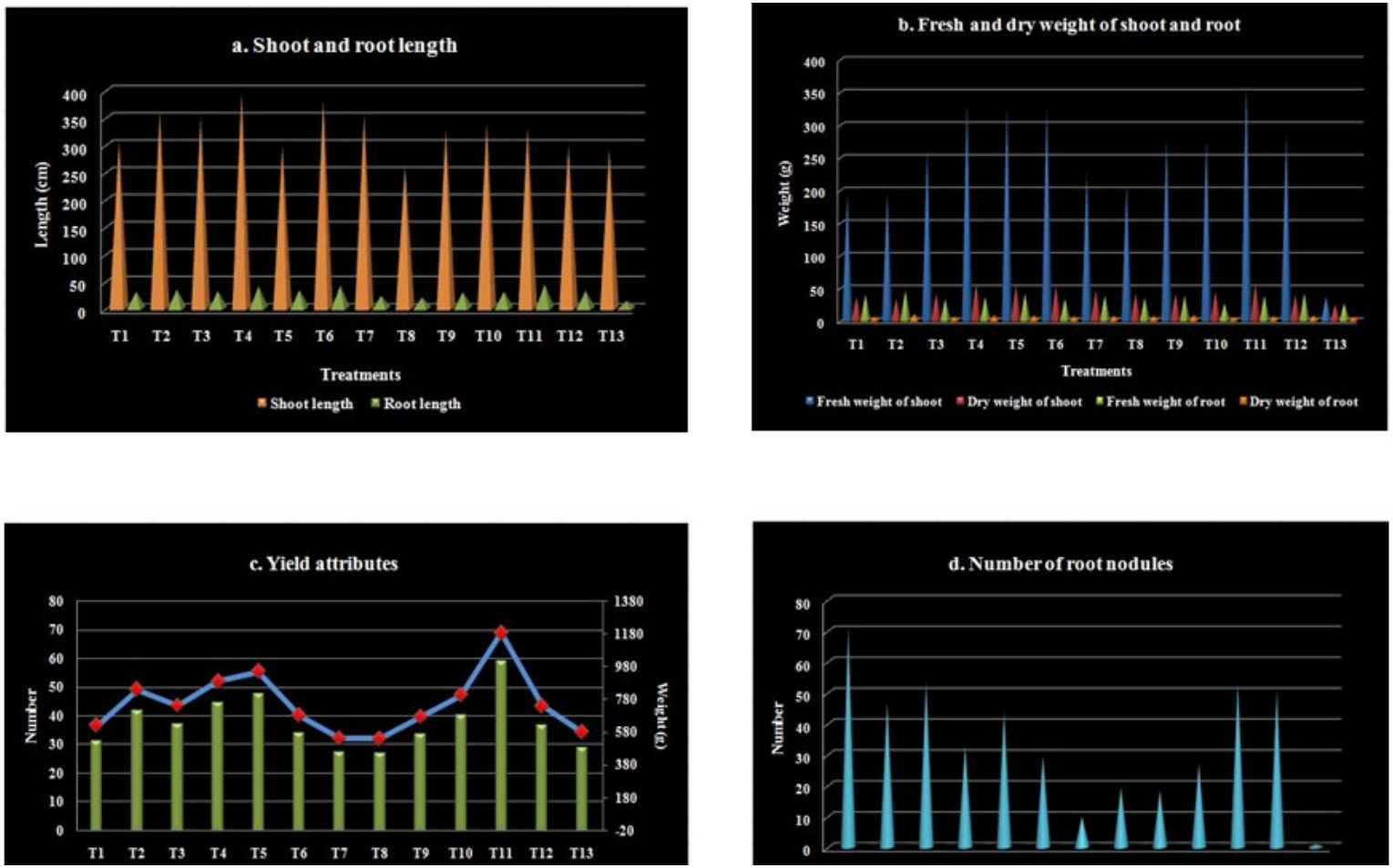


Figure 10. *In vivo* efficacy of different fungicides on the growth and yield parameters of cowpea plants inoculated with *F. oxysporum*

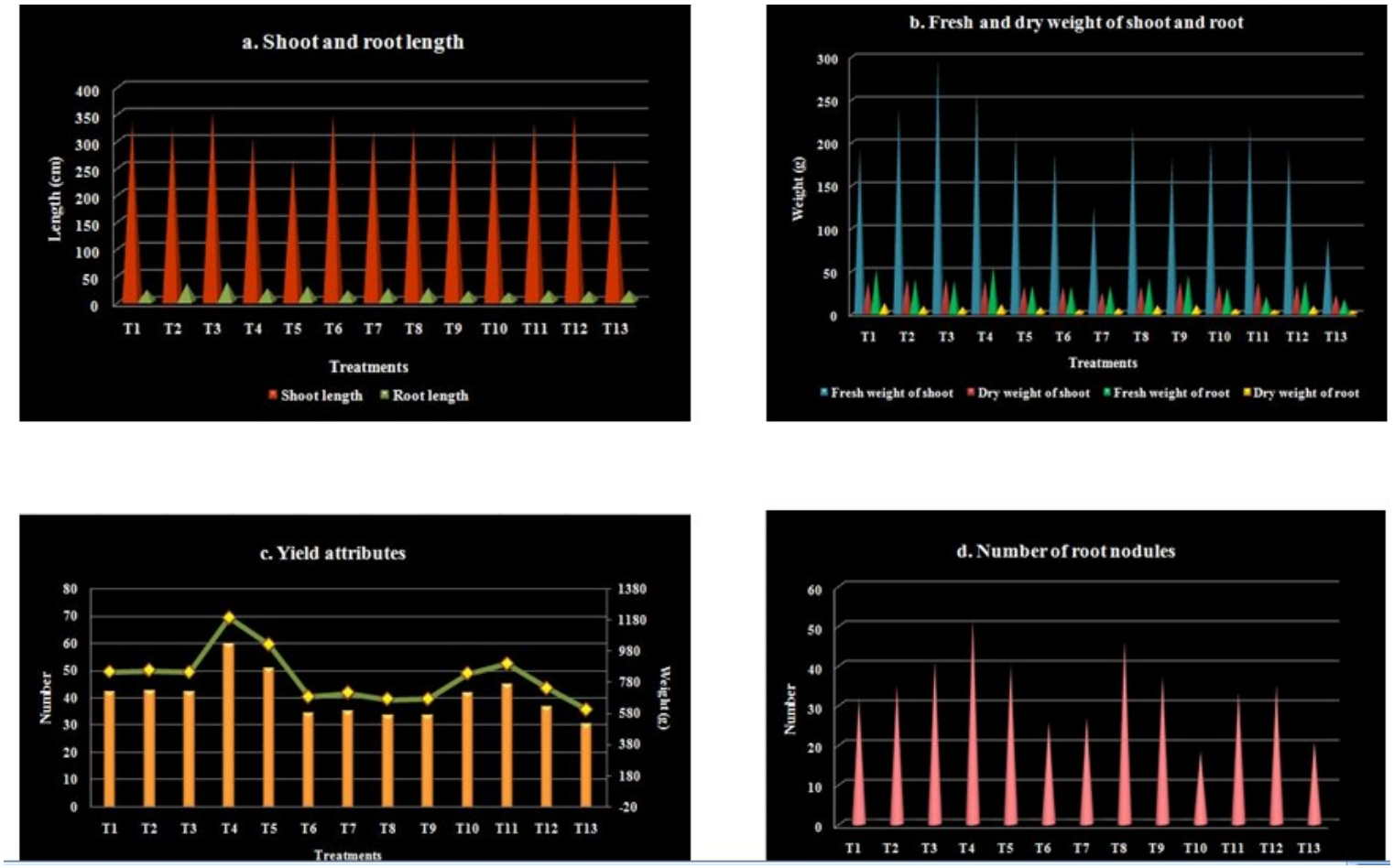


Figure 11. *In vivo* efficacy of different fungicides on the growth and yield parameters of cowpea plants inoculated with *C. gloeosporioides*

diseases along with seed treatment (carbendazim @ 2g/kg seed), soil solarization and *Trichoderma* enriched neem cake organic manure mixture were evaluated in the field to determine their efficacy in the integrated management of Fusarium wilt and anthracnose of cowpea.

The field experiment conducted to develop an IDM package for Fusarium wilt of cowpea indicated that soil solarization, application of *Trichoderma* enriched neem cake organic manure mixture and soil drenching of either tebuconazole (0.1 %) or carbendazim (0.1 %) totally suppressed the incidence of Fusarium wilt (Figure 12) and recorded the lowest (0.00) disease index followed by treatment with copper oxychloride (0.2 %) (8.33) (Figure 13).

Earlier reports also indicated that integration of soil solarization, organic amendments, biocontrol agents and fungicide application reduced wilt incidence. Jadeja and Nandoliya (2008) reported that integration of soil solarization for 15 days in summer followed by growing of sorghum in *kharif* and application of either carbendazim granules @ 10 kg/ha one month after sowing or application of *T.viride* in organic carrier @ 62.5 kg/ha was highly effective for the management of cumin wilt. Similarly, Ojha and Chatterjee (2012) also reported that combining soil solarization along with application of *T. harzianum*, neem extract and captan (0.01%) resulted in 100 % reduction of Fusarium wilt of tomato in the vegetable fields of West Bengal. Studies conducted by Senthil (2003) revealed that combination of seed treatment (4 g/kg seed) and soil application (2.5 kg/ha) of *T. viride*, soil application of neem cake 150 kg/ha and soil drenching of mancozeb (0.3 %) effectively suppressed Fusarium wilt of cowpea and also appreciably increased the biomass and pod yield of crop.

Integrated disease management of anthracnose of cowpea revealed that foliar application of either flusilazole (0.1 %) or tebuconazole (0.1 %) registered the lowest (8.33 %) anthracnose incidence (Figure 12). The data on disease index indicated that seed treatment (carbendazim @ 2g/kg seed) along with foliar application of either flusilazole (0.1 %) or tebuconazole (0.1 %) recorded the

lowest (0.93) index of anthracnose followed by treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) (4.63). Foliar application of copper oxychloride (0.2 %) recorded an index of 18.52 (Figure 13). The results were in accordance with Varaprasad (2000) who reported that seed treatment with carbendazim @ 2 g/kg seed combined with two foliar sprays of carbendazim + mancozeb (0.05 %) at 15 days interval gave maximum reduction in chickpea blight incidence. Deeksha and Tripathi (2002b) also suggested that seed treatment followed by two prophylactic sprays of either carbendazim or propiconazole @ 0.1 % each at 15 days interval showed minimum disease index of black gram anthracnose.

Soil solarization and application of *Trichoderma* enriched neem cake organic manure mixture significantly improved the growth and yield parameters of plants (Figure 14) in addition to the reduction of soil borne pathogens, which was in agreement with several previous reports.

Soil solarization suppress soil borne pathogens such as fungi, bacteria, nematodes and pests along with weed seeds leading to better growth and yield of plants. Negi and Raj (2013) observed that solarization with transparent polyethylene sheet of 25 µm thickness for 40 days inside polyhouse reduced carnation wilt incidence by 81.82 %. Patel *et al.* (2008) reported that maximum plant height, number of branches, number of pods per plant, total dry matter accumulation as well as pod and haulm yields of groundnut were registered when transparent polyethylene (TPE) sheet of 0.025 mm was kept for 45 days. Kumar *et al.* (2002) also reported that solarization of tomato fields using 0.05 mm transparent polythene sheet resulted in tallest plants (78.4 cm), large sized fruits (0.893 kg/plant), highest number of branches per plant (8.20 per plant), leaf area index (2.563) and crop yield (21.6 t/ha).

