Varietal screening and management of anthracnose of black pepper using new generation fungicides

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(2018-11-040)

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COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM - 695 522

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Abstract of the thesis

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2020

DECLARATION

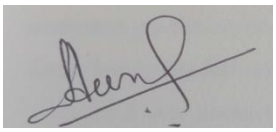
I, hereby declare that this thesis entitled "**Varietal screening and management of anthracnose of black pepper using new generation fungicides.**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

Place: Vellayani **ATHIRA K.**

Date: 27-1-2021 (2018-11-040)

CERTIFICATE

Certified that this thesis entitled "**Varietal screening and management of anthracnose of black pepper using new generation fungicides"** is a record of research work done independently by Ms. Athira K. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to her.



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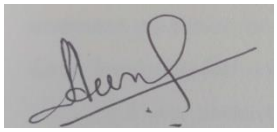

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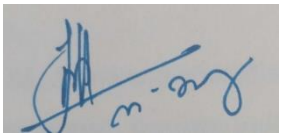

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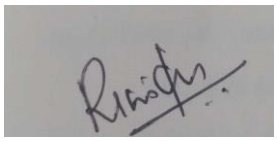

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Athira

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
^{0}C	Degree Celsius
CD	Critical difference
SE (m) ±	Standard error of mean
Cm	Centimeter
G	Gram
Ha	Hectares
µm	Micrometer
Ml	Millilitre
PDA	Potato Dextrose Agar
DTSA	Days taken for symptom appearance
DTCP	Days taken for complete coverage of petridish
Viz.	Namely
et al.	And other co-workers
sp.	Species
spp.	Several species
DAI	Days after inoculation
CRD	Completely Randomized Design
PDI	Per cent disease index

Introduction

1. INTRODUCTION

Black pepper (*Piper nigrum* L.), referred as "King of Spices" or "Black Gold", remains as one of the most precious and valuable spice in the world and the significance of pepper amongst globally traded commodities is unequivocal. Tropical evergreen forest of Western Ghats of South India is considered as the Centre of Origin and source of diversity among several *Piper* spp. More than 75 cultivars are being cultivated in India; of which Karimunda is the most popular cultivar in Kerala. Eighteen improved varieties of black pepper have been released for cultivation *viz*., Panniyur 1 to Panniyur 8, Sreekara, Subhakara, Panchami, Pournami, PLD-2, IISR Shakthi, IISR Thevam, IISR Girimunda and IISR Malabar Excel. Panniyur 1, Panniyur 3, Panniyur 8, IISR Girimunda and IISR Thevam are hybrids and Panniyur 2 has the highest piperine content (6.6%) (IISR, 2015).

Black pepper is a good source of vitamins, carotenes, flavonoids and lycopene, which help to scavenge free radicals and cleanse our body. It is valued for its pungency contributed by the alkaloid piperine and flavour by the volatile oil which impart multiple application in processed food industry, perfumery, traditional and modern medicines and beauty care in many parts of the world since ancient times (Kallupurakkal and Ravindran, 2002). Medicinal properties of *P. nigrum* include antibacterial, antifungal, antidepressant, antidiarrheal, antiapoptotic, anti-inflammatory, antimutagenic, antioxidative, antipyretic, antitumor, to improve appetite and digestive power, anti-cold, anti-cough, for curing from throat diseases, anti-colic and anti-dysentery (Ahmad, 2012; Islam, 2015).

Leading black pepper producing countries in the world are India, Indonesia, Malaysia, Sri Lanka, Thailand, Vietnam, Brazil and China. India is having largest area under black pepper (1.83 lakh ha) and accounts for 54 per cent of total area in the world followed by Indonesia (1.43 lakh ha) (Anon., 2016). Black pepper cultivation in India is mainly confined to Kerala, Karnataka, Tamil Nadu, Goa, Pondicherry and north eastern states. Karnataka contributes about 27 per cent of area and 54.6 per cent of total production of black pepper with a productivity of 471 kg ha^{-1}. But Kerala has 63 per cent

of cultivated area and contributes only 34.3 per cent of the production with a productivity of 254 kg ha^{-1} (Senthilkumar and Swarupa, 2017). The price of black pepper has been declining since 2017-18. The average price of black pepper declined to Rs 378.21 per kg (2018-19) from Rs 694.77 per kg in the domestic market during 2016 -17 (Spice Board of India, 2019).

In the recent past, India suffered a setback in black pepper production attributed to heavy rainfall, floods, landslides, pests and diseases in the pepper-growing districts of Kerala and Karnataka. Among the major constraints in the production, diseases incited by various microorganisms play a significant role. Anthracnose or fungal pollu incited by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is a wide spread and economically important disease causing significant yield loss. The disease is more prevalent during the post-monsoon season in the major black pepper growing regions. It often attains epiphytotic proportions particularly in the high elevation regions where misty conditions prevail.

C. gloeosporioides is also an important pathogen on other major spice crops (Nair *et al*., 1977; Karunakaran and Nair, 1980; Govindaraju *et al*., 1998; Kumari and Rajagopalan,, 2000; Sankar and Kumari, 2002). The fungus attacks all the aerial parts including foliage, stem, spikes and berries (Anandaraj and Sarma, 1995). Chlorotic angular leaf spot surrounded by yellowish halo is the most common symptom of anthracnose. In high ranges, circular spots are noticed and occasionally the necrotic portion drops off producing shot holes. Under favourable condition, leaf spots join together resulting in leaf blight followed by defoliation. The fungus causes necrosis on the stalk of the spike resulting in spike shedding. Infected spikes turn black, shrivel and shed. The infection starts from the tip of the spike and gradually progresses upwards. Berries at the proximal end of the spike develop to maturity except that they exhibit minute black spot. Berries close to the diseased portion are smaller in size. It also causes a mild crack on some berries and further development is affected. The affected berries dry up gradually and remain as light berries (fungal pollu).

The present management of anthracnose or pollu includes spraying of Bordeaux mixture 1% or combination fungicide carbendazim + mancozeb @ 0.2% (KAU, 2016). The decline in export to European countries has been attributed to pesticide residue due to its irrational usage. The pathogen developed resistance against many of conventional fungicides and also against systemic fungicides with single site of action. In this paradigm, a shift in the current management strategies with the introduction of new molecules of fungicides effective at low concentrations, low residual effect and low mammalian toxicity is of great importance. One of the key reasons for the success of strobilurins fungicides is that it gives better control over the fungi from all four groups of plant pathogens *i.e.*, Ascomycota, Basidiomycota, Deuteromycota and Oomycota. Even though triazoles, strobilurins and their combination fungicides are gaining attention, its complete potential has not been explored and adopted for black pepper disease management.

In this context the present study entitled with **"**Varietal screening and management of anthracnose of black pepper using new generation fungicides." is emphasised on the following objectives: To screen KAU varieties and most popular local cultivar Karimunda for resistance against anthracnose of black pepper caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and evolve management strategy using new generation fungicides.

Review of Literature

2. REVIEW OF LITERATURE

Black pepper has been designated as the most valuable spice since ancient times and occupies a unique position in spice industry. Black pepper has played a pivotal role in India's international trade. It is cultivated in about 26 countries, of which Vietnam, Brazil, Malaysia and Sri Lanka are the leading producers. In India, the cultivation is mainly confined to Karnataka, Kerala and Tamil Nadu (Ravindran, 2000). The production and productivity of black pepper has been drastically reduced recently due to incidence of various pests and diseases, high cost of production and inconstant market price for the produce which in turn lead to subsequent neglect of crop by the farmers.

Black pepper is vulnerable to many diseases like foot rot, anthracnose or fungal pollu, slow decline and stunt disease of which, anthracnose caused by ascomycetous fungi *Colletotrichum gloeosporioides* is an economically important fungal disease limiting its production and productivity in Kerala.

2.1. HISTORY, OCCURRENCE AND PREDISPOSING FACTORS

Anthracnose of black pepper was first identified in North Malabar of Kerala as "berry spot" and "berry split" by Ayyar *et al.* (1921). Later, the disease was named as 'fungal pollu' and the causal organism was identified as *C. gloeosporioides* (Rao, 1926). *C. capsici*, *C. gloeosporioides* and *C. piperis* were reported as the causal agents of anthracnose of black pepper (Kueh, 1990). Chetana *et al.* (2015) reported five new species of *Colletotrichum viz., C. syzygicola*, *C. queenslandicum*, *C. siamese*, *C. endophytica* and *C. guajavae* from anthracnose infected black pepper by multi loci phylogenetic analysis. There are several species coming under genus *Colletotrichum* of which *C. graminicola* and *C. higginsianum* genomes were completely sequenced. *C. gloeosporioides* genome is under study but various genes involve in pathogenesis and host defense mechanism have been identified.

Corda (1837) introduced the generic name *Colletotrichum* for *Colletotrichum lineola* causing anthracnose of sorghum. Penzing (1882) reported the pathogenic *C. gloeosporioides* for the first time and it was first reported in India by

Butler (1918) in coffee leaves. *C. gloeosporiodes* is a widespread and common plant pathogen world-wide; and infects about 470 different host genera (Sutton, 1992; Cannon *et al*., 2000). The fungus is widely distributed in tropical and subtropical regions than in temperate regions (CAB International, 2005) and also functions as an endophytic strain. Endophytes of *Colletotrichum* are mostly members of *C. gloeosporioides*, *C. graminicola* and *C. boninense* (Jayawardena *et al*., 2012). This fungus is designated as world's 18 most important plant pathogenic fungi due to its versatile, widespread and destructive nature (Hyde *et al*., 2009; Phoulivong *et al.,* 2010 and Dean *et al.,* 2012).

The asexual stage (anamorph) is called *C. gloeosporioides* (Schrenk and Spaulding, 1903) while *Glomerella cingulata* is the sexual stage (teleomorph). The telomorphic stage is known for their ability to cause serious disease (Cannon *et al*., 2012)

C. gloeosporioides follows two routes for germination: pathogenic and saprophytic (Barhoom and Sharon, 2004). In hemibiotrophic mode of infection, sequential biotrophic and necrotrophic phases occur. Initially pathogen establishes interaction with host by developing melanised appressorium and penetrates the host cuticle. Infection vesicles and primary hyphae formed after penetration function similar to haustoria (formed by powdery mildews and rust fungi) and do not cause any harm to host. This stage of infection is called biotrophic phase. In the final stage, necrotrophic secondary hyphae develop and spread to kill the entire host cell (Munch *et al.*, 2008).

A severe outbreak of anthracnose of black pepper was reported in Idukki district of Kerala during 1999 (Kurian *et al*., 2000). The disease was observed both in the nursey and plantations. The disease caused severe defoliation in nurseries, whereas in plantations it infected the leaves, spikes and berries.

Damage to berries has been reported to cause cent per cent yield loss. Anthracnose is a serious problem, especially during the post-monsoon season in majority of the black pepper plantations. Maximum damage due to the disease was noticed during August-September (Nair *et al*., 1987). Kurian *et al.* (2002) attributed high rainfall and

number of rainy days as key determining factors in the outbreak of anthracnose of black pepper in the high ranges of Kerala.

Nair *et al.* (1987) reported the severity of disease ranged from 28.0 to 34.0 per cent leading to a crop loss ranging from 1.9 to 9.5 per cent. Ahmed *et al*. (2014) observed that severity of anthracnose on betel vine was found to be more severe in July (80.50 %); while, the severity was recorded the lowest in December (10.87 %).

Estrada *et al*. (1993) reported that the presence of germination inhibitors in the conidial matrix of *C. gloeosporioides* often inhibit their *in situ* germination. The normal germination of the conidia was restored after dissemination by rain splash. Manandhar *et al*. (1995) observed that appressoria produces secondary conidia during wet periods which facilitated secondary spread of anthracnose disease of bell pepper.

Biju *et al*. (2013) reported a positive correlation of the disease incidence with rain fall, number of rainy days and minimum temperature; and negative correlation with maximum temperature. The disease outbreak was higher during August and September with disease incidence of 37.9 and 39.1 per cent respectively; during which the maximum temperature ranged from 23.7 to 24.3°C and minimum temperature from 17.6 to 18.7°C. The average monthly rainfall of 306.6 - 584.7 mm and 22-31 rainy days were reported to be most congenial for the disease incidence.

The annual rain fall of 2000 mm with a uniform distribution pattern was ideal for black pepper cultivation. Heavy continuous rainfall limits flowering. Reduced spike intensity in rainfed condition is due to staggered and delayed spiking. The rainfed condition also increased spike shedding due to anthracnose (Krishnamurthy *et al*., 2016).

2.2. SYMPTOMATOLOGY, ISOLATION OF PATHOGEN, PROVING PATHOGENICITY OF *Colleotrichum* spp. CAUSING ANTHRACNOSE DISEASE OF BLACK PEPPER

2.2.1. Symptomatology

Anthracnose, derived from a Greek word meaning ‘coal’, is the common name for plant diseases characterized by very dark and sunken lesions containing spores (Isaac, 1992).

Dastur (1935) reported that the symptom of anthracnose disease initiates as circular spots with brown to black centre surrounded by yellow halo on leaves whereas black lesions on stem. On later stage withering, drying and death of affected vines were also noticed.

Thomas and Menon (1939) described the symptoms of fungal pollu disease of black pepper for the first time. They observed that circular or irregular grey spots on upper leaf surface, followed by appearance of acervuli in concentric circles. Later stem infection started at tip and spread downward slowly. The spikes were also infected.

Cheeran and Mathew (1984) observed the symptoms of black pepper anthracnose initially as necrotic specks, later changed to dark brown spots. These spots enlarged gradually to form irregular circles, surrounded by yellow halo. The necrotic area became white to papery white in colour. Infection can also be found on spikes leading to spike shedding.

Chattopadhyay and Maiti (1990) described anthracnose symptoms on betel vine leaves by the presence of circular spots with brownish black centres with yellow halo. These spots later coalesced to form large lesions.

The symptoms of anthracnose of bush pepper caused by *C. gloeosporiodes* appeared initially as pin head sized spots on foliage, later enlarged to form blighted areas. The blighting was observed from tip of margin and shot hole symptoms were also observed (Kumari and Sankar, 2003).

Colletotrichum spp. affects plant parts of Proteaceae hosts *viz*., twigs, leaves, blooms and fruit, causing various symptoms such as leaf spot, leaf blight, shot hole, crown root rot, defoliation, bloom blight and fruit rot (Lubbe *et al*., 2006). Jayakumar *et al.* (2009) observed two different kinds of leaf anthracnose symptoms on black pepper leaves. The first one is angular to irregular or circular brownish lesions with a chlorotic halo and pinhead size acervuli on the leaves, later leads to berry splitting and the production of hollow berries. The novel symptom was pale green or yellowish green lesions initially on the leaves, turning to brown or black with raised margins with acervuli at the centre as the disease progressed.

Biju *et al*. (2020) noticed diverse array of foliar symptoms of anthracnose disease during the survey carried out in black pepper growing areas of Kerala and Karnataka. Symptoms include brown spot surrounded by yellow halo on the newly emerged leaves and randomly distributed spots with or without yellow halo on older leaves. Spots with grey centre along with shot hole and leaf blight symptoms were observed in few locations. In nurseries, pinhead size dark brown necrotic spots later surrounded by yellow halo were observed as a common symptom. An unusual symptom was noticed in older leaves as greyish necrotic lesion with brown-blackish margins with randomly distributed pin–head sized structures.

2.2.2. Isolation of the pathogen

Kumari and Sankar (2003) isolated the pathogen from the samples of anthracnose infected black pepper. The materials were surface sterilized with 0.1 per cent mercuric chloride followed by three washings with sterile water. These surface sterilized bits were placed on potato dextrose agar (PDA) medium containing streptomycin sulphate (30 mg 1^{-1}).

Isolation of *C. gloeosporioides* from infected leaves and berries of black pepper was done by submerging the infected plant bits in 5 per cent sodium hypochlorite for five minutes followed by a series of sterile water washings. The surface sterilised bits were

then placed on Petri dishes containing potato dextrose agar (PDA) medium and incubated at 30^0C for 48 h (Ann and Mercer, 2017).

2.2.3. Proving pathogenicity

Sankar (2002) conducted the pathogenic studies of *C. gloeosporioides* on black pepper using pin prick method. The pinpricked leaves were inoculated with 7 day old culture of the mycelium of the pathogen and conidia on both sides. A thin layer of moist cotton was placed over the inoculated area to maintain humidity required for symptom development. The fungus was re-isolated from infected part to prove Koch's postulate.

Chandrakant (2005) proved pathogenicity of *C. gloeosporioides* on black pepper seedling by making injuries with sterilized sand papers and inoculated the mycelial bit along with spore mass on leaf surface. Seedlings were kept in humid chamber for symptom development and the pathogen was re-isolated from the inoculated part.