Trichoderma spp. through the production of secondary metabolites, cell wall degrading enzymes, mycoparasitism, competitive saprophytic ability etc., can compete with soil borne pathogens and bring about their effective suppression

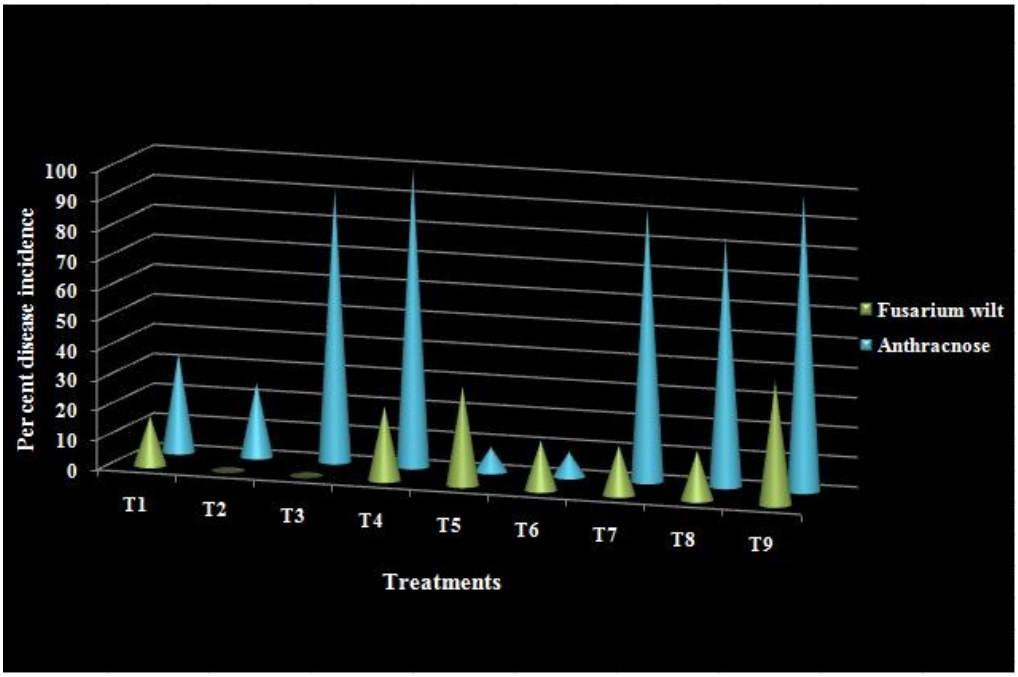


Figure 12. Effect of soil solarization, *Trichoderma* enriched neem cake organic manure mixture and chemicals on the incidence of Fusarium wilt and anthracnose of cowpea under field conditions

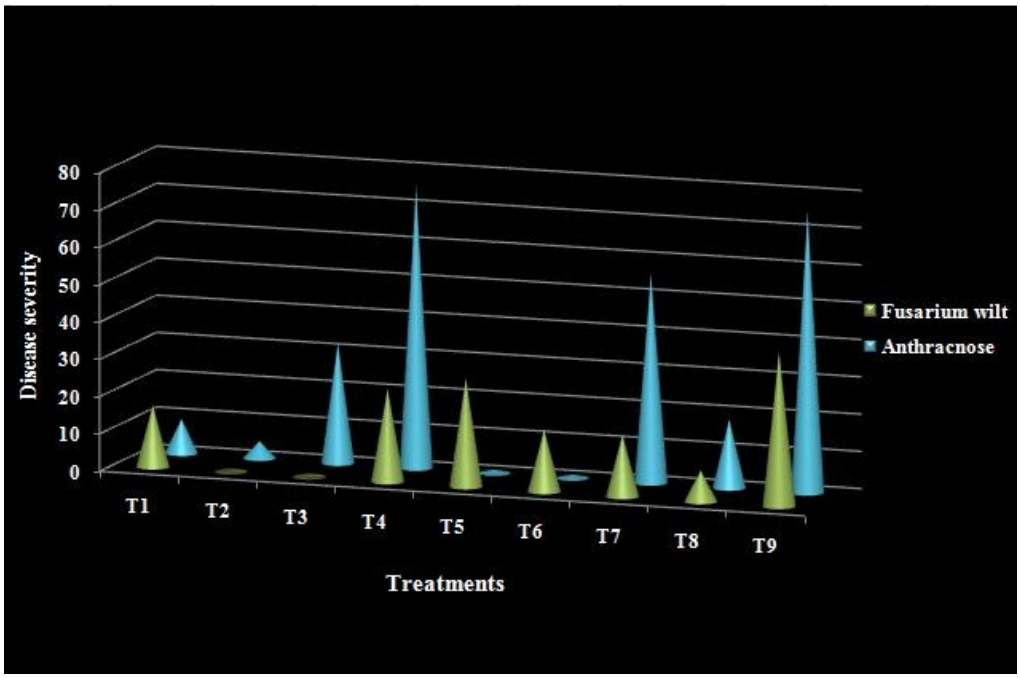


Figure 13. Effect of soil solarization, *Trichoderma* enriched neem cake organic manure mixture and chemicals on the severity (disease index) of Fusarium wilt and anthracnose of cowpea under field conditions

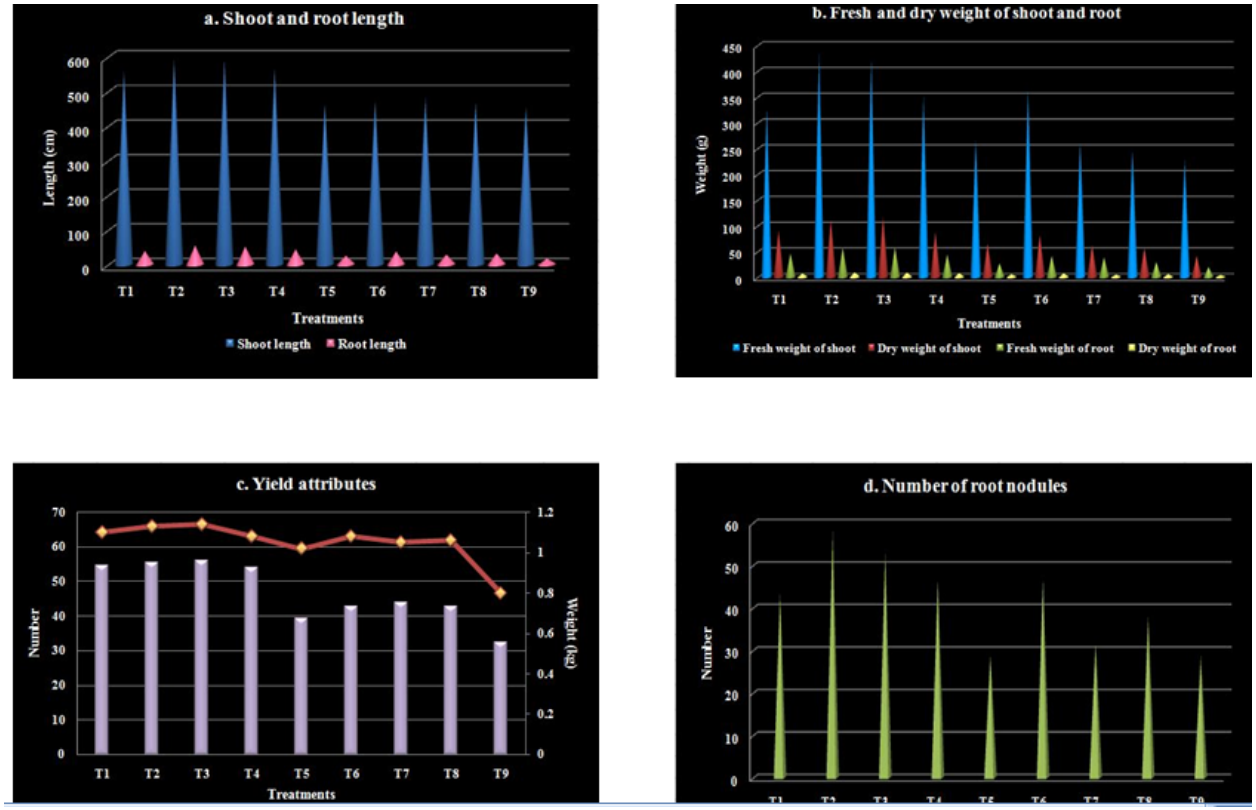


Figure 14. Effect of soil solarization, *Trichoderma* enriched neem cake organic manure mixture and chemicals on the growth and yield parameters of cowpea plants under field conditions

(Lewis and Papavizas, 1984). It has been proved to be a potential biocontrol agent for managing several soil borne diseases under green house and field conditions (Hardar *et al.,* 1979). Incorporation of organic manure and organic amendments like neem cake to the soil nourish the growth of *T. viride* in addition to the suppression of soil borne pathogens. Soil amendments also influence soil physical properties such as pore size, aeration, water retention *etc*., and thereby facilitating rapid extension of root system and better uptake of nutrients thus improving the vigor of plants.

Role of *Trichoderma* enriched neem cake organic manure mixture in plant growth promotion and suppression of soil borne pathogens has been observed earlier by other workers as well. Kulkarni and Anahosur (2011) reported that pre sowing application of organic manure + neem cake + *T. harzianum* + *T. viride* was most effective in avoiding stalk rot infection in maize and recorded the maximum plant stand (97.33 %) and highest grain yield (1363.14 kg/ha). Similarly, Latha (2013) also observed that least collar rot incidence (20.4 %) and maximum ground nut pod yield (1321 kg ha^{-1}) was recorded in the combined application of *P. fluorescens*, *T. viride*, neem cake and farmyard manure.

Since, indiscriminate use of pesticides on any crop leads to accumulation of residues, it is mandatory to study its degradation kinetics prior to the recommendation of any pesticide for field use. Pesticide residue level exceeding the maximum residue limit (MRL) reflects the improper use of pesticides and so, rectification at source is required. With this view, the dissipation pattern of the fungicides tested in the field was studied and is depicted.

Flusilazole (0.1%) when sprayed on cowpea resulted in an initial deposit of 0.61 mg kg^{-1} on fruits two hours after spraying. From the initial deposit, 96.72 % of the residues dissociated on the seventh day, whereby the concentration was recorded to be 0.02 mg kg^{-1} which reached the below detectable level on the tenth day of spraying. An initial deposit of 0.56 mg kg^{-1} residue of tebuconazole (0.1 %) was recorded two hours after spraying. The residue level was 0.02 mg kg^{-1} on

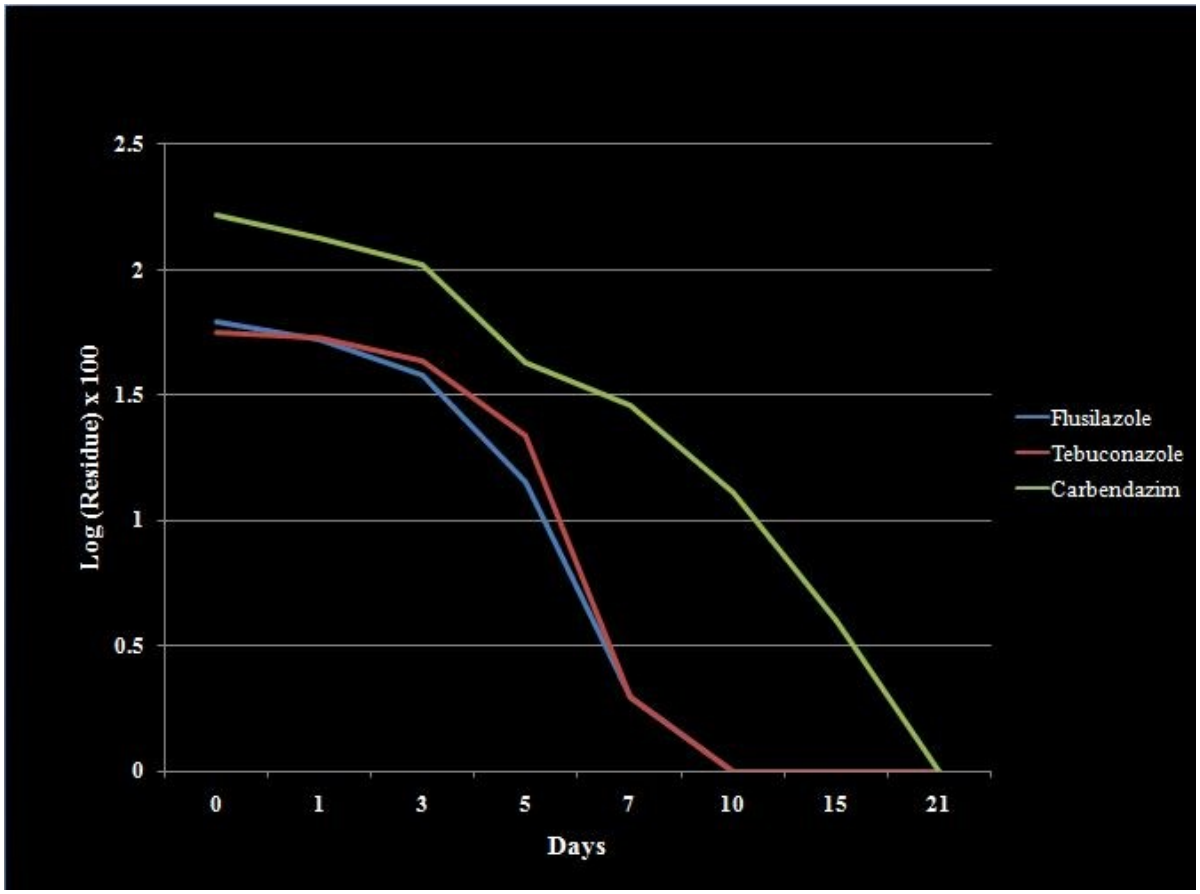


Figure 15. Dissipation pattern of fungicides in cowpea pods from treated plots

the seventh day and the dissipation percentage being 96.43 which reached the below detectable level on the tenth day of spraying. Compared to the other fungicides, a higher initial residue deposit of 1.65 mg kg^{-1} was detected in carbendazim (0.1 %). On the 15th day, the residue became 0.04 mg kg^{-1}, whereby 97.58 % of the residues dissipated which reached the below detectable level from the 21st day (Figure 15).

Pesticide free commodity could be accomplished by observing sufficient pre harvest interval for the concerned chemical. Hence, it is imperative to fix the waiting period for the fungicides used in the field. As the MRL value of the new generation fungicides tested in this study for cowpea has not been fixed in India by FSSA (Food Safety and Standards act), the values prescribed by EU (European Union) was used for calculating the waiting period.

The half life and waiting period of different fungicides calculated indicated that, flusilazole (0.1 %) when sprayed on cowpea fruits had a half life of 1.5 days and waiting period of 8.15 days. Tebuconazole (0.1 %) degraded to half its initial deposit in 3.5 days and the waiting period estimated was zero days. The half life recorded for carbendazim (0.1 %) was 2.7 days and the waiting period worked out was 8.53 days.

Though half life and waiting period of these fungicides had been worked out in crops like paddy and apple, works on vegetables are comparatively scanty. Kundu *et al.* (2011) reported that the harvest residue of tebuconazole 25.9 EC in paddy and groundnut were below the detection limit of the instrument (< 0.01 ppm) irrespective of doses and hence, the use of tebuconazole could be advocated for the control of diseases in paddy and groundnut without any residual toxicity problem. A half life of 4.23 and 7.77 days was noted in flusilazole treated apple at the recommended and high dosage levels (Shuang *et al*., 2011).

In consideration of the waiting period calculated for different fungicides, tebuconazole (0.1 %) having the shortest waiting period of zero days was identified as the safest chemical for cowpea while, flusilazole (0.1 %) and

carbendazim (0.1 %) had a waiting period of eight days which was outside the harvest interval of the crop.

Economic analysis on the integrated management of Fusarium wilt and anthracnose of cowpea using new generation fungicides revealed that the returns from treatment with carbendazim (0.1 %), copper oxychloride (0.2 %) or tebuconazole (0.1 %) alone were high being Rs. 5.9, 4.3 and 2.8 respectively for every one rupee spent. The returns from the treatments which received the integrated package of seed treatment (carbendazim @ 2g/kg seed), soil solarization, *Trichoderma* enriched neem cake organic manure mixture and application of either carbendazim (0.1 %) or tebuconazole (0.1 %) were Rs. 2.3 and 1.6 for every rupee spent. The farmers have to choose wisely the treatment options depending upon the index of the diseases, even though the benefits vary.

The concise analysis of the results indicated that integration of seed treatment with carbendazim (2 g/kg seed), soil solarization for 45 days using transparent polythene sheets during warm season, application of *Trichoderma* enriched neem cake organic manure mixture @ 1 kg/pit 15 days after seed emergence and soil drenching of either tebuconazole (0.1 %) or carbendazim (0.1 %) 30, 45 and 60 days after seed emergence was highly effective for the management of Fusarium wilt of cowpea. However, seed treatment with carbendazim (2 g/kg seed) and foliar application of either flusilazole (0.1 %) or tebuconazole (0.1 %) 30, 45 and 60 days after seed emergence was found to be the best for the control of anthracnose of cowpea.

Since, the repeated use of fungicides with single site action in target fungus is associated with problems of resistant strain development; rotation of fungicides with differing modes of action is desirable in IDM. As, copper oxychloride (0.2 %) having multi site action was also identified as a potential fungicide in the present study against Fusarium wilt and anthracnose of cowpea, its rotation with systemic fungicides will definitely provide an effective management strategy for these diseases.

Thus, the overall results of the study indicated that apart from the efficacy of fungicides against two diseases, associated yield increase, safety to the crop as well as economic benefits should also be taken into consideration while recommending an IDM package. Hence, the following integrated management package was developed for the control of Fusarium wilt and anthracnose of cowpea in disease prone areas as: 1) Seed treatment with carbendazim (2 g/kg seed) 2) Soil solarization for a period of 45 days using transparent polythene sheets during warm season 3) Application of *Trichoderma* enriched neem cake organic manure mixture @ 1 kg/pit 15 days after seed emergence 4) Application of tebuconazole (0.1%) at 30, 45 and 60 days after seed emergence. In order to counter the possibility of resistance build up due to repeated use of the triazole fungicide, the contact fungicide copper oxychloride (0.2 %) may be used in rotation.

6. SUMMARY

The present investigation on "Integrated management of Fusarium wilt and anthracnose of vegetable cowpea (*Vigna unguiculata* subsp. s*esquipedalis* (L.) Verdcourt) using new generation fungicides" was carried out in the Department of Plant Pathology, College of Agriculture, Vellayani during the year 2011 - 2014 with an aim to test the efficacy of new generation fungicides for the management of Fusarium wilt and anthracnose of vegetable cowpea and to develop an integrated management strategy using effective fungicides compatible with ecofriendly tactics. The salient findings of the study are summarized below:

- A survey conducted in the main cowpea growing areas of Thiruvananthapuram district during 2011 - 2012 revealed that among the six major fungal diseases, anthracnose was found to be the most predominant one (0 – 55 %), followed by Cercospora leaf spot (0 – 37 %), Fusarium wilt (0 – 20 %) and web blight and collar rot (0 - 10 %). Negligible incidence of powdery mildew and rust was observed at Balaramapuram and Palapoor respectively during the course of survey. With regard to disease index, anthracnose index ranged between 0 - 33.3, followed by Fusarium wilt (0 - 20), Cercospora leaf spot (0 - 14.16) and web blight (0 - 10.33). However, among the major fungal diseases, considerable yield losses were found attributable due to Fusarium wilt and anthracnose of cowpea.

- Studies on symptomatology of Fusarium wilt revealed that the affected plants showed yellowing, withering and drooping of leaves followed by defoliation and drying up of vines. The base of the stem became swollen and resembled a small tuber which later disintegrated resulting in shredding. The taproot and lateral roots were also affected. Occasionally, abnormal flattening of the stem along the growing tip, fasciation and sterility of flowers were also noticed. The symptoms of anthracnose appeared as circular to irregular brown necrotic spots on leaves, spindle shaped lesions with light grey centre and reddish brown margin on the stem and vines and irregular deep seated reddish brown spots on the pods.

- A total of 12 isolates of *Fusarium* spp. and eight isolates of *Colletotrichum* spp. were obtained from infected plants collected from major cowpea growing areas of Thiruvananthapuram.

- Based on the morphological and cultural characters the isolates of the pathogen associated with Fusarium wilt of cowpea were tentatively identified as, *F. oxysporum* Schlecht, *F. equiseti* (Corda) Sacc. and *F. solani* (Mart.) Sacc. Among the 12 isolates, *F. oxysporum* was the prevalent species and widely distributed as it was found to be present in six locations examined. On the contrary, *F. equiseti* and *F. solani* were noted as less frequent species with three and two isolates each. The isolates of the pathogen associated with anthracnose of cowpea were tentatively identified as, *C. gloeosporioides* Penz. based on morphological and cultural characterization.

- The identity of the species of both the pathogens was further confirmed through ITS- rDNA sequence analyses. Amplification of the ITS- rDNA region of the 12 different isolates of *Fusarium* spp. using universal primers ITS 1 and ITS 4 yielded a PCR product of 500 - 530 bp long. An identity of 99 – 100 % was observed between the *Fusarium* isolates obtained in this study and other sequences of *Fusarium* spp. available in NCBI database. Amplification of the ITS- rDNA region of eight different isolates of *Colletotrichum* spp. yielded an amplicon of approximately 540 bp long. Alignment of the sequences of representative isolates collected from different locations in this study with other known sequences of *Colletotrichum* spp. obtained from GenBank revealed that an identity of 99 – 100 % exist among the sequences.