Haralpatil (2006) performed the pathogenicity study of *C. gloeosporioides* on detached leaf of betel vine. Leaves were sterilized with 70 per cent alcohol and washed with sterilized water for 3-4 times. Injuries were made on leaves by using carborandum powder and spore suspensions along with mycelial bits were inoculated on abberated leaf surface. The leaves were kept in humid chamber for symptom development

Biju *et al.* (2020) conducted pathogenicity studies of *C. gloeosporioides* on black pepper. Black pepper variety Panniyur 1 at four leaf stage and second fully opened leaf were used for inoculation. The leaves were subjected to surface sterilization with 0.5 per cent sodium hypochlorite for 2 min and inoculated with spore suspension ($3 \times 10^6 ml^{-1}$). 50 µl of the spore suspension was spotted on leaf surface and covered with a moistened cotton swab. The entire plant was covered with a polythene bag to maintain humidity and monitored for symptom development.

2.3. MORPHOLOGICAL AND PATHOGENIC VARIABILITY OF *Colletotrichum* ISOLATES AND IDENTIFICATION OF VIRULENT ISOLATE

Traditionally the identification and characterization of *Colletotrichum* spp. were relied on the morphology features such as colony colour, size and shape of conidia and appressorium, optimal temperature for growth, growth rate and presence or absence of setae (Arx, 1957; Smith and Black, 1990; Gunnel and Gubler, 1992; Sutton, 1992).

2.3.1. Cultural and Morphological characterisation of different isolates

Mordue (1971) reported that conidia of *C. gloeosporioides* were hyaline, cylindrical in shape with a size ranging from 9-24 µm x 3-6 µm and phialidic conidiophore. *C. gloeosporioides* produced pale salmon coloured conidial masses with straight cylindrical conidia of size 12-17 µm x 3.5-6 µm (Sutton, 1992).

Kanapathipillai (1996) stated that *C. gloeosporioides* from black pepper had conidial size of 14.31 x 4.33 µm. The colony appeared as grey to orange coloured with regular margin and the mycelium was hyaline.

Sankar (2002) observed that *C. gloeosporioides* isolates of black pepper had a colony colour either white or light grey with a dark greyish centre. The average conidial size was 13.10 - 16.85 µm x 3.28 - 5.43 µm.

Mammooty (2003) studied the cultural and morphological character of *C. gloeosporioides* isolates of black pepper. Colony colour varied from light to dark grey. The mycelial width of the pathogen ranged about 1.25 - 4.00 µm. The spore size ranged about 13.45 - 16.45 µm x 3.86 - 5.36 µm.

Vivekananth (2006) reported that *C. gloeosporioides* from anthracnose infected papaya produced white to greyish white, cottony growth in PDA medium with the conidial size 16 µm x 4 µm. The conidia were hyaline, single celled with an oil globule at centre with diverse shapes of ovoid to cylindrical.

Poornima (2007) conducted an experiment to study the culture and morphological variability of *C. gloeosporioides* isolated from black pepper, betel vine and thippali. The black pepper isolate had fluffy white growth with grey margins, whereas, thippali and betel vine isolates showed white coloured colony. The high sporulation rate was observed in *C. gloeosporioides* isolates of betel vine compared to thippali and black pepper. Conidia of the *Colletotrichum* isolates were hyaline, cylindrical with oil globule at centre. The average size of the conidia were 12.67 µm x 4.7 µm, 14.63 µm x 4.7 µm and 15.52 µm x 4.7 µm for thippali, black pepper and betel vine isolates respectively.

Venkataravanappa and Nargund (2002) described morphological variation of six *C. gloeosporioides* isolates causing mango anthracnose obtained from different districts of Karnataka state. The conidial size were 19.95 x 7.6 µm, 18.17 x 7.23 µm, 16.96 x 6.5 µm and 17.09 x 6.53 µm from isolates of Bangalore, Raichur, Bidar and Dharwad districts respectively.

Meshram *et al*. (2014) noticed the isolates of *C. gloeosporioides* greatly varied in their colony colour and appearance. The colony colour of *C. gloesporioides* varied from white to grey, dark orange or pink grey while the reverse side was off white, dark grey orange or a mixture and with regular colony margins.

Sreeja (2014) studied colony and morphological characters of *C. gloeosporioides* isolated from cowpea. Colony nature varied from dense to sparse and produced off white to grey colour on upper side while, it appeared dark grey to black coloured colonies on reverse side. Pathogen completed growth in Petri dish within 6-10 days and growth rate ranged from 0.80 to 1.38 cm day^{-1}. The mycelium was hyaline, septate and branched; and conidia were cylindrical with a dimension of 8.6-11.3 µm x 3.5-4.3 µm.

Papade *et al.* (2019) conducted an experiment to study morphological variation of seven isolates of *C. gloeosporioides* obtained from various hosts *viz*., *Mangifera indica, Citrus aurantifolia, Catharanthus roseus, Psydium guavajava, Punica granatum, Carica papaya,* and *Glycine max*. They reported that all *C. gloeosporioides*

isolates produced white, pink and greyish coloured colony. Conidia of all isolates were oblong to cylindrical having one fat globule at centre. Acervuli had brownish to blackish coloured raised conidial mass.

2.3.2 Screening for pathogenic variability of different isolates of *C. gloeosporioides*

Sankar (2002) studied the pathogenic variability of different *C. gloeosporioides* isolates from black pepper for their virulence. Based on time taken for symptom development, nature of symptom development and the rate of lesion development, isolates were categorized into three groups as highly virulent, semi-virulent and mildly virulent.

Kumari *et al*. (2017) evaluated pathogenic variability of different *C. gloeosporioides* isolates obtained from mango infected with anthracnose. Three different inoculation methods were used to test pathogenic variability *viz*., spray, dipping, and pinprick. Time taken for symptom development and disease severity were recorded to assess the most virulent isolates. Maximum disease severity of 71.1 was recorded on mango leaf by pin prick method.

2.4. VARIETAL SCREENING

Growing resistant varieties could be the most economical, easy and reliable method to minimize crop loss due to diseases. An adequate amount of inoculum is required for successful screening. The conventional spraying method has the drawback of causing considerable variation in the distribution of spores.

Rajapakse and Ranasinghe (2002) developed varietal screening method for anthracnose disease of chilli under field conditions by spraying spore suspension of *C. capsici* (10^5 conidia ml^{-1} water) at red ripe stage. Generally, under dry conditions with low relative humidity when chilli is cultivated with irrigation, the frequency of occurrence of anthracnose was low. Therefore, water spraying, from next day after artificial inoculation is an important requirement for conidial differentiation into infective

structure on fruit surface. Percentage incidence of anthracnose affected fruits was used to identify resistant varieties.

Kurian *et al.* (2002) evaluated fourteen varieties of black pepper for yield, quality and disease resistance against anthracnose in the high range of Idukki district. They observed no significant difference between the varieties with respect to the disease. Percentage of infected leaves was highest in local cultivar Kottanadan (15.8) and the lowest in Subhakara with PDI of 8.2 per cent followed by Sreekara with PDI of 10.0 per cent.

Kumar and Rawal (2009) screened seventeen papaya cultivars against anthracnose disease caused by *C. gloeosporioides* under *in vitro* condition. Among the different methods of artificial inoculation, the lesion diameter was more in spore suspension spray plus pin prick method (13.50 mm) followed by spore injection and mycelia disc plus pin prick method which accounts 8.50 and 6.20 mm, respectively. Per cent disease index (PDI) and days taken for symptom development were calculated after the treatment. According to the PDI, different cultivars were classified into resistant, moderately resistant, susceptible and highly susceptible.

Krishnappa *et al*. (2015) screened 19 pomegranate cultivars against *C. gloeosporioides* by using detached leaf inoculation technique. Five young leaves from each genotype were collected and washed thoroughly with distilled sterile water and swabbed with 1 per cent sodium hypochlorite followed by sterile water wash and inoculated with 5 mm disc of 12 days old culture. Control was maintained by spraying sterile water alone. Moist cotton swab was placed at the base of petiole leaves and were kept in Petri plates lined with moist blotting paper to maintain humidity. Further, these plates were placed in humid chamber and incubated at 27±1°C for 12 days. After 12 days of incubation the genotypes were evaluated for their reaction on 0-5 scale. The varieties were categorized based on different grades as Grade 0 No disease (Immune), Grade 1- 0.0 to 5.0 (Resistant), Grade 2- 5.1 to 10.0 (Moderately Resistant), Grade3-10.1 to 25.0

(Moderately Susceptible), Grade 4- 25.1 to 50.0 (Susceptible) and Grade 5- >50 per cent (Highly Susceptible).

Bhagwat *et al*. (2015) conducted varietal screening of mango against anthracnose disease caused by *C. gloeosporioides* under natural condition. Uniform aged plants with 8-10 leaves were marked for screening and all the recommended agronomical practices were adopted for the crop. Observations on infection and symptom development were recorded on the basis of 0-5 scale. Based on per cent area infected, cultivars were rated from 0-5. 0-Immune, 1- Resistant (1-10 %), 2 - Moderately resistant (11-20 %), 3-Moderately susceptible (21 -30%), 4 - Susceptible (31 - 50 %), and 5-Highly susceptible (above 50%).

Adikshita (2017) screened fifteen cultivars of mango against *C. gloeosporioides.* Fruits were inoculated with virulent pathogen by pin prick method and stored at room temperature (25-28°C). Disease severity was recorded at 7, 15, 21, 28 days after inoculation by following 0 - 5 disease rating scale. Based on PDI cultivars were grouped into Immune (No disease), resistant (1-10 %) moderately resistant (10.1-20 %), moderately susceptible (20.1-30%), susceptible (30.1- 50 %), and highly susceptible (above 50%).

2.5. *IN VITRO* SCREENING OF FUNGICIDES AGAINST MOST VIRULENT ISOLATE OF *C. gloeosporioides*

Several newer fungicides with low residual effects that are quite secure to man and environment has been used against anthracnose disease (Gawade *et al*., 2009; Ganeshan *et al*., 2011). Strobilurins and triazoles are systemic fungicides with curative nature and hence used for the control of many fungal diseases in crop plants (Anon., 2016). New generation fungicides are known for their systemic mode of action, curative nature, longevity in disease control, target specificity and safer to non - target sites.

Mushrooms (*Basidiomycetes*) are the natural sources of strobilurins. Strobilurin-A is known as the first naturally isolated strobilurin compound and was originally isolated

from the mushroom *Strobilurus tenacellus* by Anke *et al*. (1977). In 1996 the German market received a first patent for a strobilurin fungicide (azoxystrobin) (Sauter *et al*., 1999; Bartett *et al*., 2001).

Structurally, the presence of toxiphoric (E)-β-methoxyacrylate group is the distinctive feature of strobilurin fungicides (Balba, 2007). It has specific mode of action against broad-spectrum of fungi, rapid and highly efficient, cost effective and rapidly degrade during plant metabolism; and these benefits have contributed to the large-scale use of these fungicides.

Strobilurins are also referred as Q_oI fungicides (quinone outside inhibitors) due to their peculiar mechanism of action. They specifically bind to the quinone oxidation (Q_o) site of cytochrome b to inhibit mitochondrial respiration. This binding blocks electron transfer between cytochrome b and cytochrome c1; thus inhibits the synthesis of nicotinamide adenine dinucleotide (NADH) oxidation and the mitochondrial membrane protein adenosine triphosphate (ATP) (Isamu and Makoto, 2005; Balba, 2007).

Triazole fungicides are sterol biosynthesis inhibitors. Sterols such as ergosterol are needed for cell membrane structure and function. Triazole fungicides interfere with fungal cell wall formation and thereby inhibit fungal growth (Leroux *et al*., 2008).

Triazole fungicides were found to be effective in managing *C. gloeosporioides* of black pepper. Propiconazole, hexaconazole and difenconazole at 0.05 per cent inhibited mycelia growth of the pathogen. Copper oxy chloride at 0.25 per cent showed 87.11 per cent inhibition; and was the least effective among the tested fungicides (Chandrakrant, 2005).

Sundravadana *et al*. (2007) reported the efficacy of azoxystrobin against mango anthracnose pathogen, under *in vitro* conditions. Azoxystrobin at 1.0, 2.0, and 4.0 ppm completely inhibited the mycelial growth of *C. gloeosporioides*.

Kurian *et al*. (2008) reported that black pepper vines treated with the combination fungicide (carbendazim + mancozeb @ 0.1 %) was found to be effective against anthracnose caused by *C. gloeosporioides* with the highest dry berry yield (742.7 g/vine).

The treatment also reduced the leaf infection by 70.8 per cent and spike infection by 70.3 per cent.

Jayalakshmi (2010) evaluated the efficacy of systemic and non-systemic fungicides under *in vitro* condition against *C. gloeosporioides* causing anthracnose of pomegranate. The study revealed that among systemic fungicides azoxystrobin and propiconazole at 0.15 per cent showed 62.77 per cent and 87.10 per cent inhibition of mycelial growth; and hexaconazole at 0.1 per cent showed 65.77 per cent inhibition under *in vitro* condition. The combination fungicide, carbendazim + mancozeb at 0.3% showed 81.88 per cent inhibition.

Pandey *et al*. (2012) found that carbendazim + mancozeb at 100 ppm resulted in maximum growth inhibition for *C. gloeosporioides* causing anthracnose of mango. Ingle *et al*. (2014) reported that tebuconazole (0.1 %) and carbendazim + mancozeb (0.1 %) were found to be most effective against *Colletotrichum* leaf spot of soybean.

Adhikary *et al*. (2013) evaluated the efficacy of azoxystrobin under *in vitro* conditions against anthracnose of mango. The application of azoxystrobin above 200 ppm completely inhibited the mycelial growth and conidial germination of *C. gloeosporioides* of mango.

Kinjal *et al.* (2016) reported that propiconazole at 1000 and 1500 ppm; carbendazim (12%) + mancozeb (63%) at 1000, 2000 and 2500 ppm completely inhibited the mycelial growth of *C. gloeosporioides causing* anthracnose of mung bean under *in vitro* conditions.

Parvathy and Girija (2016) evaluated the *in vitro* suppression of *C. gloeosporioides* causing anthracnose of black pepper by using commercially available fungicides. Tebuconazole at 0.1 per cent and the combination fungicide carbendazim + mancozeb at 0.1 per cent showed 100 per cent inhibition of pathogen over the control followed by propiconazole (0.1 %) with 93 per cent inhibition. The combination fungicide captan + hexaconazole fungicide (0.1 %) recorded 86.62 per cent inhibition

while azoxystrobin 0.15 per cent had 86 per cent inhibition of the pathogen over control. Copper hydroxide (0.25 %) was the least effective in inhibiting the pathogen (82.42 %).

Dev and Narendrappa (2016) conducted *in vitro* evaluation of fungicides against *C. gloeosporioides* causing anthracnose of pomegranate (*Punica granatum* L.). The study showed that two combination fungicides *viz.,* hexaconazole + zineb, trifloxystrobin + tebuconazole and a non-systemic fungicide captan showed cent percent inhibition at 100, 250, 500 and 1000 ppm concentrations. Systemic fungicides *viz.*, hexaconazole, propiconazole, tebuconazole and carbendazim showed cent percent mycelial inhibition at 500, 1000 and 2000 ppm concentrations.

Ann and Mercer (2017) reported the efficacy of tebuconazole and trifloxystrobin against *C. gloeosporioides* infection in black pepper under both *in vitro* and *in vivo*. The application of combination fungicide (tebuconazole + trifloxystrobin) at 0.4 g L^{-1} showed complete mycelial inhibition under *in vitro* condition; and in field condition, the disease reduction was 100 per cent over control.

Nisha (2018) reported that propiconazole, tebuconazole, carbendazim + mancozeb effectively inhibited *C. gloeosporioides* of betel vine. Propiconazole was the most effective with 100 per cent inhibition at 50, 100, 300 and 400 ppm followed by tebuconazole (97.22 %) and carbendazim + mancozeb (91.94 %) at 400 ppm.

Sharma *et al*. (2019) evaluated efficacy of systemic, non-systemic and combination fungicides against *C. gloeosporioides* causing mango anthracnose. The study revealed that among non-systemic fungicides, mancozeb showed cent percent mycelial inhibition at 1200 ppm while, copper oxy chloride showed 97.26 per cent inhibition at the same concentration. Systemic fungicides azoxystrobin, propiconazole and difenconazole showed 100 per cent inhibition at 50, 100, 250, 500 ppm concentrations. The combination fungicides namely trifloxystrobin + tebuconazole (nativo), azoxystrobin + tebuconazole (custodia) inhibited the mycelial growth of the pathogen at 50, 100, 250 and 500 ppm.

Materials and Methods

3. MATERIAL AND METHODS

The present study entitled “Varietal screening and management of anthracnose of black pepper using new generation fungicides.” was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani during the period from 2018-2020 with the objectives to screen varieties of KAU and most popular local cultivar Karimunda for resistance against anthracnose of black pepper caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and evolve management strategy using new generation fungicides.

3.1 SURVEY AND COLLECTION OF DISEASED SAMPLES,

3.1.1 Diseased sample collection

As a part of the study, infected samples were collected from three black pepper growing districts of Kerala *viz*., Thiruvanathapuram, Wayanad and Idukki during June 2019 to October 2019. Sample collections were made from two locations each in Thiruvananthapuram (Kowdiar and Vellayani) and Wayanad (Meenagadi and Ambalavayal), and four locations in Idukki (Myladumpara, Pampadumpara, Kattapana and Kambilikandam). The weather parameters *viz.*, maximum temperature, minimum temperature, relative humidity and rainfall were recorded during the survey period. Diseased fields were marked at each location and from each field, ten plants were selected randomly for scoring the disease intensity. The disease incidence in a particular field was also calculated.