- The studies on pathogenicity and comparative virulence indicated that of the 12 isolates of *Fusarium* spp. tested, the isolate F2 (*F. oxysporum*) from Palapoor took only seven days for the development of symptoms and was found to be highly pathogenic whereas the isolates F7, F8, F10 and F11 were found to be non

– pathogenic and did not produce any symptoms. Among the eight isolates of *Colletotrichum* spp. tested, the isolate C7 (*C. gloeosporioides*) from Kattakada took only four days for the initiation of symptoms and was found to be highly pathogenic while, the isolates C2, C4 and C6 did not produce any symptoms.

- *In vitro* evaluation of new generation fungicides against the most virulent isolate of the two pathogens revealed that tebuconazole (0.1 %), carboxin + thiram (0.4 %), carbendazim (0.1 %) and mancozeb (0.25 %) completely inhibited the growth of *F. oxysporum*, whereas 100 % inhibition of the mycelial growth of *C. gloeosporioides* was noticed with propiconazole (0.1 %), flusilazole (0.1 %), tebuconazole(0.1 %), carboxin + thiram (0.4 %) and mancozeb (0.25 %). The least per cent inhibition of the mycelial growth of the both the test pathogens were observed in azoxystrobin (0.15 %).

- Compatibility studies of 12 selected fungicides with the biocontrol agents (*T. viride* and *P. fluorescens*) and the root nodule bacterium (*Rhizobium* spp.) specified that propiconazole (0.1 %), flusilazole (0.1 %), tebuconazole (0.1 %) and carbendazim (0.1 %) were highly incompatible with *T. viride*, showing 100 % inhibition of the mycelial growth at their recommended dosage while, azoxystrobin (0.15 %) was found to be highly compatible and did not inhibit the mycelial growth of *T. viride*. None of the fungicides tested inhibited the growth of *P. fluorescens* and were found to be highly compatible. All the fungicides tested were highly compatible with *Rhizobium* spp. while, captan + hexaconazole (0.1 %), carboxin + thiram (0.4 %), copper hydroxide (0.25 %) and copper oxychloride (0.2 %) caused bacterial growth inhibition in the range of 25 – 45 % and were found only moderately compatible.

- *In vivo* evaluation of new generation fungicides against Fusarium wilt of cowpea indicated that, soil drenching of flusilazole (0.1 %), tebuconazole (0.1 %) or carbendazim (0.1 %) @ 30,45 and 60 days after seed emergence were highly effective in the total suppression of wilt incidence with the lowest disease index

(0.00) whereas, the pathogen inoculated control plants showed 100 % wilt incidence.

- *In vivo* evaluation of new generation fungicides against anthracnose of cowpea showed that three rounds of foliar application of flusilazole (0.1 %) @ 30, 45 and 60 days after seed emergence recorded the least disease incidence (50.00 %) followed by treatments with propiconazole (0.1 %), tebuconazole (0.1 %) or carbendazim (0.1 %) (75.00 %) whereas, the untreated control plants recorded 100 % disease incidence. The data on anthracnose index revealed that the plants treated with flusilazole (0.1 %) recorded the lowest disease index (5.60) followed by treatment with tebuconazole (0.1%) (8.30). However, the untreated control plants recorded the highest disease index (83.30).

- Based on the results of pot experiments, three effective fungicides *viz.,* flusilazole (0.1 %), tebuconazole (0.1 %) and carbendazim (0.1 %) which showed the least incidence and index of both the diseases along with seed treatment (carbendazim @ 2g/kg seed), soil solarization and *Trichoderma* enriched neem cake organic manure mixture were evaluated in the field to determine their efficacy in the management of Fusarium wilt and anthracnose of cowpea.

- The field experiment conducted to develop an integrated disease management package for Fusarium wilt of cowpea indicated that soil solarization, application of *Trichoderma* enriched neem cake organic manure mixture and soil drenching of either tebuconazole (0.1 %) or carbendazim (0.1 %) totally suppressed the incidence of Fusarium wilt and recorded the lowest (0.00) disease index followed by treatment with copper oxychloride (0.2 %) (8.33). However, the untreated control plants recorded the highest disease index (41.67).

- Integrated management of anthracnose of cowpea showed that foliar application of either flusilazole (0.1 %) or tebuconazole (0.1 %) registered the lowest (8.33 %) anthracnose incidence. The data on disease index indicated that foliar

application of either flusilazole (0.1 %) or tebuconazole (0.1 %) recorded the lowest (0.93) anthracnose index followed by treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) (4.63). Foliar application of the contact fungicide, copper oxychloride (0.2 %) recorded an index of 18.52. The untreated control plants and also the plants treated with soil solarization and *Trichoderma* enriched neem cake organic manure mixture alone without fungicide application registered the highest anthracnose index (75.9).

- Soil solarization and application of *Trichoderma* enriched neem cake organic manure mixture significantly improved the growth and yield parameters of plants compared to the untreated ones. With respect to pod yield, the plants treated with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and carbendazim (0.1 %) recorded the maximum yield (1.14 kg/plant) which was on par with the treatment with soil solarization, *Trichoderma* enriched neem cake organic manure mixture and tebuconazole (0.1 %) (1.13 kg/plant) whereas, the untreated control plants registered the minimum yield (0.80 kg/plant).

- The studies on persistence and degradation of fungicide residues indicated that flusilazole (0.1 %) when sprayed on cowpea resulted in an initial deposit of 0.61 mg kg^{-1} on fruits two hours after spraying. From the initial deposit, 96.72 % of the residues dissociated on the seventh day, whereby the concentration was recorded to be 0.02 mg kg^{-1} which reached below detectable level on the tenth day of spraying. An initial deposit of 0.56 mg kg^{-1} residue of tebuconazole (0.1 %) was recorded two hours after spraying and the residue level was 0.02 mg kg^{-1} on the seventh day, the dissipation percentage being 96.43 which reached below detectable level on the tenth day of spraying. Carbendazim (0.1 %) showed an initial deposit of 1.65 mg kg^{-1} and on the 15^{th} day, the residue became 0.04 mg kg^{-1} whereby 97.58 % of the residues got dissipated which reached below detectable level from the 21^{st} day.

- The half life and waiting period of the different fungicides calculated based on the MRL values prescribed by the European Union indicated that tebuconazole (0.1 %) was having the shortest waiting period (0 days) indicating its safety to the vegetable. While flusilazole (0.1 %) and carbendazim (0.1 %) had a waiting period of eight days which was outside the harvest interval of the crop.

- Economic analysis on the integrated management of Fusarium wilt and anthracnose of cowpea using new generation fungicides revealed that the returns from treatment with carbendazim (0.1 %), copper oxychloride (0.2 %) and tebuconazole (0.1 %) were high being Rs. 5.9, 4.3 and 2.8 respectively for every one rupee spent.

Taking into account, the overall efficacy of fungicides against two diseases, associated yield increase, safety to the crop as well as economic benefits the following integrated management package was developed for the control of Fusarium wilt and anthracnose of cowpea in disease prone areas as: 1) Seed treatment with carbendazim (2 g/kg seed) 2) Soil solarization for a period of 45 days using transparent polythene sheets during warm season 3) Application of *Trichoderma* enriched neem cake organic manure mixture @ 1 kg/pit 15 days after seed emergence 4) Application of tebuconazole (0.1 %) at 30, 45 and 60 days after seed emergence. In order to counter the possibility of resistance build up due to repeated use of the triazole fungicide, the contact fungicide copper oxychloride (0.2 %) may be used in rotation.

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Appendices

APPENDIX – I

COMPOSITION OF MEDIA USED

1. Potato Dextrose Agar

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

2. King's B Medium

Peptone	- 20 g
Dipotassium hydrogen phosphate	- 1.5 g
Magnesium sulphate	- 1.5 g
Glycerol	- 10 ml
Agar Agar	- 20.00 g
Distilled Water	- 1000 ml (to makeup)
pH	- 7 (to be adjusted)

3. Nutrient Agar

Beef extract	- 3.00 g
Peptone	- 5.00 g
Sodium chloride	- 1.00 g
Agar Agar	- 20.00 g
Distilled Water	- 1000 ml (to makeup)
pH	- 7 (to be adjusted)

APPENDIX - II

COMPOSITION OF STAIN USED

1. Lactophenol –Cotton blue

Anhydrous lactophenol - 67.0ml

Distilled water - 20.0ml

Cotton blue - 0.1g

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

APPENDIX – III

DETAILS OF FUNGICIDES USED FOR *IN VITRO* EVALUATION

Sl. No	Common name	Trade name	Formulation	Source of supply
1.	Propiconazole	Tilt	25 % EC	Syngenta India Ltd., Mumbai
2.	Chlorothalonil	Kavach	75 % WP	Syngenta India Ltd., Mumbai
3.	Thiophanate methyl	Hexastop	70 % WP	Coromandel International Ltd., Secunderabad
4.	Flusilazole	Nustar	40 EC	E. I. DuPont India Ltd., Gurgaon
5.	Azoxystrobin	Amistar	23%SC	Syngenta India Ltd., Mumbai
6.	Tebuconazole	Folicur	25.9 % m/m EC	Bayer Crop Science Ltd., Mumbai
7.	Captan 70 % + Hexaconazole 5 % WP	Taquat	-	Rallis India Ltd., Bangalore
8.	Carboxin 37.5% + Thiram 37.5%	Vitavax Power	70%WS	DhanukaAgritech Ltd., Ahemedabad
9.	Copper hydroxide	Kocide	77 % WP	E. I. DuPont India Ltd., Gurgaon
10.	Mancozeb	Indofil M 45	75 % WP	Indofil Chemical Co., Mumbai
11.	Carbendazim	Bavistin	50 % WP	BASF India ltd., Mumbai
12.	Copper oxychloride	Fyter	55% WDP	Copper Chemical Manufactures, Kerala

APPENDIX – IVa

ITS – rDNA SEQUENCE OF *FUSARIUM* ISOLATES

>F1
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APPENDIX – IVb

ITS – rDNA SEQUENCE OF *COLLETOTRICHUM* ISOLATES

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GCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACCGCTTGGCGTTGGGGCCCTACGGCTTCC
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>C6
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CGTGACCCTCCCGGCCTCCCGCCCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACG
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CATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACAGCTGATG
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>C7
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CGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCA
TTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACAGCTGATGTA
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>C8
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GGACTCTTGCCGTAAAACCCCCAATTCTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAA

APPENDIX – Va

MULTIPLE SEQUENCE ALIGNMENT OF THE ITS- rDNA REGION OF *FUSARIUM* ISOLATES USING CLUSTALW2

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KF534747.1      ------------------------------------------------------------
KJ569269        ------------------------------------------------------------
KC859450.1      ------------------------------------------------------------
KJ569272        ------------------------------------------------------------
KJ569277        ------------------------------------------------------------
JX517202.1      ------------------------------------------------------------
JQ625562.1      ------------------------------------------------------------
JX177431.1      ------------------------------------------------------------
HM756257.1      ------------------------------------------------------------
JQ954892.1      ------------------------------------------------------------
KJ569275        ------------------------------------------------------------
JN400710.1      ------------------------------------------------------------
KF963540        ------------------------------------------------------------
KJ569273        ------------------------------------------------------------
KJ569270        ------------------------------------------------------------
KJ569276        ------------------------------------------------------------
KJ569271        ------------------------------------------------------------
AY667489.1      -GTACACACCGCCCGTCGCTACTACCGATTGAATGGCTCAGTGAGGCGTCCGGACTGGCC 59
JQ690081.1      ------------------------------------------------------------
KJ569274        ------------------------------------------------------------
KJ569278        ------------------------------------------------------------
KJ569279        ------------------------------------------------------------
EU680476.1      TGTACACACCGCCCGTCGCTACTACCGATTGAATGGCTCGGTGAGGCCTTCGGACTGGCC 60

KF534747.1      ------------------------------------------------------------
KJ569269        ------------------------------------------------------------
KC859450.1      ------------------------------------------------------------
KJ569272        ------------------------------------------------------------
KJ569277        ------------------------------------------------------------
JX517202.1      ------------------------------------------------------------
JQ625562.1      ----------------------------------------TAAGAGGAAGTAAAAGTCGT 20
JX177431.1      ------------------------------------------------------------
HM756257.1      ------------------------------------------------------------
JQ954892.1      ------------------------------------------------------------
KJ569275        ------------------------------------------------------------
JN400710.1      ------------------------------------------------------------
KF963540        ------------------------------------------------------------
KJ569273        ------------------------------------------------------------
KJ569270        ------------------------------------------------------------
KJ569276        ------------------------------------------------------------
KJ569271        ------------------------------------------------------------
AY667489.1      CAGAGAGGTGGGCAACTACCACTCAGGGCCGGAAAGCTCTCCAAACTCGGTCATTTAGAG 119
JQ690081.1      ------------------------------------------------------------
KJ569274        ------------------------------------------------------------
KJ569278        ------------------------------------------------------------
KJ569279        ------------------------------------------------------------
EU680476.1      CAGGAGGGTTGGCAACGACCCCCCAGGGCCGGAAAGTTGGTCAAACCCGGTCATTTAGAG 120

KF534747.1      --------TCCGTAGGGTAACCTGCGGAGGG---------------ATCATTACCGAGTT 37
KJ569269        ---------------------------AGGG---------------ATCATTACCGAGTT 18
KC859450.1      ------------------------------------------------GTCGGAATT 9
KJ569272        ---------------------------AGGG---------------ATCATTACCGAGTT 18
KJ569277        ---------------------------AGGG---------------ATCATTACCGAGTT 18
JX517202.1      -------------------------GAGGG---------------ATCATTACCGAGTT 19
JQ625562.1      AACAAGGTTTCGTTGGTGAACCAGCGGAGGG---------------ATCATTACCGAGTT 65
JX177431.1      ------------------------------------------------------------
HM756257.1      ----------------------TTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTT 38
JQ954892.1      ---------------------------TTTG-----------------CAT--CCGACT- 13
KJ569275        ---------------------------AGGG---------------ATCATTACCGAGTT 18
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JN400710.1      ----------------------------CCTG--------CGGAGGGATCATTACCGAGTT 25
KF963540        -------------------------------------------AGGGATCATTACCGAGTT 18
KJ569273        -------------------------------------------AGGGATCATTACCGAGTT 18
KJ569270        ----------------------------AGGG----------------ATCATTACCGAGTT 18
KJ569276        ----------------------------AGGG----------------ATCATTACCGAGTT 18
KJ569271        ----------------------------AGGG----------------ATCATTACCGAGTT 18
AY667489.1      GAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTT 179
JQ690081.1      --------------------GACCTGCGGAGGG---------------ATCATTACCGAGTT 27
KJ569274        ----------------------------AGGG----------------ATCATTACCGAGTT 18
KJ569278        ----------------------------AGGG----------------ATCATTACCGAGTT 18
KJ569279        ----------------------------AGGG----------------ATCATTACCGAGTT 18
EU680476.1      GAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTG 180


KF534747.1      TA---------------CAACTCCCAAACCCCTGTGAACATACCAATTG--TTGCCTCGG 80
KJ569269        TA---------------CAACTCCCAAACCCCTGTGAACATACCAATTG--TTGCCTCGG 61
KC859450.1      TT---------------CAACTCCCAAACCCCTGTGAACATACCAATTG--TTGCCTCGG 52
KJ569272        AT---------------ACAACTCATCAACCCTGTGAACATACCTATAACGTTGCCTCGG 63
KJ569277        AT---------------ACAACTCATCAACCCTGTGAACATACCTATAACGTTGCCTCGG 63
JX517202.1      AT---------------ACAACTCATCAACCCTGTGAACATACCTAAAACGTTGCCTCGG 64
JQ625562.1      AT---------------ACAACTCATCAACCCTGTGAACATACCTATAACGTTGCCTCGG 110
JX177431.1      -------------------------AACCCCTGTGAACATACCTTTAT-GTTGCCTCGG 33
HM756257.1      TA---------------CAACTCCCAAACCCCTGTGAACATACCACTT--GTTGCCTCGG 81
JQ954892.1      --------------------CACTCCAACCCCTGTG-ACATACCACTT--GTTGCCTCGG 50
KJ569275        TA---------------CAACTCCCAAACCCCTGTGAACATACCACTT--GTTGCCTCGG 61
JN400710.1      TA---------------CAACTCCCAAACCCCTGTGAACATACCACTT--GTTGCCTCGG 68
KF963540        TA---------------CAACTCCCAAACCCCTGTGAACATACCACTT--GTTGCCTCGG 61
KJ569273        TA---------------CAACTCCCAAACCCCTGTGAACATACCACTT--GTTGCCTCGG 61
KJ569270        TA---------------CAACTCCCAAACCCCTGTGAACATACCACTT--GTTGCCTCGG 61
KJ569276        TA---------------CAACTCCCAAACCCCTGTGAACATACCACTT--GTTGCCTCGG 61
KJ569271        TA---------------CAACTCCCAAACCCCTGTGAACATACCACTT--GTTGCCTCGG 61
AY667489.1      TA---------------CAACTCCCAAACCCCTGTGAACATACCACTT--GTTGCCTCGG 222
JQ690081.1      TA---------------CAACTCCCAAACCCCTGTGAACATACCTATAC-GTTGCCTCGG 71
KJ569274        TA---------------CAACTCCCAAACCCCTGTGAACATACCTATAC-GTTGCCTCGG 62
KJ569278        TA---------------CAACTCCCAAACCCCTGTGAACATACCTATAC-GTTGCCTCGG 62
KJ569279        TA---------------CAACTCCCAAACCCCTGTGAACATACCTATAC-GTTGCCTCGG 62
EU680476.1      TAGGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCTTAG--TTGCTTCGG 238
                                    .*.** ***  **: :.*  ::   **** ****

KF534747.1      CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 122
KJ569269        CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 103
KC859450.1      CGG-ATCAACCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCCGAA 94
KJ569272        CGGGAACAGACG---------GCCCCG-------TAACACGGGCCG--CCCCCGCCAGAG 105
KJ569277        CGGGAACAGACG---------GCCCCG-------TAACACGGGCCG--CCCCCGCCAGAG 105
JX517202.1      CGGGAACAGACG---------GCCCCG-------TAACACGGGCCG--CCCCCGCCAGAG 106
JQ625562.1      CGGGAACAGACG---------GCCCCG-------TAACACGGGCCG--CCCCCGCCAGAG 152
JX177431.1      CGG-ATCAGCCCG-------CGCCCCG-------TAAAACGGGACG--GCCCGCCGCAGG 76
HM756257.1      CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 123
JQ954892.1      CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 92
KJ569275        CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 103
JN400710.1      CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 110
KF963540        CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 103
KJ569273        CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 103
KJ569270        CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 103
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KJ569271        CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 103
AY667489.1      CGG-ATCAGCCCG-------CTCCCGG-------TAAAACGGGACG--GCCCG-CCAGAG 264
JQ690081.1      CGG-ATCAGCCCG-------CGCCCCG-------TAAAACGGGACG--GCCCG-CCCGAG 113
KJ569274        CGG-ATCAGCCCG-------CGCCCCG-------TAAAACGGGACG--GCCCG-CCCGAG 104
KJ569278        CGG-ATCAGCCCG-------CGCCCCG-------TAAAACGGGACG--GCCCG-CCCGAG 104
KJ569279        CGG-ATCAGCCCG-------CGCCCCG-------TAAAACGGGACG--GCCCG-CCCGAG 104
EU680476.1      CGG-GCCCGCCATTCATGGCCGCCGGGGGCTCTCAGCCCCGGGCCCGCGCCCG-CCGGAG 296
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KJ569269        GACCCCTAAACTCTGTTTCTATAT-GTAACTTCTGAGT---AAAACCATAAATAAATCAA 159
KC859450.1      GACCCCCAAACTCTGTTTCTATAT-GTAACTTCTGAGT---AAAACCATAAATAAATCAA 150
KJ569272        GACCCCCTAACTCTGTTTCTATAATGTTTCTTCTGAGT---AAACAAGCAAATAAATTAA 162
KJ569277        GACCCCCTAACTCTGTTTCTATAATGTTTCTTCTGAGT---AAACAAGCAAATAAATTAA 162
JX517202.1      GACCCCCTAACTCTGTTTCTATAATGTTTCTTCTGAGT---AAACAAGCAAATAAATTAA 163
JQ625562.1      GACCCCCTAACTCTGTTTCTATAATGTTTCTTCTGAGT---AAACAAGCAAATAAATTAA 209
JX177431.1      AACCACAAAACTCTGAT--TTTAGTGTAACTTCTGAGTC--TAAAAAACAAATAAATCAA 132
HM756257.1      GACCCCTAAACTCTGTTT-CTATATGTAACTTCTGAGTA--AAACCAT-AAATAAATCAA 179
JQ954892.1      GACCCCTAAACTCTGTTT-CTATATGTAACTTCTGAGTA--AAACCAT-AAATAAATCAA 148
KJ569275        GACCCCTAAACTCTGTTT-CTATATGTAACTTCTGAGTA--AAACCAT-AAATAAATCAA 159
JN400710.1      GACCCCTAAACTCTGTTT-CTATATGTAACTTCTGAGTA--AAACCAT-AAATAAATCAA 166
KF963540        GACCCCTAAACTCTGTTT-CTATATGTAACTTCTGAGTA--AAACCAT-AAATAAATCAA 159
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KJ569270        GACCCCTAAACTCTGTTT-CTATATGTAACTTCTGAGTA--AAACCAT-AAATAAATCAA 159
KJ569276        GACCCCTAAACTCTGTTT-CTATATGTAACTTCTGAGTA--AAACCAT-AAATAAATCAA 159
KJ569271        GACCCCTAAACTCTGTTT-CTATATGTAACTTCTGAGTA--AAACCAT-AAATAAATCAA 159
AY667489.1      GACCCCTAAACTCTGTTT-CTATATGTAACTTCTGAGTA--AAACCAT-AAATAAATCAA 320
JQ690081.1      GACCCCTAAACTCTGTTT-TTAG-TGGAACTTCTGAGTA--AAACAAACAAATAAATCAA 169
KJ569274        GACCCCTAAACTCTGTTT-TTAG-TGGAACTTCTGAGTA--AAACAAACAAATAAATCAA 160
KJ569278        GACCCCTAAACTCTGTTT-TTAG-TGGAACTTCTGAGTA--AAACAAACAAATAAATCAA 160
KJ569279        GACCCCTAAACTCTGTTT-TTAG-TGGAACTTCTGAGTA--AAACAAACAAATAAATCAA 160
EU680476.1      ACACCACGAACTCTGTCT-GATCTAGTGAAGTCTGAGTTGCTTGTATCGCAATCAGTTAA 355
                ...*..  *******:    ::   *  :. *******   ::. .   .***.*.* **