The per cent infected leaves indicated the prevalence of disease in selected locations. Incidence of anthracnose of black pepper was calculated by using the formula given by Singh (2002)

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

The disease severity was estimated by calculating per cent disease index based on a score chart. Ten leaves were selected randomly and graded based on 0 - 7 disease scale (Sankar, 2002).

Percentage disease index was calculated by the formula given by Mayee and Datar (1986)

$$PDI = \frac{\text{Sum of grades of all the leaves}}{\text{Total no. of leaves observed}} \times \frac{100}{\text{Maximum grade used}}$$

Score chart used for assessing the disease severity of anthracnose of black pepper

Grade of the leaf	% of the infected are of leaf
0	No infection
1	1 - 10%
3	11 - 25%
5	26 - 50%
7	> 50%

3.2 SYMPTOMATOLOGY, ISOLATION OF PATHOGEN, PROVING PATHOGENICITY OF *C. gloeosporioides* CAUSING ANTHRACNOSE DISEASE OF BLACK PEPPER

3.2.1 Symptomatology

The symptoms of anthracnose of black pepper varied in different locations depending on the prevailing climatic conditions. The fungus attacks almost all aerial parts including foliage, stem, spikes and berries. *C. gloeosporioides* produces the major symptom of anthracnose as dark brown sunken leaf spot with yellow halo, leaf blight, spike shedding and berry splitting.

3.2.2. Isolation of the pathogen

The black pepper infected with anthracnose were collected from various locations; Thiruvananthapuram district (Kowdiar and Vellayani), Wayanad district (Ambalavayal and Meenagadi), and Idukki district (Myladumpara, Pampadumpara, Kattappana and Kambalikandam). The infected leaf samples were initially washed thoroughly under running tap water. The leaves were cut in to small bits of diseased portion along with the healthy portion. The bits were then surface sterilized with 0.1 per cent mercuric chloride for 30 seconds followed by washings with sterile water two times. The excess moisture was removed by placing over a sterile filter paper. The surface sterilised bits were transferred on to potato dextrose agar medium (PDA) in a sterile Petri dish by using a forceps.

The inoculated plates were incubated at room temperature of (28 ± 3^0C). After mycelial growth was observed, subculturing of the fungus was done on to the PDA plates. The cultures were purified by hyphal tip method and maintained on to PDA slants. The same procedure was followed to isolate the pathogen from different locations.

3.2.3. Purification and Maintenance of the isolates

Dhingra and Sinclair (1985) have developed single spore isolation technique to purify isolated fungal culture. The spore suspension of *C. gloeosporioides* was prepared by placing the spore mass into test tube containing sterile water and serially diluted to obtain the desired concentration of spores. One ml of conidial suspension was taken and poured in the sterile Petri dish by using a micropipette. Molten plain agar (2 %) was then poured over previously transferred conidial suspension and allowed to solidify. The plates were incubated under room temperature (28 ± 3^0C) for conidial germination. The spores were marked by using the fine marker; the growing hyphal tip was taken and placed aseptically on to the solidified PDA medium. The plates were then incubated at room temperature (28 ± 3^0C).

The purified isolates were maintained by repeated subculturing. The cultures were allowed to grow under room temperature and kept in a refrigerator at 4°C for storage (Nakasone *et al*., 2004). The virulence of the isolates were maintained by transferring into the host plant and the re-isolated cultures were used for further studies.

3.2.4. Proving Koch's postulates for the isolates of *Colletotrichum* sp.

The pathogenicity studies were carried out on excised leaves of Panniyur 1, Panniyur 3 and Karimunda varieties of black pepper. The leaves were washed thoroughly under running tap water and surface sterilized with 70 per cent ethanol. Gentle aberrations were given by pin pricks on the leaf surface for the easy colonization of pathogen. The pin pricked area was inoculated with mycelial disc of the actively growing seven days old culture taken using cork borer. These mycelial discs on the leaves were then covered with a thin layer of moist cotton to provide humidity.

The inoculated leaves were incubated in moist humid chamber at room temperature for symptom development. A control treatment was also maintained without inoculation of the pathogen. The observations *viz*., days taken for symptom development, nature of symptom developed and lesion size were recorded. The pathogen was reisolated from the symptom developed and compared with the original isolated culture for proving the pathogenicity. The re-isolated cultures were maintained for further studies.

3.3 MORPHOLOGICAL AND PATHOGENIC VARIABILITY OF *Colletotrichum* ISOLATES AND IDENTIFICATION OF VIRULENT ISOLATE

3.3.1. Morphological characterization of different Colletotrichum isolates from different locations

The morphological variations of different isolates with respect to the appearance, colour, and growth were studied. The molten PDA medium was poured in to sterile Petri dishes and allowed to solidify. Mycelial discs of 5 mm diameter were cut from the seven-day old culture of all the *Colletotrichum* isolates. The Petri plates were inoculated with the mycelial disc at the centre of the dish. Four replications of each isolates were

maintained. The plates were incubated at room temperature of 25±3°C. Observations like colony colour, appearance, nature of growth of the mycelium, radial growth of the mycelium and days taken for covering the entire Petri dish were recorded.

3.3.2. Mycelial, Conidial and Acervular characters of *Colletotrichum* sp.

The morphological characters of the various isolates *viz.*, the mycelial characters, sporulation, size and shape of conidia and appressoria of all eight isolates were studied by using slide culture technique.

The slide culture unit consist of a glass slide with a cover slip kept over two glass rod bits. A blotting paper was kept inside the Petri plate and the entire unit was sterilized. Molten 2 per cent agar was poured in another sterile petri dish in a thin layer and allowed to solidify. Using a sterile inoculation needle, the solidified 2 per cent agar was cut into squares bits of size similar with to that of the cover slip and placed the agar block over the glass slide. With the help of the heat sterilised inoculation needle, conidial mass from the seven-day old culture was inoculated on to the agar block by touching the four edges of the block under aseptic conditions. The coverslip was then placed carefully over the agar block and the moisture was maintained by wetting the blotting paper with sterile water. The unit was incubated for three to five days. The cover slips were then lifted up carefully by using the forceps and placed over a slide containing a drop of lactophenol cotton blue stain. The slides were mounted with DPX mount and observed under the microscope by using the LEICA software with magnification of 400x and 1000x. The observations were made on the size of the mycelium, conidia and appressoria etc.

3.3.3. Screening for pathogenic variability of different isolates of *C. gloeosporioides*

The eight isolates were subjected to virulence rating to assess the pathogenic variability and the most virulent isolate of the pathogen was identified. The virulence rating was carried out in three different varieties (Panniyur-1, Panniyur-3 and Karimunda) of black pepper. The uniform sized healthy leaves of three varieties collected

from farmer's field were used for the artificial inoculation of the pathogen. The leaves were washed separately under running tap water and surface cleaned with a cotton swab dipped in 70 per cent ethanol. After drying, pinpricks were given on the surface of the leaves in order to create aberrations to facilitate the easy entry of the pathogen. Mycelial discs of 5 mm diameter were taken from the seven-day old culture and placed over the surface of the abberated leaves. The mycelial discs were covered with a thin layer of moist cotton and the inoculated leaves of Panniyur-1, Panniyur-3 and Karimunda varieties were incubated in a moist chamber. Three replications were maintained for each variety and for each isolate. The un - inoculated leaves were kept as control. Observations on the days taken for symptom development, nature of symptom developed, lesion size and rate of lesion development were recorded separately for three varieties.

3.4. SCREENING OF BLACK PEPPER VARIETIES AGAINST ANTHRACNOSE

Rooted cuttings of KAU varieties (Panniyur 1 to 8) and local cultivar Karimunda were tested against the virulent pathogen from 9.2. An experiment was laid out in CRD using one year old black pepper cuttings under poly bags raised as per POP, KAU (2016).

Five leaves were selected from each variety and surface sterilized with 70 per cent alcohol. Aberrations were made on the foliage using carborundum powder to facilitate the entry of the pathogen. Spore suspension (10^6 spores ml^{-1}) of seven days old culture was prepared and sprayed on fully opened leaves using an atomizer. Then leaves were covered with polythene cover to maintain humidity for symptom development. Three replications of each variety were maintained. Days taken for symptom development, and lesion size (cm) were recorded at regular intervals. Based on lesion size developed different grades were given and PDI was calculated.

Grading and reaction of black pepper varieties

Grade	Leaf area affected	Reaction category
0	No infection	Immune (I)
1	1-10%	Highly resistant (HR)
3	11-25%	Resistant (R)
5	26-50%	Susceptible (S)
7	> 50%	Highly susceptible (HS)
0	No infection	Immune (I)

3.5 EVALUATION OF NEW GENERATION FUNGICIDES AGAINST *C. gloeosporioides*

In vitro suppression of most virulent isolate of *C. gloeosporioides* after virulence rating was done with commercially available new generation fungicides *i.e.*, two triazoles (hexaconazole, tebuconazole), two strobilurins (azoxyystrobin, kresoxim methyl) and four combination fungicides (azoxystrobin + tebuconazole, carbendazim + mancozeb, trifloxystrobin + tebuconazole, captan + hexaconazole). *In vitro* mycelial suppression was assessed by poisoned food technique (Nene and Thapliyal, 1993) (Table1).

In this method double strength PDA of 50 ml and 50 ml of sterile water were prepared separately for the respective fungicides of different concentrations and autoclaved. The fungicides were pre-weighed and added into conical flask containing 50 ml sterile water under aseptic condition and shaken thoroughly until mixture attained required concentrations. The molten medium amended with the fungicide was poured into the

sterile Petri dishes and allowed to solidify. The same procedure was repeated for all the concentrations of each fungicide.

After the solidification of the media, mycelial discs of 5 mm diameter of seven days old culture of *C. gloeosporioides* were cut using the sterile cork borer and placed on the centre of the solidified fungicide amended PDA medium using the flame sterilized inoculation needle. Three replications for each concentration of different fungicides were maintained. A control treatment was maintained by inoculating the pathogen without fungicide amendment. The Petri dishes were then wrapped and incubated at the room temperature of 28±3°C. The growth in diameter was recorded at regular intervals and the per cent inhibition by the fungicide was calculated.

Percent inhibition of the pathogen by the fungicide over the control is calculated by the formula:

Percent inhibition = C-T/C *100

Where C= Growth of the pathogen in control plate in cm

T= Growth of the pathogen in the treatment plate in cm

Table 1: Fungicides used for the assay

Sl. No.	Fungicides		Formulation	Concentration used
	Chemical name	Trade name		
1	Azoxystrobin	Amistar	23 SC	10, 25, 50, 100 ppm
2	Kresoxim methyl	Ergon	44.3 SC	10, 25, 50, 100 ppm
3	Hexaconazole	Contaf	5 EC	10, 25, 50, 100 ppm
4	Tebuconazole	Orius	25 EC	10, 25, 50, 100 ppm
5	Azoxystrobin + Tebuconazole	Custodia	11% +18.3%SC	10, 25, 50, 100 ppm
6	Trifloxystrobin + Tebuconazole	Nativo	25% + 55% WP	10, 25, 50, 100 ppm
7	Captan + Hexaconazole	Taqat	70% WP + 5% WP	10, 25, 50, 100 ppm
8	Carbendazim+ Mancozeb	Saaf	12%+63% WP	10, 25, 50, 100 ppm
9	Copper oxy chloride	Blitox	50% WP	10, 25, 50, 100 ppm

Results

4. RESULTS

The present study entitled “Varietal screening and management of anthracnose of black pepper using new generation fungicides.” was conducted at Department of Plant Pathology, College of Agriculture, Vellayani during 2018 – 2020 with the objective to screen KAU varieties and most popular local cultivar Karimunda for resistance against anthracnose of black pepper caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and evolve management strategy using new generation fungicides. The results obtained are summarized below.

4.1. SURVEY AND COLLECTION OF DISEASED SAMPLES

4.1.1. Collection of diseased samples

A survey was conducted in black pepper growing tracts of Kerala; Thiruvananthapuram, Wayanad and Idukki districts during June 2019 - October 2019 to assess the disease incidence and severity of anthracnose and for collection of diseased samples. The survey was carried out in two locations in Thiruvananthapuram district (Kowdiar and Vellayani), two locations in Wayanad district (Ambalavayal and Meenangadi) and four locations in Idukki district (Myladumpara, Pampadumpara, Kattappana and Kambalikandam) (Plate 1). The weather parameters like maximum and minimum temperature, relative humidity and rainfall were recorded, which are the most prerequisite factors in disease development. The maximum temperature varied from 22.1°C to 31.9°C and minimum temperature from 12.4°C to 25.3°C. The two locations of Thiruvananthapuram district had the highest maximum temperature of 31.9°C while the lowest maximum temperature of 22.1°C was reported from Myladumpara of Idukki district. The relative humidity of the surveyed areas ranged from 87 - 91 per cent. The maximum relative humidity (91 %) was recorded at Thiruvananthapuram district and minimum (87 %) at Myladumpara and Kattapana of Idukki district. The maximum rainfall was observed at Myladumpara (178.6 mm) and minimum at Vellayani (10.7 mm) (Table 2).

Table 2: Weather parameters of the surveyed locations

Sl. No.	Location	Period of collection	Temperature (^{o}C)		Relative humidity (%)	Rainfall (mm)
			Max	Min		
1.	Kowdiar, Thiruvananthapuram	June, 2019	31.9	25.3	90	10.7
2.	College of Agriculture, Vellayani	June, 2019	31.9	25.3	91	10.7
3.	Meenangadi, Wayanad	August,2019	24.9	19.2	88	124.3
4.	Ambalavayal, Wayanad	August, 2019	24.9	19.2	88	118.7
5.	Myladumpara, Idukki	October, 2019	22.1	12.4	87	178.6
6.	Pampadumpara, Idukki	October, 2019	23.1	13.2	88	177.4
7.	Kattapana, Idukki	October, 2019	22.3	13.5	87	170.2
8.	Kambalikandam, Idukki	October, 2019	22.8	12.5	88	168.3

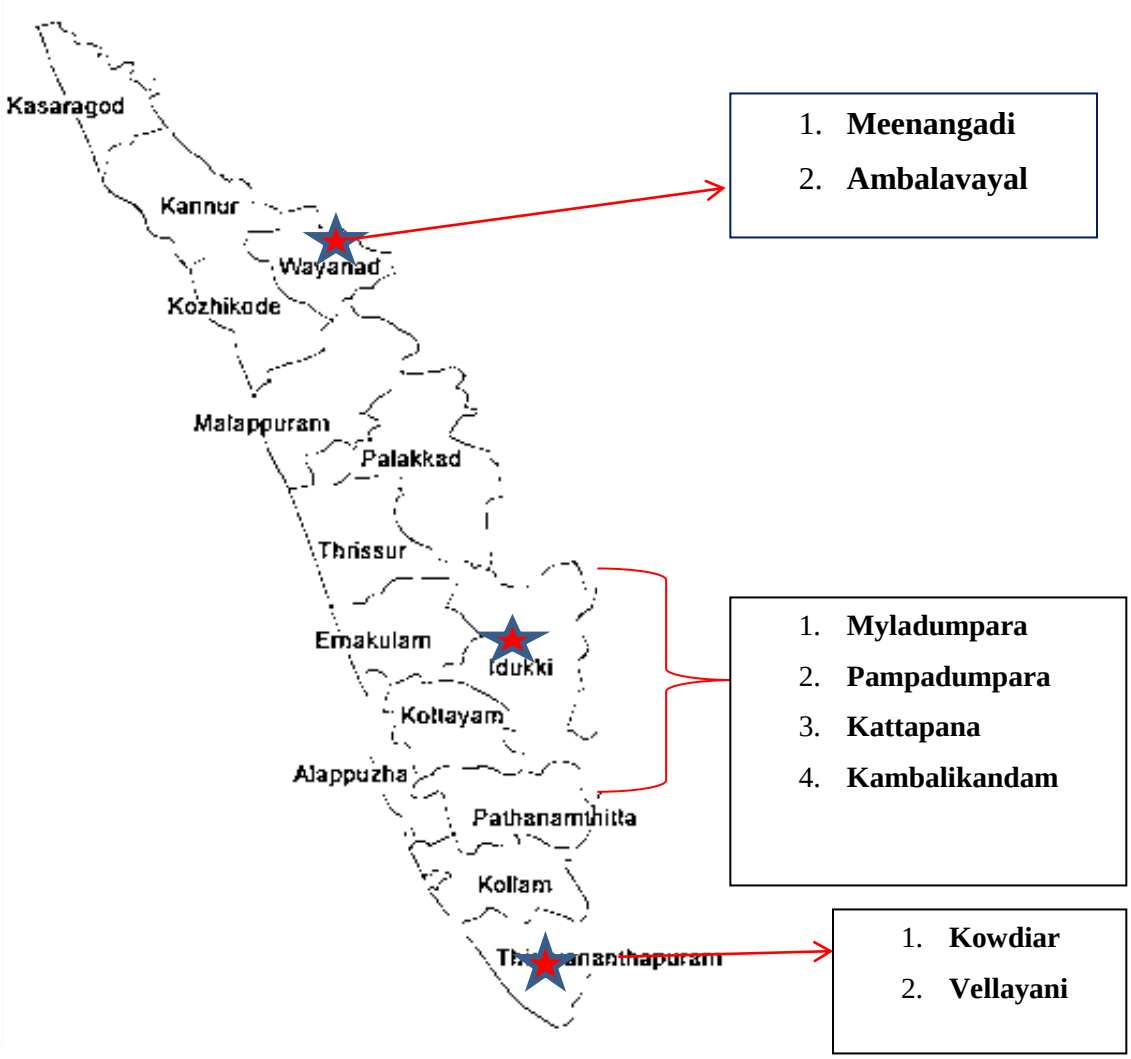


Plate 1. Map of Kerala showing locations of black pepper anthracnose sample collection

Table 3: Score chart for anthracnose of black pepper

Scale	Leaf area affected
0	No infection
1	1-10%
3	11-25%
5	26-50%
7	>50%

The disease severity was assessed in terms of per cent disease index based on a score chart (0-7 scale) developed by Sankar (2002) (Table 3) (Plate2). Disease incidence and severity of anthracnose disease of black pepper were recorded from surveyed locations. The disease incidence was assessed as percentage of number of plants infected to total number of plants observed. In each location ten plants were observed for calculating the disease incidence while ten leaves from each plant were scored for disease severity. The disease incidence of anthracnose ranged from 10 - 60 per cent. The highest disease incidence of 60 per cent was recorded in Myladumpara followed by Kattapana (50%), Pampadumpara (40%) and Kambalikandam (30%). The lowest DI was recorded at Kowdiar (10%). The disease severity ranged from 13.75 to 50.28 per cent. The highest PDI was recorded in Myladumpara (50.28 %) (Table 4). The survey revealed a positive correlation between weather parameters and disease severity. Idukki district with the most congenial weather parameters for the disease recorded the maximum severity.

Table 4: Disease incidence and severity of anthracnose of black pepper in different locations

Sl. No.	Locations	Disease incidence (%)*	Per cent disease index (%)*
1.	Kowdiar, Thiruvananthapuram	10.00	13.75
2.	College of Agriculture, Vellayani	20.00	16.80
3.	Meenagadi, Wayanad	20.00	34.28
4.	Ambalavayal, Wayanad	20.00	43.50
5.	Myladumpara, Idukki	60.00	50.28
6.	Pampadumpara, Idukki	40.00	47.14
7.	Kattapana, Idukki	50.00	48.62
8.	Kambalikandam, Idukki	30.00	21.50

Total number of plants surveyed for disease incidence in each location = 10

Total number of leaves surveyed for disease severity in each plant = 10

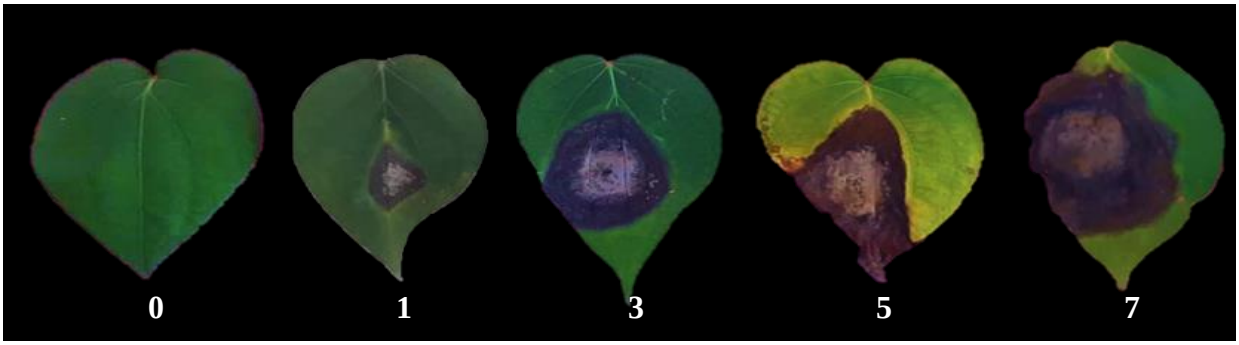


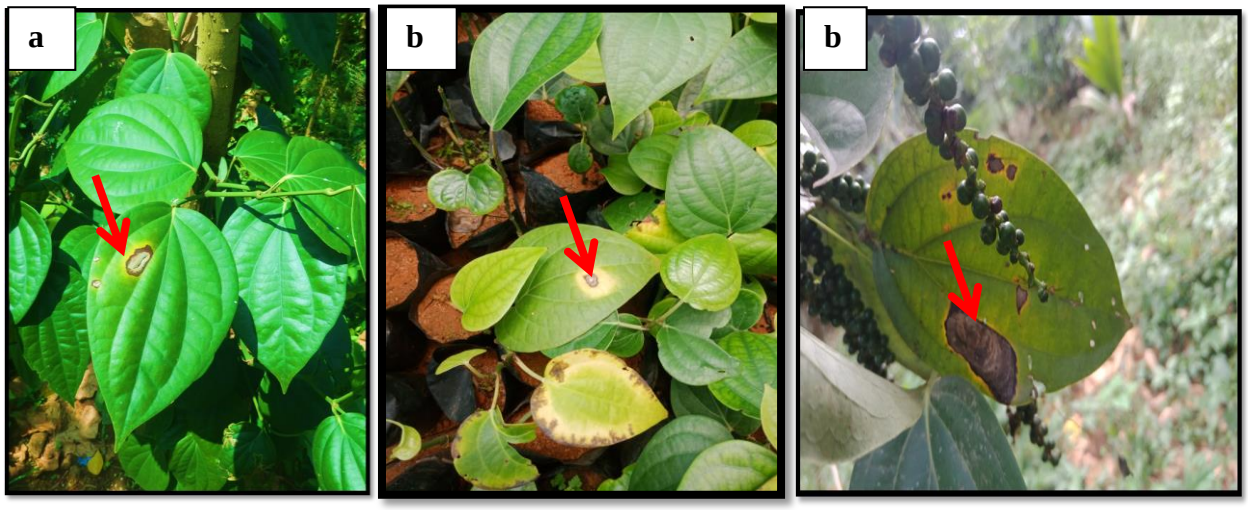
Plate 2. Score chart for anthracnose disease in leaves of black pepper

4.2 SYMPTOMATOLOGY, ISOLATION OF PATHOGEN, PROVING PATHOGENICITY OF *Colletotrichum* sp. CAUSING ANTHRACNOSE DISEASE OF BLACK PEPPER

4.2.1. Symptomatology - Symptom variation was noticed at surveyed locations *viz*., necrotic spots with a yellow halo, leaf blight and spike infection. The nature of symptoms of anthracnose in various black pepper varieties cultivated at surveyed locations was recorded (Table 5). A characteristic anthracnose symptom of necrotic spot with a prominent yellow halo was observed at College of Agriculture, Vellayani while in Kowdiar necrotic spots with acervuli at the centre (Plate 3). Panniyur 1, Panniyur 2, Panniyur 5 and Karimunda were the most widely cultivated varieties in surveyed regions. In Wayanad district, the two locations Meenangadi and Ambalavayal, anthracnose symptom was observed as leaf blight, necrotic spots with yellow halo and drying of the margin from tip (Plate 4). Necrotic spots on the foliage along with yellow halo, leaf blight and spike infection leading to hollow (pollu) berries were observed at Myladumpara and Pampadumpara respectively (Plate 5). Similarly typical necrotic spots with yellow halo and leaf blight symptoms were widely noticed in Kambilikandum and Kattapana of Idukki district (Plate 6).

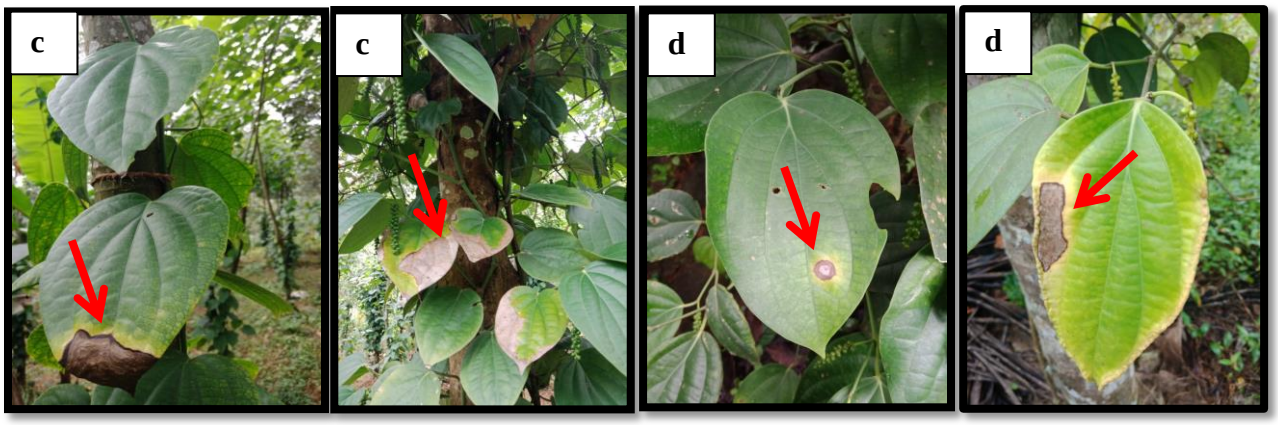
Table 5: Nature of symptoms of anthracnose in different varieties of black pepper at the surveyed locations

Sl. No.	Locations	Variety	Nature of symptoms
1.	Kowdiar, Thiruvananthapuram	Panniyur 2	Necrotic spot with a yellow halo, acervuli seen at the centre of the necrotic spot
2.	College of Agriculture, Vellayani,	Panniyur 2	Necrotic spot with a prominent yellow halo
3.	Meenangadi, Wayanad	Panniyur 1	Leaf blight
4.	Ambalavayal,Wayanad	Local	Necrotic spots with a yellow halo and drying of the margins
5.	Myladumpara, Idukki	Panniyur 2	Necrotic spots with a yellow halo and leaf blight
6.	Pampadumpara, Idukki	Local	Necrotic areas on the leaves and spike infection
7.	Kattapana, Idukki	Panniyur 5	Leaf blight
8.	Kambalikandam, Idukki	Karimunda	Necrotic spots with reddish brown margin and a yellow halo



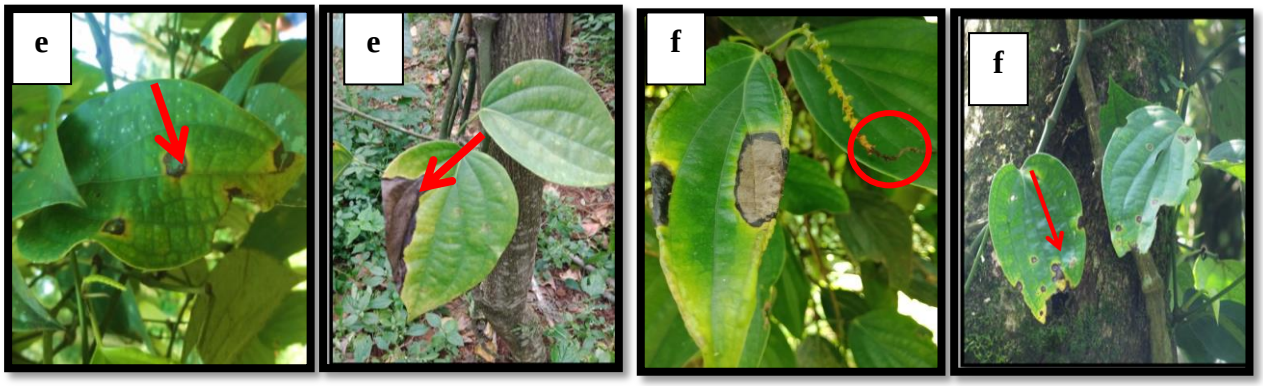
Necrotic spot **Necrotic spots with prominent yellow halo and leaf blight**

Plate3. Symptoms of anthracnose of black pepper observed at Thiruvananthapuram district (a) Kowdiar (b) Vellayani



Leaf blight **Leaf spot and blight**

Plate 4. Symptoms of anthracnose of black pepper observed at Wayanad district (c) Meenangadi (d) Ambalavayal



Leaf spot and blight **Spike infection** **Leaf spot**

Plate 5. Symptoms of anthracnose of black pepper observed at Idukki district (e) Myladumpara (f) Pampadumpara

Leaf spot and blight **Leaf spot**

Plate 6. Symptoms of anthracnose of black pepper observed at Idukki district

(g) Kattapana (h) Kambalikandam

4.2.2. Isolation of black pepper anthracnose pathogen

Leaves with typical anthracnose symptoms were collected from the surveyed locations. The pathogen was isolated from each samples as per the standard procedure described in 3.1. Potato dextrose agar (PDA) medium was used for the isolation of the pathogen. The mycelial growth from isolated tissue initiated within 2 days after inoculation. Eight isolates of *Colletotrichum* sp. were obtained from the surveyed areas. The isolates obtained from Kowdiar, Vellayani, Ambalavayal, Meenangadi, Myladumpara, Pampadumpara, Kattapana and Kambalikandam were labelled as C1, C2, C3, C4, C5, C6, C7 and C8 respectively. The pure culture of the isolates was maintained on PDA slants.

4.2.3 Proving Koch's postulates for the isolates of *Colletotrichum* sp.

The eight isolates of *Colletotrichum* sp. were artificially inoculated to the healthy leaves of black pepper (Panniyur 1) by using pinprick method. The pathogen produced symptom within 2 to 3 days of inoculation (Plate 7). The isolates C1, C2, C3, C4, C6 and C8 were on par with respect to the days taken for symptom expression. The isolates C5 and C7 were on par with respect to the lesion size produced. PDI produced by the different isolates ranged from 5.71-14.28. The maximum PDI was observed in C7 isolate followed by C2, C5 and C8. The isolates C1, C4 and C6 produced PDI of 8.57. The lowest PDI was noticed in C3 isolate (5.71) (Table 6). The pathogen was re-isolated from the artificially inoculated leaves on to PDA medium. The original culture was compared with the re-isolated cultures for their similarity to prove the Koch's postulates (Plate 8 and Plate 9).

Table 6. Pathogenicity testing of the collected isolates from different locations on excised shoot of black pepper

Isolates	Locations	Days taken for symptom development	Lesion size (cm)*(3DAI)	PDI (%)
C1	Kowdiar	3	0.70 ± 0.06^{b}	8.57
C2	Vellayani	2	$0.87\pm 0.06a^{b}$	11.40
C3	Meenangadi	2	0.63 ± 0.08^{b}	5.71
C4	Ambalavayal	3	0.70 ± 0.06^{b}	8.57
C5	Myladumpara	2	1.00 ± 0.08^{a}	11.40
C6	Pampadumpara	2	0.84 ± 0.012^{b}	8.57
C7	Kattapana	2	1.03 ± 0.08^{a}	14.28
C8	Kambalikandam	2	0.93 ± 0.05^{ab}	11.40
CD			0.242	
SEm±			0.080	

*Mean ± SD of three replications; DAI: Days after inoculation; PDI- Per cent disease index

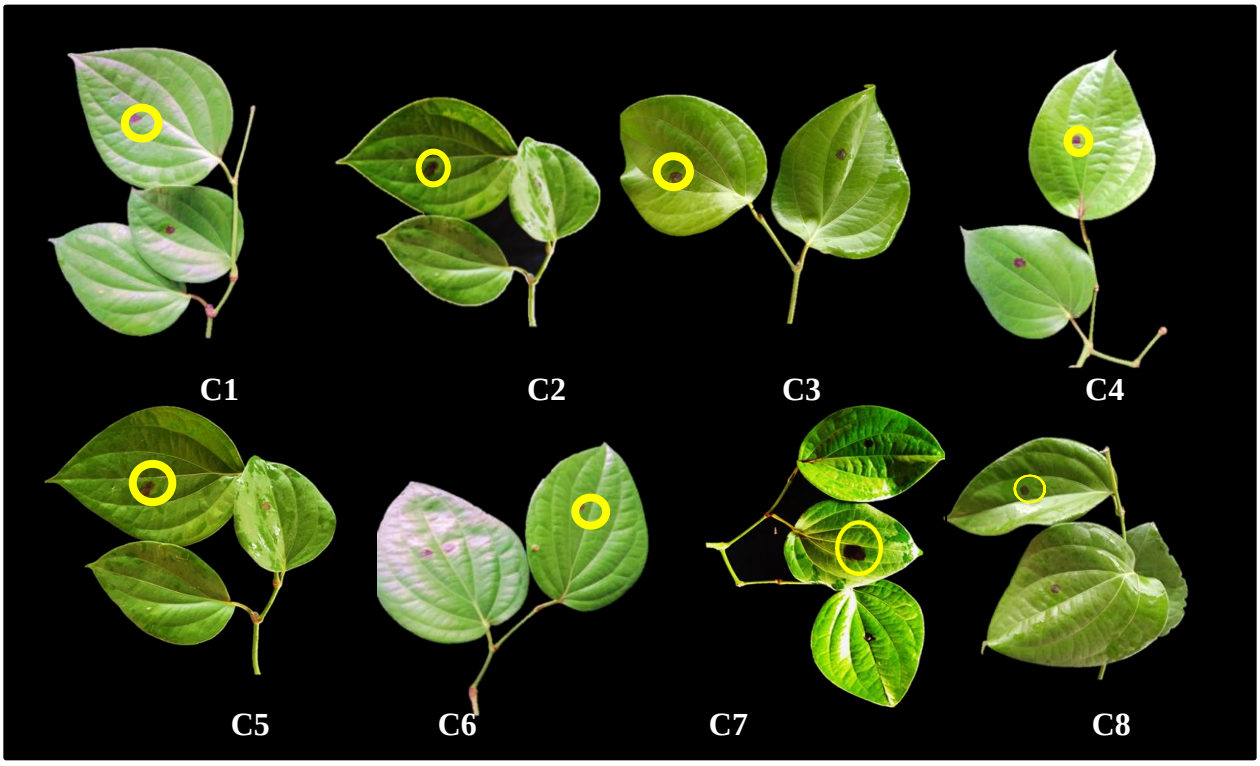


Plate 7. Symptom appearance on artificial inoculation of Colletotrichum isolates in Panniyur 1 (3 DAI)