KF534747.1      AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 238
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KJ569277        AACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA 222
JX517202.1      AACTTTCAACAACGGATCTCTTGGCTCCGGCATCGATGAAGAACGCAGCGAAATGCGATA 223
JQ625562.1      AACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA 269
JX177431.1      AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 192
HM756257.1      AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 239
JQ954892.1      AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 208
KJ569275        AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 219
JN400710.1      AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 226
KF963540        AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 219
KJ569273        AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 219
KJ569270        AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 219
KJ569276        AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 219
KJ569271        AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 219
AY667489.1      AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 380
JQ690081.1      AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 229
KJ569274        AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 220
KJ569278        AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 220
KJ569279        AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATA 220
EU680476.1      AACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATA 415
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KF534747.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 298
KJ569269        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 279
KC859450.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 270
KJ569272        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 282
KJ569277        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 282
JX517202.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 283
JQ625562.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 329
JX177431.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 252
HM756257.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 299
JQ954892.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 268
KJ569275        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 279
JN400710.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 286
KF963540        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 279
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KJ569273        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 279
KJ569270        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 279
KJ569276        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 279
KJ569271        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 279
AY667489.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 440
JQ690081.1      AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 289
KJ569274        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 280
KJ569278        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 280
KJ569279        AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGC 280
EU680476.1      ACTAGTGTGAATTGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCC 475
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KF534747.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCCCCCGGGTTT 357
KJ569269        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCCCCCGGGTTT 338
KC859450.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCCCCCGGGTTT 329
KJ569272        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTACAACCCT-CAGGCCCCCGGGCCT 341
KJ569277        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTACAACCCT-CAGGCCCCCGGGCCT 341
JX517202.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTACAACCCT-CAGGCCCCCGGGCCT 342
JQ625562.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTACAACCCT-CAGGCCCCCGGGCCT 388
JX177431.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCCCCCGGGTTT 311
HM756257.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCACAG---CTT 355
JQ954892.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCACAG---CTT 324
KJ569275        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCACAG---CTT 335
JN400710.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCACAG---CTT 342
KF963540        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCACAG---CTT 335
KJ569273        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCACAG---CTT 335
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KJ569276        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCACAG---CTT 335
KJ569271        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCACAG---CTT 335
AY667489.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCACAG---CTT 496
JQ690081.1      CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCTCAG---CTT 345
KJ569274        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCTCAG---CTT 336
KJ569278        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCTCAG---CTT 336
KJ569279        CAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCT-CAAGCTCAG---CTT 336
EU680476.1      TGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGG---CTT 532
                 .****** ** ************ *********** *:.***: **.** *       *
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KF534747.1      G-GTGTTGGGGA-TCGG-CGAGCCCTTGCGGCAAG-------CCGGCCCCGAAATCTAGT 407
KJ569269        G-GTGTTGGGGA-TCGG-CGAGCCCTCGCGGCAAG-------CCGGCCCCGAAATCTAGT 388
KC859450.1      G-GTGTTGGGGA-TCGG-CGAG-CCTCACGGCAAG-------CCGGCCCCGAAATACAGT 378
KJ569272        G-GCGTTGGGGA-TCGG-CGGAAGCCCCCTGCGGGCACAACGCCGTCCCCCAAATACAGT 398
KJ569277        G-GCGTTGGGGA-TCGG-CGGAAGCCCCCTGCGGGCACAACGCCGTCCCCCAAATACAGT 398
JX517202.1      G-GCGTTGGGGA-TCGG-CGGAAGCCCCCTGCGGGCACAACGCCGTCCCCCAAATACAGT 399
JQ625562.1      G-GCGTTGGGGA-TCGG-CGGAAGCCCCCTGCGGGCACAACGCCGTCCCCCAAATACAGT 445
JX177431.1      G-GTGTTGGGGA-TCGGGCTGTA--CTCCAGC----------CCGGCCCCGAAATCTAGT 357
HM756257.1      G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCTCAAATTGATT 396
JQ954892.1      G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCTCAAATTGATT 365
KJ569275        G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCTCAAATTGATT 376
JN400710.1      G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCTCAAATTGATT 383
KF963540        G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCTCAAATTGATT 376
KJ569273        G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCTCAAATTGATT 376
KJ569270        G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCTCAAATTGATT 376
KJ569276        G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCCCAAATTGATT 376
KJ569271        G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCCCAAATTGATT 376
AY667489.1      G-GTGTTGGGAC-TCGC-------GTTAATTCG----------CGTTCCTCAAATTGATT 537
JQ690081.1      G-GTGTTGGGAC-TCGC-------GGTAACCCG----------CGTTCCCCAAATCGATT 386
KJ569274        G-GTGTTGGGAC-TCGC-------GGTAACCCG----------CGTTCCCCAAATCGATT 377
KJ569278        G-GTGTTGGGAC-TCGC-------GGTAACCCG----------CGTTCCCCAAATCGATT 377
KJ569279        G-GTGTTGGGAC-TCGC-------GGTAACCCG----------CGTTCCCCAAATCGATT 377
EU680476.1      GTGTGTTGGGTCGTCGT------CCCCTCTCCGGG---GGGGACGGGCCCCAAAGGCAGC 583
                * * ****** . ***           .  *          **  **  ***   *

KF534747.1      GGCGGTCTCGCTGCAGCTTCCATTGCGTAGTAG-TAAAACCCTCGCAACTGGTACGCGGC 466
KJ569269        GGCGGTCTCGCTGCAGCTTCCATTGCGTAGTAG-TAAAACCCTCGCAACTGGTACGCGGC 447
KC859450.1      GGCGGTCTCGCTGCAGCTTCCATTGCGTAGTAG-TAAAACCCTCGCAACTGGTACGCGGC 437
KJ569272        GGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAG-CTAACACCTCGCAACTGGAGAGCGGC 457
KJ569277        GGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAG-CTAACACCTCGCAACTGGAGAGCGGC 457
JX517202.1      GGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAG-CTAACACCTCGCAACTGGAGAGCGGC 458
JQ625562.1      GGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAG-CTAACACCTCGCAACTGGAGAGCGGC 504
JX177431.1      GGCGGTCTCGCTGCAGCCTCCATTGCGTAGTAG-CTAACACCTCGCAACTGGAACGCGGC 416
HM756257.1      GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 454
JQ954892.1      GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 423
KJ569275        GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 434
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JN400710.1      GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 441
KF963540        GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 434
KJ569273        GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 434
KJ569270        GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 434
KJ569276        GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 434
KJ569271        GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 434
AY667489.1      GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAGTAAAAC-CCTCGTTACTGGTAATCGTC 595
JQ690081.1      GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAATCATACACCTCGTTACTGGTAATCGTC 445
KJ569274        GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAATCATACACCTCGTTACTGGTAATCGTC 436
KJ569278        GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAATCATACACCTCGTTACTGGTAATCGTC 436
KJ569279        GGCGGTCACGTCG-AGCTTCCATAGCGTAGTAATCATACACCTCGTTACTGGTAATCGTC 436
EU680476.1      GGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGG 643
                ***** . **    .*.  * . :*****  ..  :::  *. *   :*** :.  *

KF534747.1      GCGGC-CAAGCCGTTAAACCCCCAA--CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 523
KJ569269        GCGGC-CAAGCCGTTAAACCCCCAA--CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 504
KC859450.1      GCGGC-CAAGCCGTTAAACCCCCAA--CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 494
KJ569272        GCGGC-CACGCCGTAAAACACCCAA--CTTCTGAATGTTGACCTCGAATCAGGTAGGAAT 514
KJ569277        GCGGC-CACGCCGTAAAACACCCAA--CTTCTGAATGTTGACCTCGAATCAGGTAGGAAT 514
JX517202.1      GCGGC-CACGCCGTAAAACACCCAA--CTTCTGAATGTTGACCTCGAATCAGGTAGGAAT 515
JQ625562.1      GCGGC-CACGCCGTAAAACACCCAA--CTTCTGAATGTTGACCTCGAATCAGGTAGGAAT 561
JX177431.1      GCGGC-CAAGCCGTTAAACCCCC------------------------------------ 438
HM756257.1      GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 510
JQ954892.1      GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 479
KJ569275        GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 490
JN400710.1      GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 497
KF963540        GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 490
KJ569273        GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 490
KJ569270        GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 490
KJ569276        GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 490
KJ569271        GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 490
AY667489.1      GCGGC-CACGCCGTTN-AACCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 650
JQ690081.1      GCGGC-CACGCCGTAAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 501
KJ569274        GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 492
KJ569278        GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 492
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KJ569279        GCGGC-CACGCCGTTAAACCCCAA---CTTCTGAATGTTGACCTCGGATCAGGTAGGAAT 492
EU680476.1      CCGGCGCTTGCCGAACGCAAATCAATCTTTTTCCAGGTTGACCTCGGATCAGGTAGGGAT 703
                 **** *: ****::  .... .

KF534747.1      ACCCGCTGAACTTAAGCATATCAAAAG--CGGGAGGAATTGCAGAAT------------ 568
KJ569269        ACCCGCTGAACTTAA---------------------------------------------- 519
KC859450.1      ACCCGCTGAACTTAAGCATATCAATAAGGCCGGAGGAA---------------------- 532
KJ569272        ACCCGCTGAACTTAA---------------------------------------------- 529
KJ569277        ACCCGCTGAACTTAA---------------------------------------------- 529
JX517202.1      ACCCGCTGAACTTAAGCATA---------------------------------------- 535
JQ625562.1      ACCCGCTGAACTTATGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCCA 621
JX177431.1      ------------------------------------------------------------
HM756257.1      ACCCGCTGAACTTAAGCATATCAATAAGCGG--AGGAA---------------------- 546
JQ954892.1      ACCCGCTGAACTTAAGCATATCAATAAGCGG--AGGA----------------------- 514
KJ569275        ACCCGCTGAACTTAA---------------------------------------------- 505
JN400710.1      ACCCGCTGAACTTAAGCATATCAA-AAGCGGGAAGGAAGAG------------------- 537
KF963540        ACCCGCTGAACTTAA---------------------------------------------- 505
KJ569273        ACCCGCTGAACTTAA---------------------------------------------- 505
KJ569270        ACCCGCTGAACTTAA---------------------------------------------- 505
KJ569276        ACCCGCTGAACTTAA---------------------------------------------- 505
KJ569271        ACCCGCTGAACTTAA---------------------------------------------- 505
AY667489.1      ACCG-CTGAACTTAAGC------------------------------------------- 666
JQ690081.1      ACCCGCTGAACTTAAGCATATCAATA---------------------------------- 527
KJ569274        ACCCGCTGAACTTAA---------------------------------------------- 507
KJ569278        ACCCGCTGAACTTAA---------------------------------------------- 507
KJ569279        ACCCGCTGAACTTAA---------------------------------------------- 507
EU680476.1      ACCCGCTGAACTTAAGCATAT--------------------------------------- 724