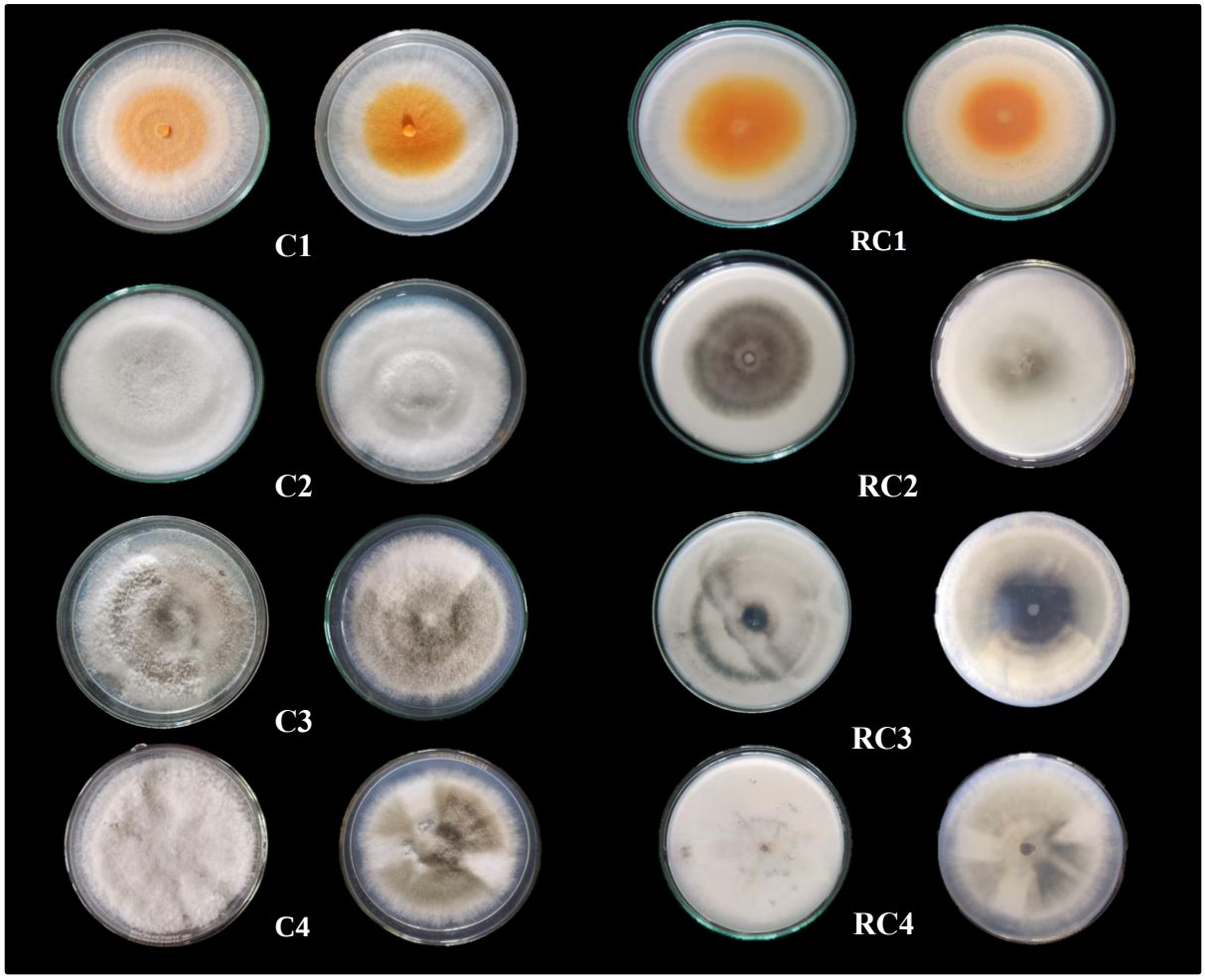


Plate 8. Growth of isolated and re-isolated Colletotrichum isolates C1 - C4 (front view), RC1 - RC4 (rear view)

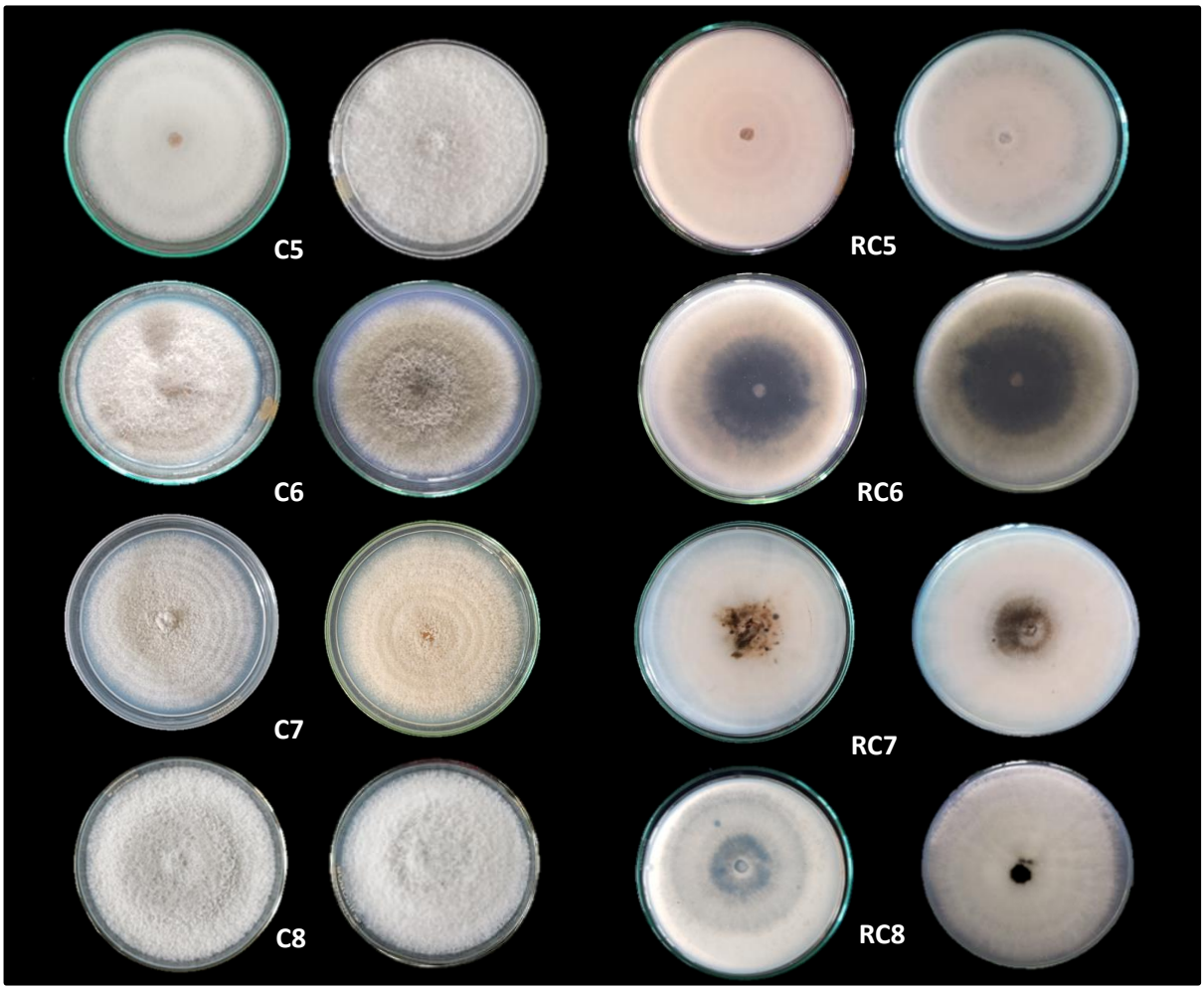


Plate 9. Growth of isolated and re-isolated Colletotrichum isolates C5 - C8 (front view), RC5 - RC8 (rear view)

4.3. MORPHOLOGICAL AND PATHOGENIC VARIABILITY OF Colletotrichum ISOLATES AND IDENTIFICATION OF VIRULENT ISOLATE

4.3.1. Morphological characterization of different *Colletotrichum* isolates from different locations

All the eight isolates of *Colletotrichum* sp. were grown on PDA medium and morphological characters were studied by observing the colony growth. Each isolates of *Colletotrichum* sp. exhibited variations in mycelial colour and growth pattern. The mycelium of C1 isolate was white with yellowish orange centre on its front and reverse side with a sparse growth and regular margin. The isolate C2 appeared as off white with greyish centre on front view, off white with greenish grey centre in the rear view and it had a fluffy growth with an irregular margin. C3 isolate was greyish with a white zone on front side whereas on reverse side it appeared as greyish with dark greyish centre; and it had a fluffy growth with regular margin. Mycelium of isolate C4 was greyish with dark greyish centre in the front side and greyish on the reverse side. The C5 isolate was whitish in colour both in front and reverse view and had a fluffy growth with regular margin. Mycelium of C6 isolate had a greyish centre with whitish regular margin on front view and greyish with dark grey centre on reverse side with a sparse growth. The C7 isolate appeared as whitish to light pink colour on front view and salmon pink on rear view with a regular margin with a sparse growth whereas in the case of C8 isolate it appeared as off white colour on both sides and had sparse growth with regular margin. (Table 7)

Table 7: Growth pattern of different isolates of *Colletotrichum* sp. causing anthracnose of black pepper on PDA medium

Isolates	Growth pattern	Characteristics of the mycelium on PDA after 7 days	
		Front view	Rear view
C1	Sparse	Whitish with yellowish orange centre and regular margins	Whitish with orange centre
C2	Fluffy	Off white with greyish centre and irregular margins	Off white with greenish grey centre
C3	Fluffy	Greyish with a white zone	Greyish with dark greyish centre
C4	Fluffy	Alternate white and greyish zones with regular margins	Greyish
C5	Fluffy	Whitish with regular margins	Whitish
C6	Sparse	Greyish centre with whitish regular margin	Greyish with dark grey centre
C7	Sparse	Whitish to light pink with regular margins	Salmon pink
C8	Sparse	Off white	Off white

The mycelial growth of the eight isolates was recorded on 3rd, 5th and 7th day. On 3rd day, the maximum mycelial growth was recorded by C5 isolate (4.77 cm) which was on par with C7 (4.68 cm). The isolates C3, C8, C6 and C2 were on par. The lowest mycelial growth was observed for C1 isolate (4.08 cm) was on par with C4 (4.10 cm).

The isolate C5 had the maximum mycelial growth (7.75 cm) at 5 DAI which was on par with C7 (7.6 cm); while minimum growth was observed in the isolate C1 (5.35 cm) which was on par with C4 (5.37 cm). The isolates C3, C8, C6, and C2 were statistically on par.

The mycelial growth of the eight isolates was compared and result showed that the maximum mycelial growth of 8.93 cm was recorded by C5 isolate (7 DAI) which was on par with C7 (8.85 cm). The isolate C1 had the minimum mycelial growth of 7.15 cm. The isolates C4, C3, C8, C6 and C2 were on par with respect to the mycelial growth (7 DAI).

Days taken to grow the entire Petri dish (9 cm) ranged from 7.25 to 9.75 days and the rate of growth of Colletotrichum isolates varied between 1.02 - 1.28 cm day^{-1}. The isolate C5 was comparatively a fast grower with a rate growth of 1.28 cm day^{-1} and took 7.25 days for complete growth in Petri dish followed by C7 with an average growth rate of 1.25 cm day^{-1} and took 7.75 days to complete Petri dish. The isolate C1 was a slow grower which took 9.75 days to complete growth in Petri dish with the lowest growth rate of 1.02 cm day^{-1}. The isolates C2, C6, C8, C3 and C4 took 8.0, 8.0, 9.0, 9.25 and 9.5 days respectively to complete growth in Petri dish (Table 8, Plate 10).

Table 8: Growth characteristics of the different isolates of *Colletotrichum* sp. causing anthracnose of black pepper on PDA medium

Isolates	Growth in petri dish (cm)*			Rate of growth (cm day^{-1}) *	DTCP
	3^{rd} day	5^{th} day	7^{th} day		
C1	4.08 (2.08)^{e}	5.35 (2.42)^{d}	7.15 (2.67)^{d}	1.02	9.75
C2	4.45 (2.11)^{bc}	5.85 (2.52)^{b}	8.52 (2.91)^{b}	1.22	8.00
C3	4.15 (2.04)^{de}	5.57 (2.47)^{c}	8.28 (2.87)^{bc}	1.14	9.25
C4	4.10 (2.02)^{de}	5.37 (2.42)^{d}	8.10 (2.85)^{c}	1.15	9.50
C5	4.77 (2.19)^{a}	7.75 (2.87)^{a}	8.93 (3.00)^{a}	1.28	7.25
C6	4.33 (2.08)^{cd}	5.73 (2.49)^{bc}	8.45 (2.90)^{b}	1.21	8.00
C7	4.68 (2.16)^{ab}	7. 60 (2.85)^{a}	8.85 (2.98)^{a}	1.25	7.75
C8	4.25 (2.06)^{cde}	5.72 (2.49)^{bc}	8.40 (2.89)^{b}	1.18	9.00
CD (0.05)	0.059	0.040	0.105	0.032	1.121

* Mean of four replications; DTCP-Days taken to complete 9 cm growth in petri plate; Values in parenthesis are square root transformed values

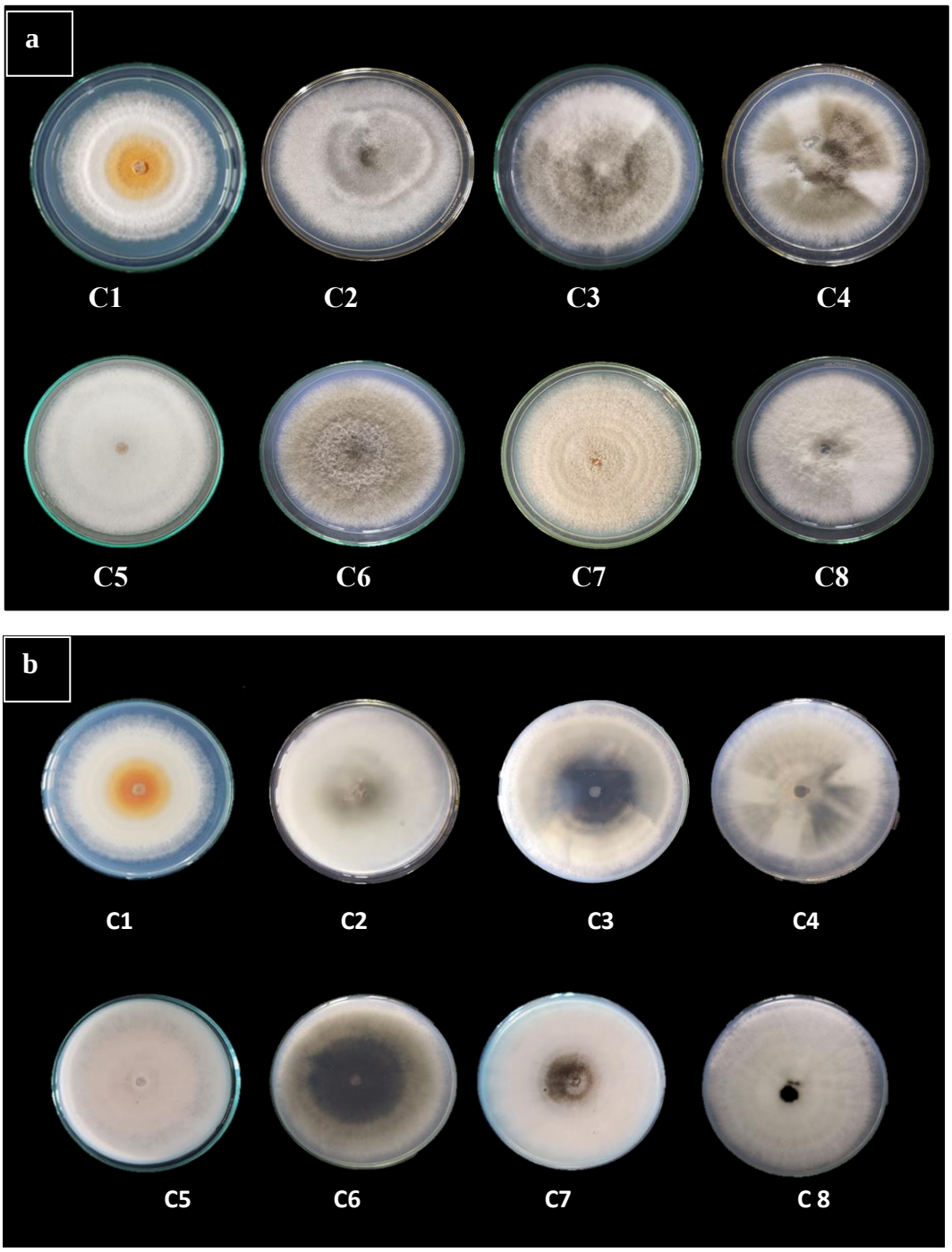


Plate 10. Growth pattern of different isolates of *Colletotrichum* sp. on PDA media on 7th day. (a) Front view (b) Rear view

4.3.2. Mycelial, Conidial and Acervular characters of *Colletotrichum* sp.

The microscopic characters of collected isolates were studied by slide culture technique and observed under microscope (100x). The mycelium of the fungus was hyaline and septate. The mycelial width of the different isolates varied from 2.21 µm to 3.45 µm. The isolate C7 recorded the highest mycelial width of 3.45 µm and the lowest width for the isolate C1 (2.21 µm). The mycelial width of 2.65µm, 3.31 µm, 2.54 µm, 2.56 µm, 2.21 µm, 3.23 µm and 2.52 µm were recorded for the isolates C1, C2, C3, C4, C5, C6 and C8 respectively. Similarly the septal distance of the Colletotrichum isolates varied from 8.50 to 21.23 µm. The maximum septal distance of 21.23 µm was recorded for C8 isolate and the minimum for the isolate C5 (8.50µm) (Table 9) (Plate 11).