KF534747.1      ---
KJ569269        ---
KC859450.1      ---
KJ569272        ---
KJ569277        ---
JX517202.1      ---
JQ625562.1      GTA 624
JX177431.1      ---
HM756257.1      ---
JQ954892.1      ---
KJ569275        ---
JN400710.1      ---
KF963540        ---
KJ569273        ---
KJ569270        ---
KJ569276        ---
KJ569271        ---
AY667489.1      ---
JQ690081.1      ---
KJ569274        ---
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KJ569278        ---
KJ569279        ---
EU680476.1      ---
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APPENDIX – Vb

MULTIPLE SEQUENCE ALIGNMENT OF THE ITS- rDNA REGION OF *COLLETOTRICHUM* ISOLATES USING CLUSTALW2

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KJ584648        ---------------------------------------------AGGGATCATTATCGA 15
KJ584649        ---------------------------------------------AGGGATCATTATCGA 15
KJ584650        ---------------------------------------------AGGGATCATTATCGA 15
KJ584652        ---------------------------------------------AGGGATCATTATCGA 15
FJ172225.1      -------------------------CGTAGGGGTGAACCTGCGGAGGGATCATTATCGA 34
JX669450.1      --GGAAGTAAAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATCATTATCGA 58
KJ584654        ---------------------------------------------AGGGATCATTACTGA 15
JX161648.1      ----------------TAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACTGA 44
KJ584655        ---------------------------------------------AGGGATCATTACTGA 15
KJ584651        ---------------------------------------------AGGGATCATTACTGA 15
KF053199.1      ----------------------CCTTCCGTAGGTGAACCTGCGGAGGGATCATTACTGA 37
KJ584653        ---------------------------------------------AGGGATCATTACTGA 15
JX258732.1      GAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACTGA 60
KF154738.1      ----------------------CCTCTGCTG------ACGCGGAGGGA-CATTACTGA 29
EU070913.1      -----------------------------TTGGTGAACCAGCGGAGGGATCATTACTGA 30
JX567509.1      ----------------------TCTCCGTAGGTGAACCAGCGGAGGGATCATTACTGA 36
KJ002768.1      -------------------------------------ACCAGCGGAGGGATCATTACTGA 23
FN555114.1      ---------------GTAACAAGGTTTCCGTAGGTGAACCAGCGGAGGGATCATTACCGA 45
                                                     ***** *****  **

KJ584648        GTTACCGCTCCTTATAACCCTTTGTGAACATACCT-CAAACGTTGCCTCGGCGGGCAGCC 74
KJ584649        GTTACCGCTCCTTATAACCCTTTGTGAACATACCT-CAAACGTTGCCTCGGCGGGCAGCC 74
KJ584650        GTTACCGCTCCTTATAACCCTTTGTGAACATACCT-CAAACGTTGCCTCGGCGGGCAGCC 74
KJ584652        GTTACCGCTCCTTATAACCCTTTGTGAACATACCT-CAAACGTTGCCTCGGCGGGCAGCC 74
FJ172225.1      GTTACCGCTCCTTATAACCCTTTGTGAACATACCT-CAAACGTTGCCTCGGCGGGCAGCC 93
JX669450.1      GTTACCGCTCCTTATAACCCTTTGTGAACATACCC-CAAACGTTGCCTCGGCGGGCAGCC 117
KJ584654        GTTTACGCTC--TATAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGG- 72
JX161648.1      GTTTACGCTC--TATAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGG- 101
KJ584655        GTTTACGCTC--TATAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGG- 72
KJ584651        GTTTACGCTC--TATAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGG- 72
KF053199.1      GTTTACGCTC--TATAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGG- 94
KJ584653        GTTTACGCTC--TACAACCCTTTGTGAACATACCTACAACTGTTGCTTCGGCGGGTAGG- 72
JX258732.1      GTTTACGCTC--TACAACCCTTTGTGAACATACCTACAACTGTTGCTTCGGCGGGTAGG- 117
KF154738.1      GTTACCGCTC--ATCAACCCTTTGTGAACATACCT-TAACTGTTGCTTCGGCGGGTAGGC 86
EU070913.1      GTTACCGCTC--TACAACCCTTTGTGAACATACCT-CAACTGTTGCTTCGGCGGGCAGGA 87
JX567509.1      GTTACCGCTC--TATAACCCTTTGTGAACATACCT--AACTGTTGCTTCGGCGGGCAGGG 92
KJ002768.1      GTTTACGCTC--TATAACCCTTTGTGAACATACCA--AACCGTTGCTTCGGCGGGCGGGA 79
FN555114.1      GTTGAAAAAC--TCCAACCCCT-GTGAACATAACCTCTGTCGTTGCTTCGGCGG------ 96
                *** ....:*  :  ***** * *********.*   :.  ***** *******

KJ584648        GG----AGCCCAGCTCCG---------GCGCCCGGAG-----CCG--CCTTCTCGGCGCG 114
KJ584649        GG----AGCCCAGCTCCG---------GCGCCCGGAG-----CCG--CCTTCTCGGCGCG 114
KJ584650        GG----AGCCCAGCTCCG---------GCGCCCGGAG-----CCG--CCTTCTCGGCGCG 114
KJ584652        GG----AGCCCAGCTCCG---------GCGCCCGGAG-----CCG--CCTTCTCGGCGCG 114
FJ172225.1      GG----AGCCCAGCTCCG---------GCGCCCGGAG-----CCG--CCTT-ACGGCGCG 132
JX669450.1      GG----AGCCCAGCTCCG---------CCGCCCGGAG-----CCG--CCGTCTCGGCGCG 157
KJ584654        G------TCTCCGTGACC----------CTCCCGGCC---TCCCG--CCCCCGGGCGGGT 111
JX161648.1      G------TCTCCGTGACC----------CTCCCGGCC---TCCCG--CCCCCGGGCGGGT 140
KJ584655        G------TCTCCGTGACC----------CTCCCGGCC---TCCCG--CCCCCGGGCGGGT 111
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KJ584651        G------TCTCCGTGACC----------CTCCCGGCC---TCCCG--CCCCCGGGCGGGT 111
KF053199.1      G------TCTCCGTGACC----------CTCCCGGCC---TCCCG--CCCCCGGGCGGGT 133
KJ584653        G------TCCCCGTGACC----------CTCCCGGCC---TCCCGCCCCCCCGGGCGGGT 113
JX258732.1      G------TCCCCGTGACC----------CTCCCGGCC---TCCCGCCCCCCCGGGCGGGT 158
KF154738.1      G------TCCCCTAAAAAGGACG----TCTCCCGGCCCTCTCCCG--TCCGCGGGTGG-- 132
EU070913.1      GGACAACCCCCCTCGGGGGGCGGTCCCCCTCCCGGCC-----GCG-CCCT-CACGGG-CG 139
JX567509.1      G-----TTCCCCTCGGG--ACG----CCCTCCCGGCC-----ACG-CCCTTCGCGGGGCG 135
KJ002768.1      G-------GTCCGCCTC-----------CCCCCGGCC-----CCG----CTCGCGGG--- 109
FN555114.1      ----------------------------------GCT---------------------- 99
                                                  *.

KJ584648        CCCCACCCGCCGGCGGACCACTAAACTCTATTGCAACGACGTCTCTTCTGAGTGGTACAA 174
KJ584649        CCCCACCCGCCGGCGGACCACTAAACTCTATTGCAACGACGTCTCTTCTGAGTGGTACAA 174
KJ584650        CCCCACCCGCCGGCGGACCACTAAACTCTATTGCAACGACGTCTCTTCTGAGTGGTACAA 174
KJ584652        CCCCACCCGCCGGCGGACCACTAAACTCTATTGCAACGACGTCTCTTCTGAGTGGTACAA 174
FJ172225.1      CCCCACCCGCCGGCGGACCACTAAACTCTATTGCAACGACGTCTCTTCTGAGTGGTACAA 192
JX669450.1      CCCCACCCGCCGGCGGACCACCAAACTCTATTTAAACGACGTCTCTTCTGAGTGGCACAA 217
KJ584654        CGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAA 171
JX161648.1      CGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAA 200
KJ584655        CGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAA 171
KJ584651        CGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAA 171
KF053199.1      CGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAA 193
KJ584653        CGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAA 173
JX258732.1      CGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAA 218
KF154738.1      -GGCGCCCGCCTGAGGATGACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGACACAA 191
EU070913.1      TGGCGCCCGCCGGAGGAT-ACCAAACTCTATTTTAACGACGTTTCTTCTGAGTGGCACAA 198
JX567509.1      AGGCGCCCGCCGGAGGAT-ACCAAACTCTATTTTAACGACGTTTCTTCTGAGTGCCACAA 194
KJ002768.1      --GCGCCCGCCGGAGGAAAACCCAACTCTATTTTAACGACGTCTCTTCTGAGTGGCACAA 167
FN555114.1      ---CGCCCGCCGGAGGTT--ACAAACTCTTGTGTATTTTTTTAGCATCTCTGAGCCTAGA 154
                   *.****** *.**:   . .******  *  *:  :  *  *:*** :*:*  :..*

KJ584648        G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 232
KJ584649        G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 232
KJ584650        G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 232
KJ584652        G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 232
FJ172225.1      G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 250
JX669450.1      G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 275
KJ584654        G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 229
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JX161648.1      G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 258
KJ584655        G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 229
KJ584651        G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 229
KF053199.1      G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 251
KJ584653        G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 231
JX258732.1      G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 276
KF154738.1      G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 249
EU070913.1      G--CAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 256
JX567509.1      G--CAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 252
KJ002768.1      G--CAAATAGTCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 225
FN555114.1      AGACAAATAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC 214
                .  ******.* ******** ************************************

KJ584648        AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 292
KJ584649        AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 292
KJ584650        AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 292
KJ584652        AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 292
FJ172225.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 310
JX669450.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 335
KJ584654        AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 289
JX161648.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 318
KJ584655        AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 289
KJ584651        AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 289
KF053199.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 311
KJ584653        AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 291
JX258732.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 336
KF154738.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 309
EU070913.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 316
JX567509.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 312
KJ002768.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 285
FN555114.1      AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 274
                ************************************************************

KJ584648        CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 352
KJ584649        CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 352
KJ584650        CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 352
KJ584652        CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 352
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FJ172225.1      CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 370
JX669450.1      CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 395
KJ584654        CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 349
JX161648.1      CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 378
KJ584655        CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 349
KJ584651        CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 349
KF053199.1      CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 371
KJ584653        CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 351
JX258732.1      CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 396
KF154738.1      CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 369
EU070913.1      CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 376
JX567509.1      CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 372
KJ002768.1      CACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 345
FN555114.1      CACATTGCGCCCGCCGGTATTCCGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCA 334
                ***************.* **** ************************************