Sporulation was observed in eight isolates under lab condition and conidia were borne on acervular conidiomata. The conidial shape varied from dumbbell, cylindrical to oblong with an oil globule at centre. The conidial size varied from 9.4 to 12.1 µm in length, 3.6 to 4.6 µm in breadth among the isolates (Table 10) (Plate 12). The C1, C2, C5 and C7 isolates had dumbbell shaped conidia with a dimension of ranging from 9.4 - 10.1 and 3.6 - 3.8 µm. The isolates C3 and C8 produced oblong shaped conidia with a size of 11.2 – 12.1 µm x 3.6 µm. The isolates C4 and C6 exhibited cylindrical conidia with a dimension of 11.4 x 4.6 µm and 11.3 x 3.6 µm respectively. The maximum appressorial size of 11.2 x 4.3 µm was recorded for the isolate C7; while the isolates C1, C2, C3 had 9.5 x 4.2 µm, 9.2 x 3.5 µm and 8.7 x 4.5 µm respectively. Similarly, the isolate C4 had an appressorial size of 10.8 x 3.8 µm. C5, C6 and C7 isolates produced appressoria of size 8.5 x 3.8 µm and 10.5 x 4.3 µm and 9.8 x 3.8 µm respectively (Plate 13). The above studies on the morphological and microscopic characters identified all the isolates as *C. gloeosporioides*.

Table 9: Mycelial characteristics of *Colletotrichum* isolates causing anthracnose of black pepper on PDA medium (7 DAI)

Isolates	Mycelial width (µm*)	Septal distance (µm*)	Colour	Nature of mycelium
C1	2.65	20.54	Hyaline	Septate
C2	3.31	19.21	Hyaline	Septate
C3	2.54	13.86	Hyaline	Septate
C4	2.56	9.80	Hyaline	Septate
C5	2.21	8.50	Hyaline	Septate
C6	3.23	9.30	Hyaline	Septate
C7	3.45	14.21	Hyaline	Septate
C8	2.52	21.23	Hyaline	Septate

Table 10: Conidial characteristics of the different Colletotrichum isolates causing anthracnose of black pepper

Isolates	Conidia		Appressoria
	Size (µm*)	Shape	Size (µm*)
C1	9.7 x 3.8	Dumbbell	9.5 x 4.2
C2	9.5 x 3.7	Dumbbell	9.2 x 3.5
C3	11.2 x 3.6	Oblong	8.7 x 4.5
C4	11.4 x 4.6	Cylindrical	10.8 x 3.8
C5	9.4 x 3.6	Dumbbell	8.5 x 3.8
C6	11.3 x 3.6	Cylindrical	10.5 x 4.3
C7	10.1 x 3.6	Dumbbell	11.2 x 4.3
C8	12.1 x 3.6	Oblong	9.8 x 3.8

*Mean of three replications

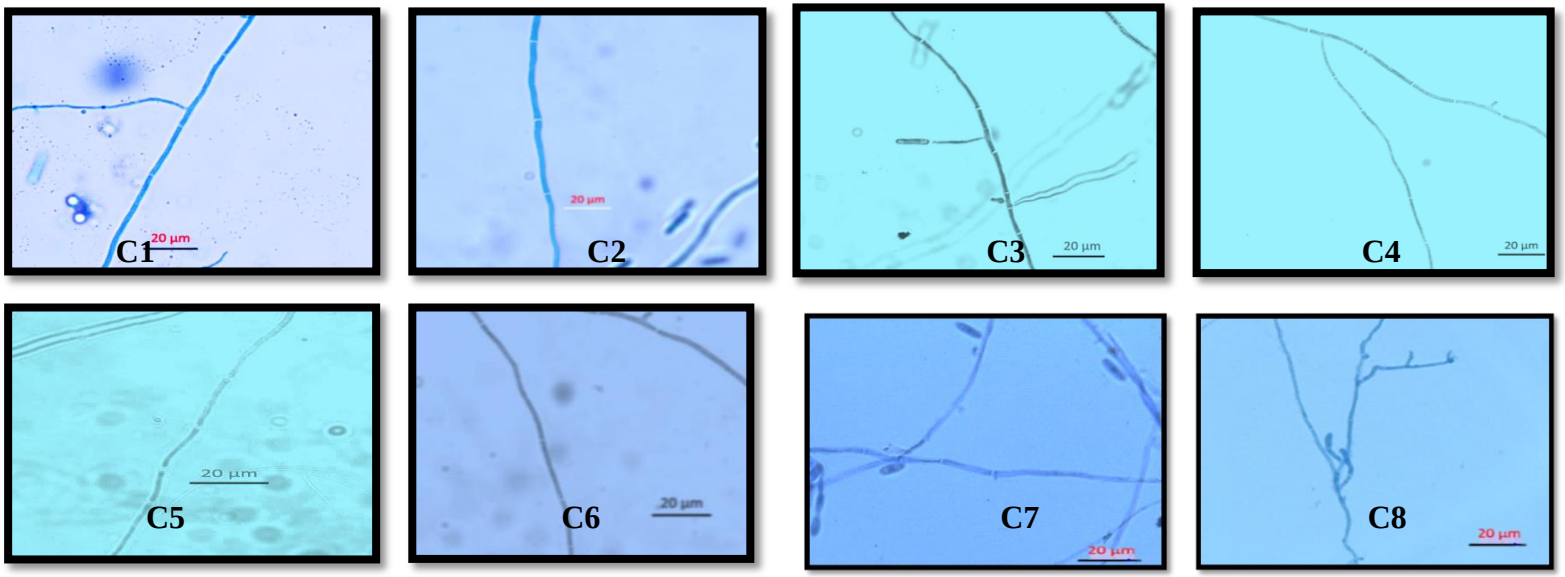


Plate 11. Mycelial characteristics of Colletotrichum isolates from black pepper at 7 DAG

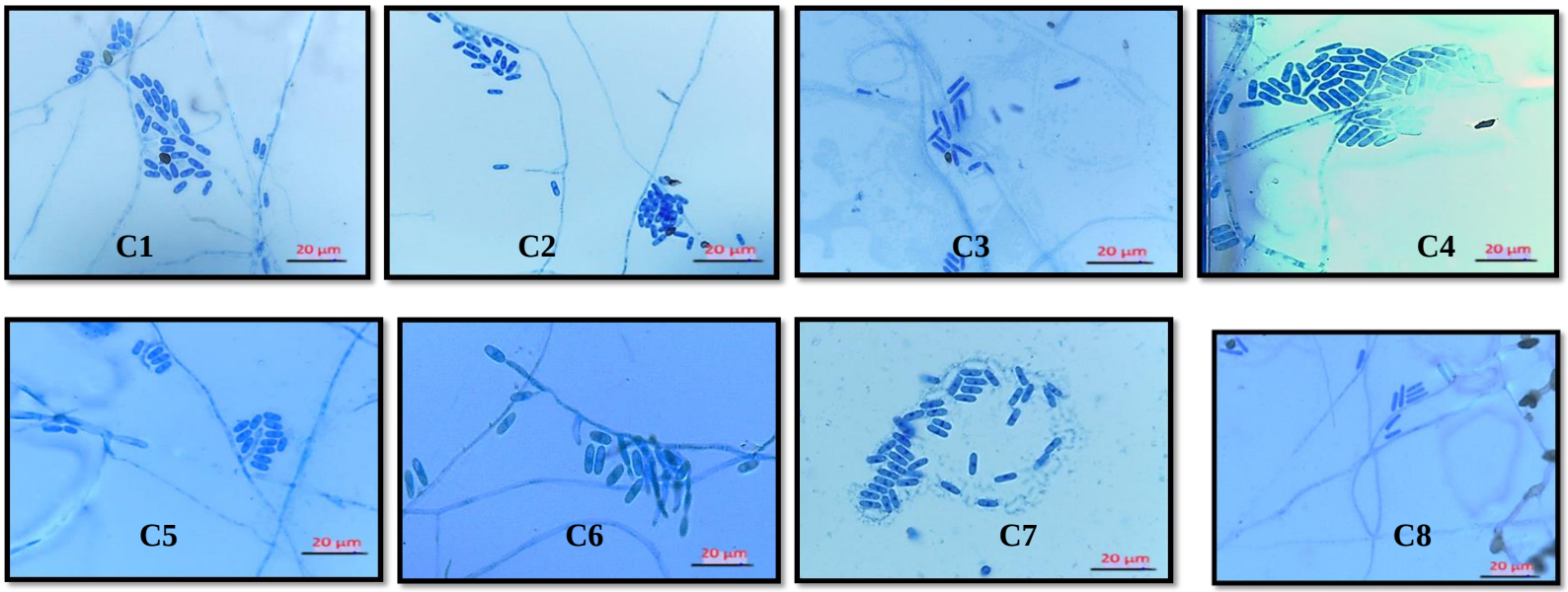


Plate 12. Microscopic view of conidia of Colletotrichum isolates at 7 DAG

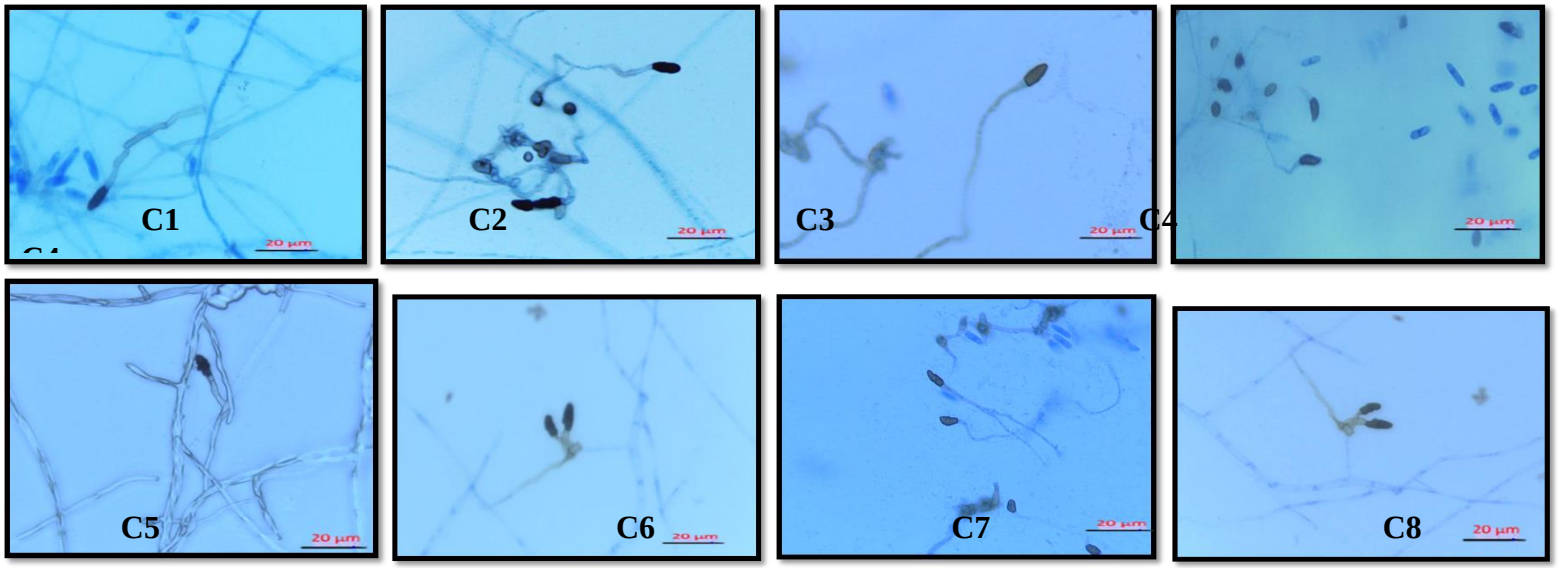


Plate 13. Formation of appressoria in Colletotrichum isolates at 7 DAG

4.3.3. Screening of virulent isolate of *C. gloeosporioides*

In vitro screening was carried out for identifying the most virulent isolate among the eight isolates of *C. gloeosporoides* by artificial inoculation on to the detached leaves of three black pepper varieties *viz*., Panniyur 1, Panniyur 3 and Karimunda. Virulence rating was assessed based on the days taken for symptom development, size of lesion developed and rate of lesion development.

4.3.3.1. Leaf inoculation

Seven day old culture of eight *C. gloeosporioides* isolates *viz.*, C1, C2, C3, C4, C5, C6, C7 and C8 were artificially inoculated on detached leaves of Panniyur1, Panniyur 3 and Karimunda following the pin prick method.

In Panniyur 1, the days taken for symptom appearance ranged from 2-4 days. The isolates C2, C7 and C8 took minimum (2) days for symptom appearance while C1and C3 took the maximum period for symptom expression (4 day). The days for symptom appearance was 3 in the isolates C4, C5 and C6.

The C7 isolate produced maximum lesion of 1.00 cm (5 DAI) and 1.92 cm (7 DAI). C8 isolate produced a lesion size of 0.75 cm (5 DAI) and 1.05 cm (7 DAI). The isolates C4, C5 and C6 produced a lesion size of 0.33, 0.38 and 0.28 cm respectively (5 DAI). These isolates followed the same trend at 7 DAI. Isolate C2 produced 0.53 and 0.98 cm lesion at 5 DAI and 7 DAI respectively. C1 and C3 did not initiate infection on the third day after inoculation; but produced lesion size of 0.73 and 0.80 cm (7 DAI).

The rate of lesion development cm day^{-1} varied from 0.15 to 0.40 between the different Colletorichum isolates. The maximum rate was observed in C7 (0.40) and minimum in C1 isolate (0.15). Isolates C3, C4 and C6 were on par with respect to the rate of lesion development. The isolate C2, C5 and C8 had a rate of lesion development 0.19 and 0.21 respectively (Table 11, Plate 14)

Table 11: Characteristics of the lesion produced by isolates of *C. gloeosporioides* causing anthracnose of black pepper on detached leaf of Panniyur 1 (Virulence rating)

Isolates	Days taken for symptom appearance	Lesion size (cm) *		Rate of lesion development(cm day^{-1}) *
		3^{rd} DAI	5^{th} DAI	
C1	4	0.00 (1.00)^{d}	0.73 (1.10)^{d}	0.15
C2	2	0.35 (1.16)^{c}	0.98 (1.21)^{bc}	0.19
C3	4	0.00 (1.00)^{d}	0.80 (1.14)^{cd}	0.17
C4	3	0.33 (1.15)^{c}	0.93 (1.19)^{bc}	0.18
C5	3	0.38 (1.17)^{c}	0.95 (1.20)^{bc}	0.19
C6	3	0.28 (1.12)^{c}	0.90 (1.18)^{bc}	0.18
C7	2	1.00 (1.41)^{a}	1.92 (1.56)^{a}	0.40
C8	2	0.75 (1.32)^{b}	1.05 (1.24)^{b}	0.21
CD (0.05)		0.056	0.145	0.033
SEm±		0.019	0.049	0.011

* Mean of four replications; Values in parenthesis are square root transformed values

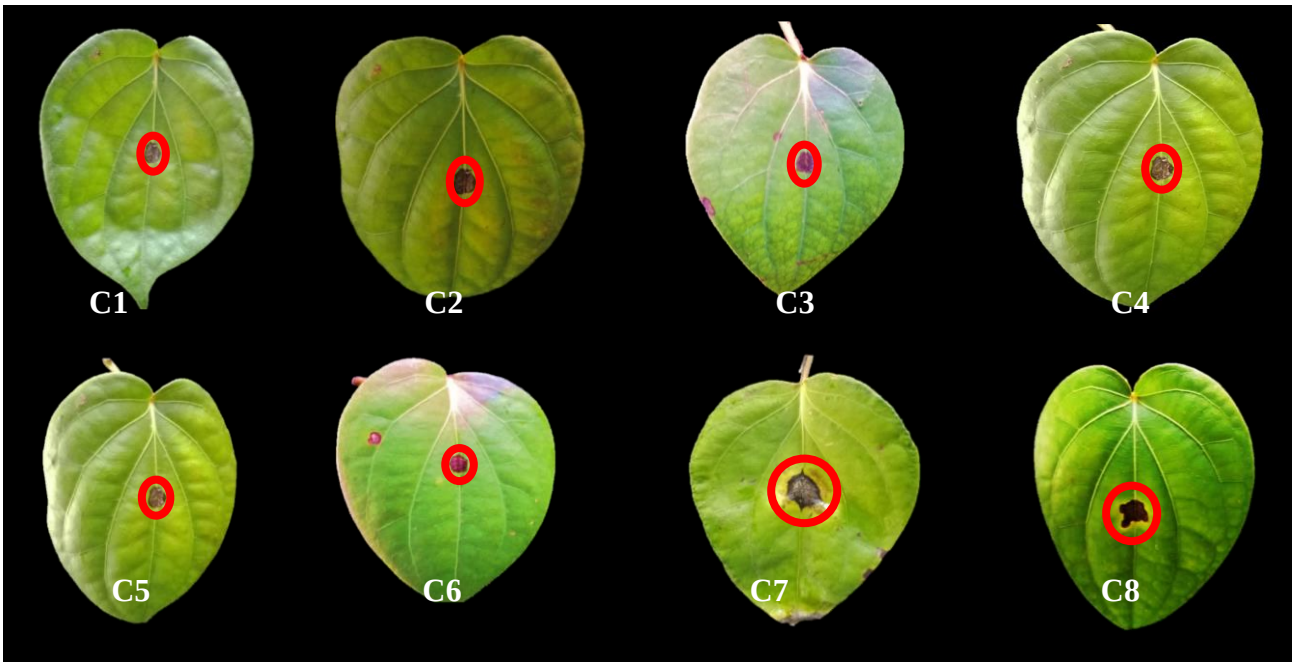


Plate 14. Lesion development on artificial inoculation of *C. gloeosporioides* isolates on detached leaf in variety Panniyur 1 (5 DAI)

The virulence rating studies carried out in Panniyur 3 revealed that the days taken for symptom development ranged from 2 - 4 days. The isolates C5, C6, C7 and C8 produced symptom within 2 days after inoculation. The isolates C1 and C3 developed symptoms only after 4 days of inoculation. C2 and C4 isolates exhibited symptom 3 days after inoculation.

The lesion size produced by isolates C5 and C7 (3 DAI) were on par. The isolates C8, C6, C2 and C4 produced a lesion size of 0.95, 0.85, 0.38 and 0.34 cm respectively. C1 and C3 did not develop symptoms at 3 DAI. The isolate C7 produced maximum lesion size of 2.40 cm (5 DAI) with a rate of lesion development of 0.49 cm day^{-1}. There was a rapid increase in lesion size from 1.05 (3 DAI) to 2.40 (5 DAI). The isolates C1, C2, C3 and C4 were on par. The isolates C6 and C5 were statistically different which produced a lesion size of 1.35 and 1.95 cm respectively. The highest rate of lesion development was for isolate C7 (0.49 cm day^{-1}) and lowest for isolates C1 and C3 (0.15 cm day^{-1}). The other isolates had a rate of lesion development ranging from 0.18 to 0.40. (Table12) (Plate15)

The local variety Karimunda was also subjected to the virulence rating. The days taken for symptom appearance were either 2 or 3 days after inoculation. . The study revealed that the isolate C7 produced symptom 2 days after inoculation and attained a maximum lesion size of 3.22 cm at 5 DAI. The days taken for symptom development ranged from 2 to 3 days. The rate of lesion development among the various *Colletotrichum* isolates ranged from 0.15- 0.66 cm $day^{-1.}$ The highest rate of lesion development was observed for isolate C7 (0.66 cm day^{-1}) and lowest for the isolates C1 *ie*., 0.15 cm day^{-1}(Table14) (Plate 16).

C7 isolate was found to be the most virulent among the screened ones with highest rate of lesion development and minimum days for symptom appearance on three black pepper varieties tested *viz*., Panniyur 1, Panniyur 3 and Karimunda. Hence the isolate C7 was used for further studies.

Table 12 : Characteristics of the lesion produced by isolates of *C. gloeosporioides* causing anthracnose of black pepper on detached leaf of Panniyur 3 (Virulence rating)

Isolates	Days taken for symptom appearance	Lesion size (cm) *		Rate of lesion development(cm day^{-1}) *
		3rd DAI	5th DAI	
C1	4	0.00 (1.00)^{d}	0.73 (0.85)^{e}	0.15
C2	3	0.38 (1.17)^{c}	0.92 (0.96)^{d}	0.18
C3	4	0.00 (1.00)^{d}	0.75 (0.86)^{de}	0.15
C4	3	0.37 (1.17)^{c}	0.90 (0.94)^{de}	0.18
C5	2	1.00 (1.41)^{a}	1.95 (1.40)^{b}	0.39
C6	2	0.85 (1.36)^{c}	1.35 (1.16)^{c}	0.27
C7	2	1.05 (1.43)^{a}	2.40 (1.55)^{a}	0.49
C8	2	0.95 (1.39)^{b}	2.03 (1.42)^{b}	0.40
CD (0.05)		0.065	0.240	0.026
SEm±		0.031	0.082	0.009

* Mean of four replications; Values in parenthesis are square root transformed values

Table 13 Characteristics of the lesion produced by isolates of *C. gloeosporioides* causing anthracnose of black pepper on detached leaf of Karimunda (Virulence rating)

Isolates	Days taken for symptom appearance	Lesion size (cm) *		Rate of lesion development (cm day^{-1}) *
		3^{rd} DAI	5^{th} DAI	
C1	3	0.20 (1.09)^{d}	0.78 (1.13)^{e}	0.15
C2	3	0.43 (1.19)^{c}	0.93 (1.19)^{e}	0.19
C3	3	0.33 (1.15)^{cd}	0.85 (1.16)^{e}	0.17
C4	2	0.83 (1.35)^{b}	1.25 (1.32)^{d}	0.25
C5	2	0.95 (1.39)^{ab}	1.57 (1.44)^{c}	0.33
C6	3	0.25 (1.12)^{d}	0.82 (1.15)^{e}	0.16
C7	2	1.13 (1.45)^{a}	3.22 (1.93)^{a}	0.66
C8	2	0.95 (1.39)^{ab}	2.02 (1.59)^{b}	0.40
CD (0.05)		0.071	0.211	0.035
SEm±		0.024	0.072	0.012

* Mean of four replications; Values in parenthesis are square root transformed values

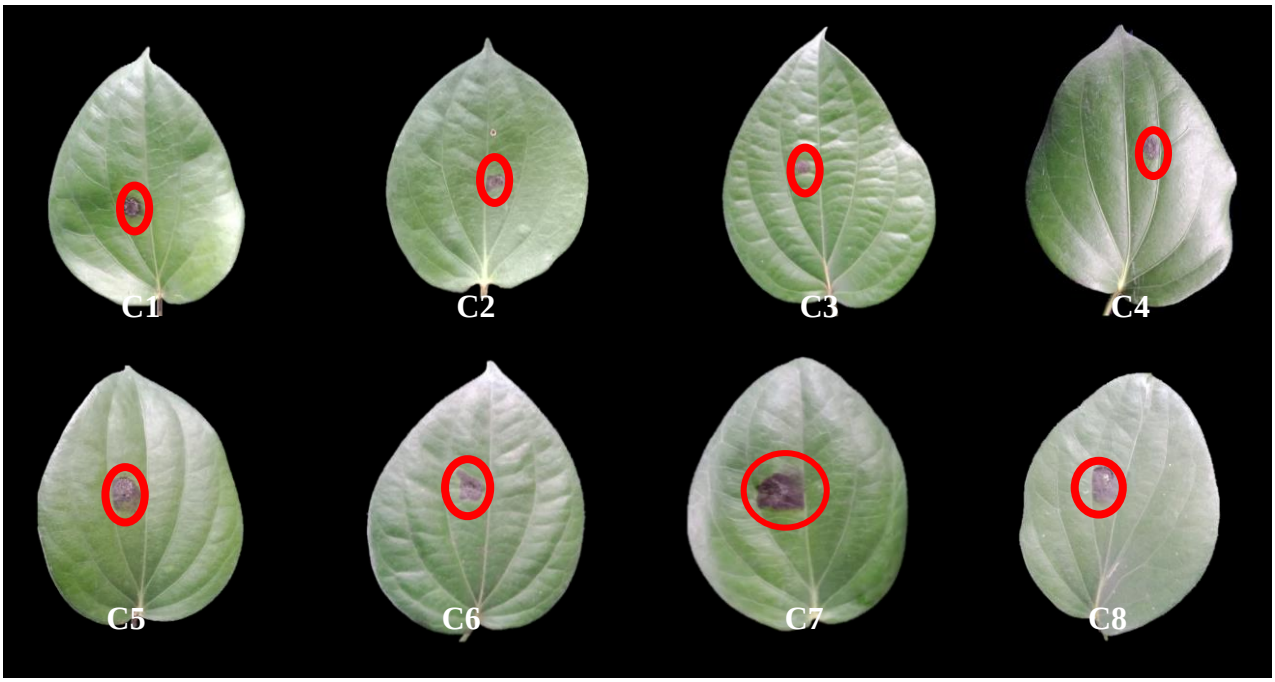


Plate15. Lesion development on artificial inoculation of *C. gloeosporioides* isolates on detached leaf in Panniyur 3 (5 DAI)

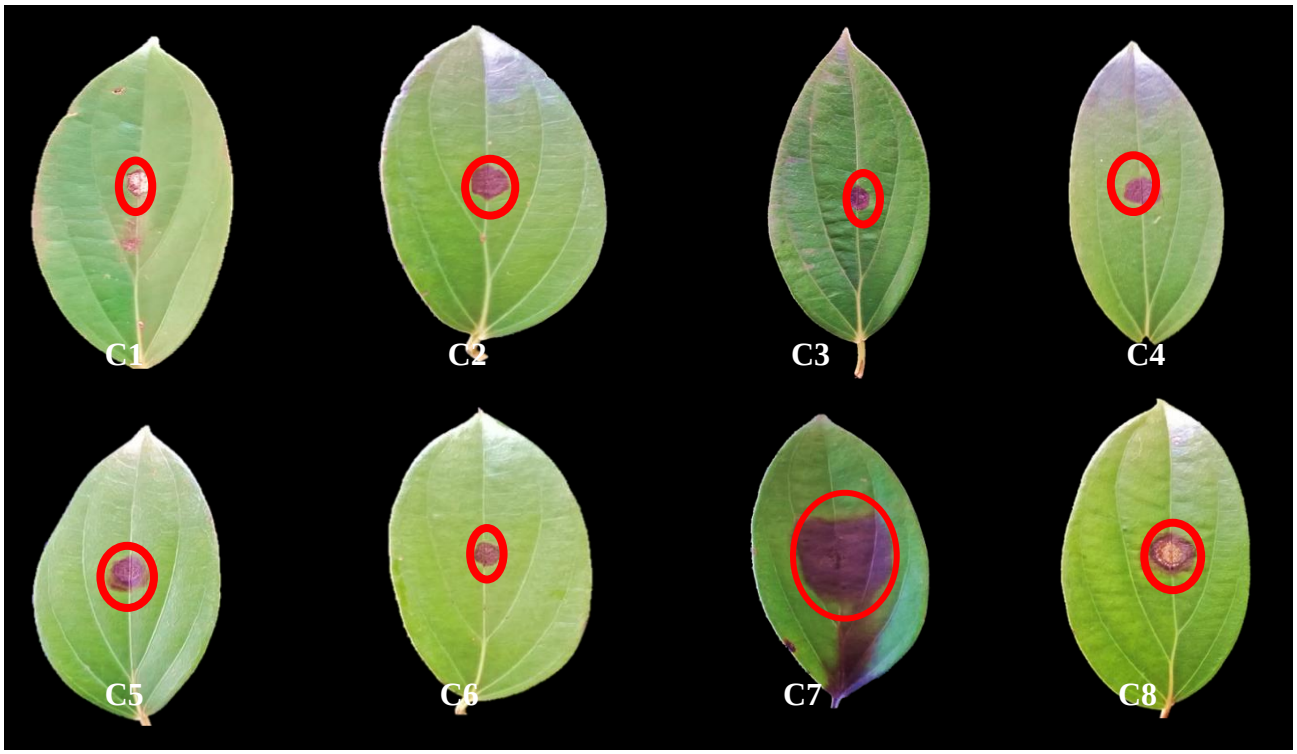


Plate16. Lesion development on artificial inoculation of *C. gloeosporioides* isolates on detached leaf in variety Karimunda (5 DAI)

4.4. SCREENING OF BLACK PEPPER VARIETIES AGAINST ANTHRACNOSE

The varietal screening was done on the basis of days taken for symptom appearance, lesion size produced and per cent disease index. Rooted cuttings of Panniyur 1 to Panniyur 8 and local cultivar Karimunda with three replications were raised in poly bags as per POP, KAU (2016) and the experiment was laid out in CRD design. The pathogen was inoculated on fully opened leaves abberated with carborandum powder followed by spray of spore suspension (10^6 spores ml^{-1}) using an atomizer.

All the varieties *viz*., Panniyur1 to Panniyur 8 and local cultivar Karimunda produced symptom within 2 to 4 days of inoculation with *C. gloeosporioides*. Panniyur 4 and Karimunda took 2 days for symptom appearance; whereas Panniyur 3, Panniyur 5 and Panniyur 7 expressed symptom 3 days after inoculation. Panniyur 2 and Panniyur 8 took maximum days (4) for symptom appearance. Panniyur 2 produced a lesion size of 5.88 cm^2 (5 DAI) which was significantly different from the other varieties. The varieties Panniyur 8, Panniyur 1, Panniyur 7, Panniyur 6, Panniyur 3 and Karimunda were on par. Panniyur 5 produced a lesion size of 18.99 cm^2; while Panniyur 4 developed the maximum lesion size of 25.82 cm^2 (5 DAI). Similarly seven days after inoculation, Panniyur 2 produced a minimum lesion size of 6.44 cm^2. The varieties Panniyur 8, 6, 1, 3, Karimunda and Panniyur 7 were on par. Panniyur 4 produced maximum lesion size of (27.51 cm^2) followed by Panniyur 5 (21.08 cm^2)

Per cent disease index was calculated based on a score chart ranged from 0 - 7 scale developed by Sankar (2002). Panniyur 4 recorded the highest PDI of 51.43 followed by P5 (41.43). This variety showed a rapid increase in PDI from 36.6 (5 DAI) to 51.43 (7 DAI). The lowest PDI was recorded in Panniyur 2 (14.28) followed by Panniyur 8 (20.00). The varieties Panniyur 6, 3, Karimunda, Panniyur1 and 7 recorded PDI of 24.28, 30.00, 34.28, 35.71 and 37.14 respectively (Table 14, Plate 17 and Plate 18).

Table 14: Lesion size, days taken for symptom appearance and PDI of different varieties of black pepper inoculated with *C. gloeosporioides* (C4)

Varieties	DTSA	Lesion size (l*b) (cm^2)*		PDI at 5^{th} day	PDI at 7^{th} day
		5^{th} day	7^{th} day		
Panniyur 1	3	11.87 ± 1.49^{c}	15.12 ± 2.22^{cd}	32.85	35.71
Panniyur 2	4	5.88 ± 0.96^{d}	6.44 ± 1.24^{e}	11.42	14.28
Panniyur 3	3	13.28 ± 2.48^{c}	18.04 ± 1.08^{bcd}	20.12	30.00
Panniyur 4	2	25.82 ± 2.46^{a}	27.51 ± 0.27^{a}	36.6	51.43
Panniyur 5	3	18.99 ± 0.33^{b}	21.08 ± 0.34^{b}	22.85	41.43
Panniyur 6	4	13.12 ± 0.82^{c}	14.36 ± 0.73^{d}	20.21	24.28
Panniyur 7	3	12.87 ± 2.89^{c}	19.24 ± 3.93^{bc}	25.00	37.14
Panniyur 8	4	11.36 ± 0.95^{cd}	12.88 ± 2.92^{d}	18.57	20.00
Karimunda	2	$13.71 \pm 1.29b^{c}$	18.25 ± 0.55^{bcd}	27.14	34.28
CD (0.05)		5.197	5.708		
SEm±		1.736	1.906		

* Mean of three replications: DTSA- Days taken for symptom appearance

Based on PDI grading was done and categorized varieties into Immune (I) - No infection, Highly resistant (HR) – 1 to 10%, Resistant (R) -11 to 25 % Susceptible (S) – 26 to 50%, Highly susceptible (HS) - >50% (Table 14). According to the grade given, Panniyur 2 and Panniyur 8 were categorized as resistant, whereas Panniyur 1, Panniyur 3, Panniyur 5, Panniyur 6, Panniyur 7 and Karimunda as susceptible and Panniyur 4 as highly susceptible to *C. gloeosporioides* when artificially inoculated under lab condition.