KJ584648        AGCACCG-CTTGGCGTTGGGGCC-------CTACGGCTTCCGTAGGCCCCGAAATACAGT 404
KJ584649        AGCACCG-CTTGGCGTTGGGGCC-------CTACGGCTTCCGTAGGCCCCGAAATACAGT 404
KJ584650        AGCACCG-CTTGGCGTTGGGGCC-------CTACGGCTTCCGTAGGCCCCGAAATACAGT 404
KJ584652        AGCACCG-CTTGGCGTTGGGGCC-------CTACGGCTTCCGTAGGCCCCGAAATACAGT 404
FJ172225.1      AGCACCG-CTTGGCGTTGGGGCC-------CTACGGCTTCCGTAGGCCCCGAAATACAGT 422
JX669450.1      AGCACCG-CTTGGCGTTGGGGCC-------CTACGGCTTCCGTAGGCCCCGAAATACAGT 447
KJ584654        AGCTCTG-CTTGGTGTTGGGGCC-------CTACAGCTGATGTAGGCCCTCAAAGGTAGT 401
JX161648.1      AGCTCTG-CTTGGTGTTGGGGCC-------CTACAGCTGATGTAGGCCCTCAAAGGTAGT 430
KJ584655        AGCTCTG-CTTGGTGTTGGGGCC-------CTACAGCTGATGTAGGCCCTCAAAGGTAGT 401
KJ584651        AGCTCTG-CTTGGTGTTGGGGCC-------CTACAGCTGATGTAGGCCCTCAAAGGTAGT 401
KF053199.1      AGCTCTG-CTTGGTGTTGGGGCC-------CTACAGCTGATGTAGGCCCTCAAAGGTAGT 423
KJ584653        AGCTCTG-CTTGGTGTTGGGGCC-------CTACAGCTGATGTAGGCCCTCAAAGGTAGT 403
JX258732.1      AGCTCTG-CTTGGTGTTGGGGCC-------CTACAGCTGATGTAGGCCCTCAAAGGTAGT 448
KF154738.1      AGCTCTG-CTTGGTGTTGGGGCT-------CTACGGTTGACGTAGGCCCTTAAAGGTAGT 421
EU070913.1      AGCCCAG-CTTGGTGTTGGGGCC-------CTACGGTCGACGTAGGCCCTTAAAGGTAGT 428
JX567509.1      AGCTCTG-CTTGGTGTTGGGGCC-------CTACGGTTGACGTAGGCCCTTAAAGGTAGT 424
KJ002768.1      AGCACCG-CTTGGCGTTGGGGCTT------CCACGGCTGACGTGGGCCCTCAAAGACAGT 398
FN555114.1      AGCCCCGGCTTGGTGTTGGGGCGCCCGGGTCCCCCGCGGCCCGGGGCCCCCAAGTTCATC 394
                *** * * ***** ********       * .* *   .   .*****  **.   *

KJ584648        GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACATACCACCTCGCACT 454
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KJ584649        GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACATACCACCTCGCACT 454
KJ584650        GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACATACCACCTCGCACT 454
KJ584652        GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACATACCACCTCGCACT 454
FJ172225.1      GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACATACCACCTCGCACT 472
JX669450.1      GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACATACCACCTCGCACT 497
KJ584654        GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACTTTACGTCTCGCACT 451
JX161648.1      GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACTTTACGTCTCGCACT 480
KJ584655        GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACTTTACGTCTCGCACT 451
KJ584651        GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACTTTACGTCTCGCACT 451
KF053199.1      GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACTTTACGTCTCGCACT 473
KJ584653        GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACTTTACGTCTCGCACT 453
JX258732.1      GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACTTTACGTCTCGCACT 498
KF154738.1      GGCGGACCC------TCTCGGAGC----CTCCTTTGCGTAGTAACATTTCGTCTCGCATT 471
EU070913.1      GGCGGACCC------TCCCGGAGC----CTCCTTTGCGTAGTAACTTAACGTCTCGCACT 478
JX567509.1      GGCGGACCC------TCTCGGAGC----CTCCTTTGCGTAGTAACTAACGTCCTCGCATT 474
KJ002768.1      GGCGGACCC------TCGCGGAGC----CTCCTTTGCGTAGTAACATACCACCTCGCACC 448
FN555114.1      GGCGGGCTCGTCGGTACACTGAGCGCAGTAAAAACGCGGTAAAACGCGAACCCTCGCTCG 454
                *****.* *      :* * ****     :..:: *** :.:***       *****:

KJ584648        G---GGATCCGGAGGG-ACTCCTGCCGTAAAACCCCCCAATTTATCAAGG-TTGACCTCG 509
KJ584649        G---GGATCCGGAGGG-ACTCCTGCCGTAAAACCCCCCAATTTATCAAGG-TTGACCTCG 509
KJ584650        G---GGATCCGGAGGG-ACTCCTGCCGTAAAACCCCCCAATTTATCAAGG-TTGACCTCG 509
KJ584652        G---GGATCCGGAGGG-ACTCCTGCCGTAAAACCCCCCAATTTATCAAGG-TTGACCTCG 509
FJ172225.1      G---GGATCCGGAGGG-ACTCCTGCCGTAAAACCCCCCAATTTATCAAAGGTTGACCTCG 528
JX669450.1      G---GGATCCGGAGGG-ACTCCTGCCGTAAAACCCCCCAATTTTCCAAAGGTTGACCTCG 553
KJ584654        G---GGATCCGGAGGG-ACTCTTGCCGTAAAACCCCC-AATTCTCCAAAGGTTGACCTCG 506
JX161648.1      G---GGATCCGGAGGG-ACTCTTGCCGTAAAACCCCC-AATTCTCCAAAGGTTGACCTCG 535
KJ584655        G---GGATCCGGAGGG-ACTCTTGCCGTAAAACCCCC-AATTCTCCAAAGGTTGACCTCG 506
KJ584651        G---GGATCCGGAGGG-ACTCTTGCCGTAAAACCCCC-AATTTTCCAAAGGTTGACCTCG 506
KF053199.1      G---GGATCCGGAGGG-ACTCTTGCCGTAAAACCCCC-AATTTTCCAAAGGTTGACCTCG 528
KJ584653        G---GGATCCGGAGGG-ACTCTTGCCGTAAAACCCCCCAATTTTCCAAAGGTTGACCTCG 509
JX258732.1      G---GGATCCGGAGGG-ACTCTTGCCGTAAAACCCCCCAATTTTCCAAAGGTTGACCTCG 554
KF154738.1      G---GGATTCGGAGGG-ACTCTAGCCGTAAAACCCCC-AATTTTACTAAGGTTGACCTCG 526
EU070913.1      G---GGATCCGGAGGG-ACTCTTGCCGTAAAACCCCCAAAACTTTTACAGGTTGACCTCG 534
JX567509.1      G---GGATTCGAAGGG-ACTCTTGCCGTAAAACCCCCAAATTTTCTAATGGTTGACCTCG 530
KJ002768.1      G---GGACCCGCAGGGCACTCCTGCCGTAAACCCCCCCAA------------------- 485
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FN555114.1      GCCCGTCACCGGCGCG--CACCAGCCGCTAAACCCCCAAAACTTTCAAAGGTTGACCTCG 512
                *   * .  ** .* *  *:* :**** :**.***** **

KJ584648        GATCAGGTAGGAATACCCGCTGAACTTAA-------------------------- 538
KJ584649        GATCAGGTAGGAATACCCGCTGAACTTAA-------------------------- 538
KJ584650        GATCAGGTAGGAATACCCGCTGAACTTAA-------------------------- 538
KJ584652        GATCAGGTAGGAATACCCGCTGAACTTAA-------------------------- 538
FJ172225.1      GATCAGGTAGGAATACCCGCTGAACTTAAGCATATC------------------- 564
JX669450.1      GATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAGG--- 605
KJ584654        GATCAGGTAGGAATACCCGCTGAACTTAA-------------------------- 535
JX161648.1      GATCAGGTAGGAATACCCGCTGAACTTAAGCATA--------------------- 569
KJ584655        GATCAGGTAGGAATACCCGCTGAACTTAA-------------------------- 535
KJ584651        GATCAGGTAGGAATACCCGCTGAACTTAA-------------------------- 535
KF053199.1      GATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGGAGGAA---- 579
KJ584653        GATCAGGTAGGAACACCCGCTGAACTTAA-------------------------- 538
JX258732.1      GATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA----- 604
KF154738.1      GATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA----- 576
EU070913.1      GATCAGGTAGGAATACCCGC---------------------------------- 554
JX567509.1      GATCAGGTAGGAATACCCGCTGAACTTAA-------------------------- 559
KJ002768.1      ----------------------------------------------------
FN555114.1      GATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAA 567
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INTEGRATED MANAGEMENT OF FUSARIUM WILT AND ANTHRACNOSE OF VEGETABLE COWPEA (*Vigna unguiculata* subsp. s*esquipedalis* (L.) Verdcourt) USING NEW GENERATION FUNGICIDES

by

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(2011 - 21 - 112)

ABSTRACT OF THE THESIS

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DOCTOR OF PHILOSOPHY IN AGRICULTURE

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DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

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ABSTRACT

The study entitled "Integrated management of Fusarium wilt and anthracnose of vegetable cowpea (*Vigna unguiculata* subsp. s*esquipedalis* (L.) Verdcourt) using new generation fungicides" was carried out in the Department of Plant Pathology, College of Agriculture, Vellayani during 2011 - 2014 with an objective to test the efficacy of new generation fungicides for the management of Fusarium wilt and anthracnose of vegetable cowpea and to develop an integrated management strategy using effective fungicides compatible with ecofriendly tactics.

Isolation, characterization and identification of the pathogens revealed that, *Fusarium oxysporum*, *F. equiseti* and *F. solani* were associated with wilt of cowpea while, *Colletotrichum gloeosporioides* was associated with anthracnose.

In vitro evaluation of new generation fungicides indicated that tebuconazole (0.1 %), carboxin + thiram (0.4 %), and mancozeb (0.25 %) completely inhibited the mycelial growth of *F. oxysporum* and *C. gloeosporioides.*

Pot culture experiments conducted to evaluate the efficacy of new generation fungicides for the management of Fusarium wilt and anthracnose revealed that soil drenching of flusilazole (0.1 %), tebuconazole (0.1 %) or carbendazim (0.1 %) totally suppressed the incidence of wilt while, the foliar application of flusilazole (0.1 %) recorded the lowest index (5.60) of anthracnose which was on par with the treatment with tebuconazole (0.1 %).

A field experiment conducted to develop an integrated disease management package Fusarium wilt and anthracnose of cowpea indicated that soil solarization, application of *Trichoderma* enriched neem cake organic manure mixture and soil drenching of either tebuconazole (0.1 %) or carbendazim (0.1 %) recorded the lowest (0.00) disease index of Fusarium wilt whereas, three rounds of foliar application of either flusilazole (0.1 %) or tebuconazole (0.1 %) recorded the lowest (0.93) anthracnose index. The studies on persistence and degradation of

fungicide residues indicated that tebuconazole (0.1 %) was having the shortest waiting period (0 days) indicating its safety to the vegetable.

Considering the overall efficacy of fungicides against two diseases, associated yield increase, safety to the crop as well as economic benefits the following integrated management package was developed for the control of Fusarium wilt and anthracnose of cowpea in disease prone areas as: 1) Seed treatment with carbendazim (2 g/kg seed) 2) Soil solarization for a period of 45 days using transparent polythene sheets during warm season 3) Application of *Trichoderma* enriched neem cake organic manure mixture @ 1 kg/pit 15 days after seed emergence 4) Application of tebuconazole (0.1 %) at 30, 45 and 60 days after seed emergence. In order to counter the possibility of resistance build up due to repeated use of the triazole fungicide, the contact fungicide copper oxychloride (0.2 %) may be used in rotation.