Table 15: Grading and reaction of black pepper varieties to *C. gloeosporioides* causing anthracnose

Grade	Leaf area affected	Reaction category
0	No infection	Immune (I)
1	1-10%	Highly resistant (HR)
3	11-25%	Resistant (R)
5	26-50%	Susceptible (S)
7	> 50%	Highly susceptible (HS)

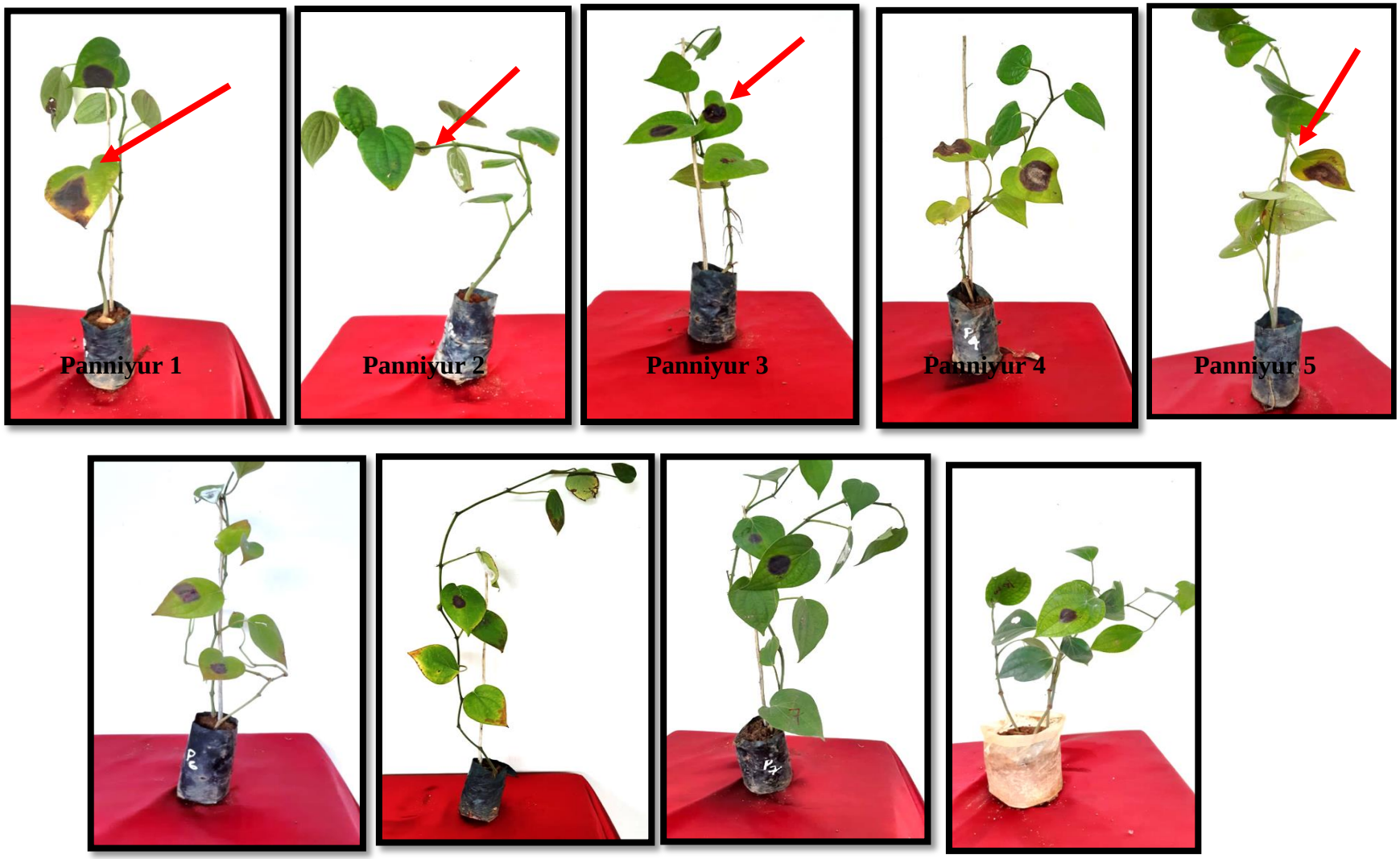


Plate 17. Symptom development in Panniyur varieties P1 to P8 and Karimunda by artificial inoculation of *C. gleosporioides* in one year old cuttings (5 DAI)

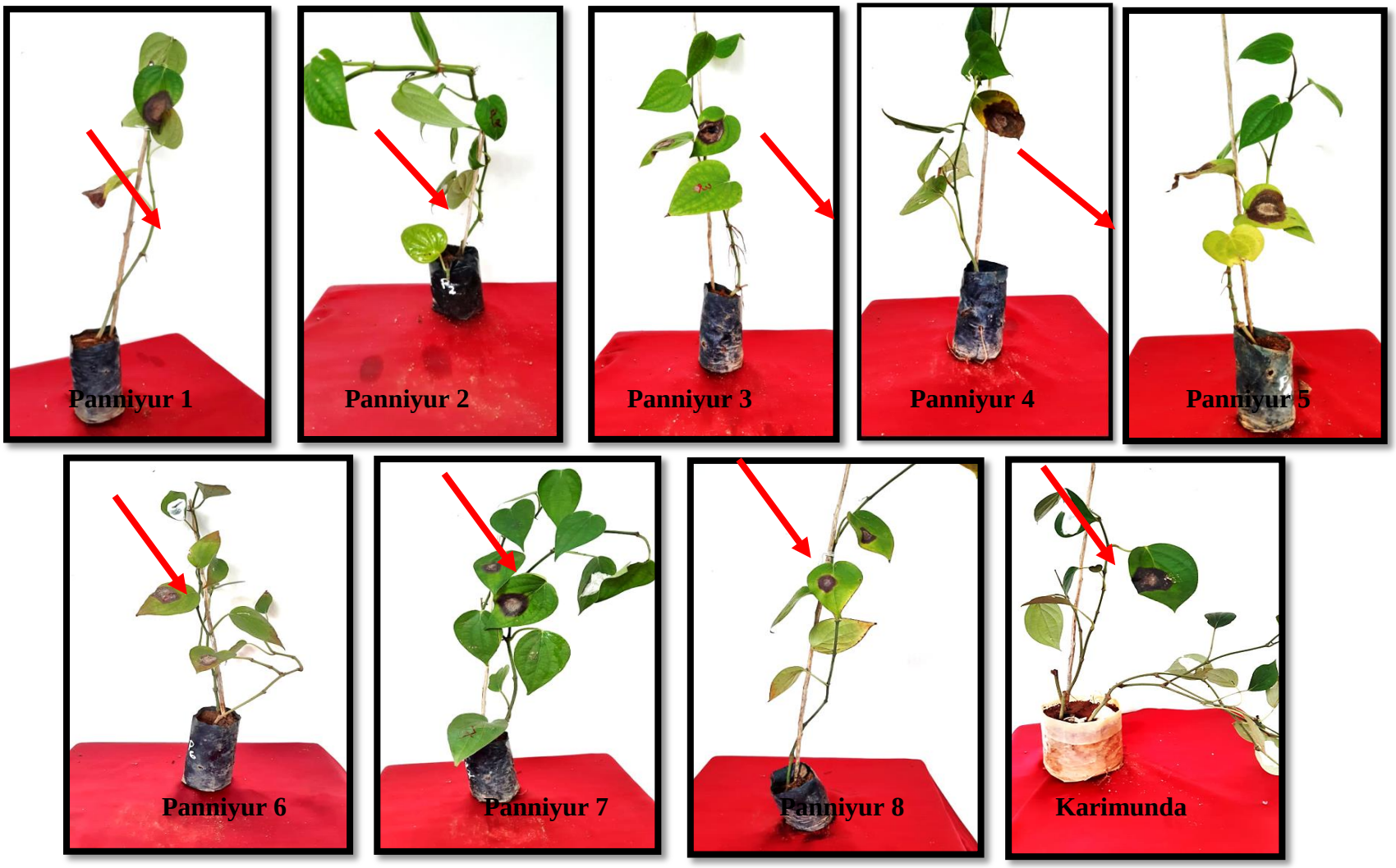


Plate 17. Symptom development in Panniyur varieties P1 to P8 and Karimunda by artificial inoculation of *C. gleosporioides* in one year old cuttings (5 DAI)

4.5. EVALUATION OF NEW GENERATION FUNGICIDES *viz*., STROBILURINS, AZOLES AND COMBINATION FUNGICIDES AGAINST *C. gloeosporioides*

The efficacy of new generation fungicides *viz.*, strobilurins, triazoles, and their combinations and contact fungicides were tested against *C. gloeosporioides*. *In vitro* evaluation was carried out by poisoned food technique in PDA medium.

4.5.1. *In vitro* evaluation of fungicides against *C. gloeosporioides*

In vitro evaluation was conducted with nine different fungicides *viz*., azoxystrobin 23 SC, kresoxim methyl 44.3 SC, hexaconazole 5 EC, tebuconazole 25EC, azoxystrobin 11% + tebuconazole 18.3% SC, trifloxystrobin 25% + tebuconazole 55% WP, captan 70% + hexaconazole 5% WP, carbendazim 12% + mancozeb 63% WP, copper oxy chloride 50% WP to screen out the most effective fungicide against the mycelial growth of *C. gloeosporioides* by poisoned food technique.

The nature of mycelium and mycelial growth (cm) of *C. gloeosporioides* at different concentrations (10, 25, 50 and 100 ppm) were recorded at different intervals (5^{th}, 7^{th} and 10^{th} day). The nature of mycelial growth was fluffy, cottony or sparse. Among the strobilurin fungicides tested kresoxim methyl showed a mycelial growth of 1.2, 2.2 and 3.23 cm at 5, 7 and 10 DAI respectively. The mycelial growth in the triazole fungicide Tebuconazole was 0.23, 0.6 and 1.63 cm at 5, 7 and 10 DAI. Of the various combination fungicides tested carbendazim 12% + mancozeb 63% WP recorded a minimal mycelial growth 0.30, 0.53, 0.87 cm respectively at different days of observation. azoxystrobin 11% + tebuconazole 8.3% SC, trifloxystrobin 25% + tebuconazole 55% WP, and captan 70% + hexaconazole 5% WP recorded a mycelial growth of 2.3, 2.3 and 3.43 respectively at 10 DAI. The contact fungicide Copper oxy chloride 50% WP (8.30 cm) had the maximum mycelial growth at 5, 7 and 10 DAI *viz*., 5.43, 6.6, and 8.3 cm respectively. azoxystrobin 23% SC (7.53 cm) had a higher mycelial growth at 10 ppm (Table 16).

At 25 ppm concentration carbendazim 12% + mancozeb 63% WP completely inhibited the mycelial growth whereas tebuconazole 25% EC (0.47cm), azoxystrobin

11% + tebuconazole 18.3% SC (0.73cm) and trifloxystrobin 25% + tebuconazole 55% WP (1.50 cm) on 10th day after incubation were found to be statistically significant over other treatments regarding mycelial inhibition. (Table17).

Table 16: *In vitro* evaluation of new generation fungicides (10ppm) against *C. gloeosporioides* causing anthracnose of black pepper (Poisoned food technique)

Treatments (Fungicides)		Mycelial growth in cm (10 ppm)*			Nature of mycelial growth
		5th day	7th day	10th day	
T1	Azoxystrobin 23% SC	5.30 ± 0.06^{b}	6.57 ±0.01^{b}	7.53 ± 0.15^{c}	Fluffy
T2	Kresoxim methyl 44.3%SC	1.20 ± 0.12^{d}	2.20 ± 0.12^{d}	3.23 ± 0.09^{e}	Cottony
T3	Hexaconazole 5% EC	1.33 ± 0.09^{cd}	3.13 ± 0.09^{b}	3.53 ±0.09^{d}	Fluffy
T4	Tebuconazole 25% EC	0.23 ± 0.03^{e}	0.63 ± 0.15^{f}	1.13 ± 0.09^{h}	Brownish sparse
T5	Azoxystrobin11% +Tebuconazole 18.3% SC	0.83 ± 0.07^{e}	1.27 ± 0.09^{e}	2.30 ± 0.06^{g}	Fluffy aerial
T6	Trifloxystrobin25%+Tebuconazole55%WP	1.13 ± 0.09^{d}	1.50 ± 0.06^{e}	2.63 ± 0.12^{f}	Fluffy
T7	Captan 70% + Hexaconazole 5%WP	1.47 ± 0.09^{c}	2.4 ± 0.09^{d}	3.43 ± 0.12^{de}	Fluffy
T8	Carbendazim 12%+Mancozeb63%WP	0.30 ± 0.06^{f}	0.53 ± 0.03^{g}	0.87 ± 0.03^{h}	Fluffy
T9	Copper oxy chloride 50%WP	5.43 ± 0.15^{b}	6.6 ± 0.12^{b}	8.30 ± 0.06^{b}	Pinkish white fluffy
T10	Control	7.14 ± 0.06^{a}	8.89 ± 0.06^{a}	9 ± 0.00^{a}	Fluffy
	CD (0.05)	0.264	0.301	0.284	
	SEm±	0.125	0.101	0.095	

*Mean ± SD of three replications

Table 17: *In vitro* evaluation of new generation fungicides (25ppm) against *C. gloeosporioides* (Poisoned food technique)

Treatments (Fungicides)	Mycelial growth in cm (25ppm)*			Nature of mycelial growth
	5th day	7th day	10th day	
Azoxystrobin 23% SC	4.30 (2.30±0.030)[c]	5.80 (2.61 ±0.006)[b]	6.50 (2.74±0.015)[c]	Fluffy
Kresoxim methyl 44.3% SC	1.23 (1.49±0.029)[e]	2.03 (1.74±0.025)[d]	2.50 (1.87±0.015)[e]	Cottony
Hexaconazole 5% EC	2.40 (1.84±0.016)[d]	3.13 (2.03±0.106)[c]	3.40 (2.09±0.028)[e]	Fluffy
Tebuconazole 25% EC	0.23 (1.11 ±0.039)[g]	0.37 (1.16±0.014)[g]	0.47 (1.21±0.014)[g]	Brownish sparse
Azoxystrobin11% +Tebuconazole18.3% SC	0.27 (1.09 ±0.026)[g]	0.50 (1.22±0.024)[f]	0.73 (1.32±0.046)[g]	Fluffy
Trifloxystrobin 25% + Tebucona-zole 55% WP	0.80 (1.34 ±0.02)[f]	1.13 (1.46±0.030)[f]	1.50 (1.58±0.018)[f]	Fluffy
Captan 70% + Hexaconazole 5% WP	1.27 (1.50±0.040)[f]	2.17 (1.77±0.009)[d]	3.23 (2.06±0.021)[d]	Fluffy
Carbendazim 12% + Mancozeb 63% WP	0.00 (1.00±0.000)[h]	0.00 (1.00±0.000)[h]	0.00 (1.00±0.000)[h]	No growth
Copper oxy chloride 50% WP	4.87 (2.42±0.013)[b]	6.03 (2.65±0.011)[b]	7.23 (2.87±0.011)[b]	Fluffy
Control	7.14 (2.85±0.08)[a]	8.89 (3.1±0.001)[a]	9 (3.16±0.00)[a]	Fluffy
CD (0.05)	0.080	0.124	0.054	
SEm±	0.027	0.041	0.018	

*Mean ± SD of three replications; Values in parenthesis are square root transformed values

At 50 ppm carbendazim 12% + mancozeb 63% WP showed complete inhibition over mycelial growth. Whereas tebuconazole 25% EC (0.40 cm), azoxystrobin 11% + tebuconazole 18.3% SC (0.43 cm) and trifloxystrobin 25% + tebuconazole 55% WP (1.07 cm) were significantly inhibited mycelial growth on 10^{th} day after incubation. Copper oxy chloride 50%WP had a mycelial growth of 7.23 cm (10 day after incubation) was found to be least effective in growth suppression (Table 18).

In higher concentration (100 ppm) carbendazim 12% + mancozeb 63% WP, tebuconazole 25% EC, azoxystrobin 11% + tebuconazole 18.3% SC and trifloxystrobin 25% + tebuconazole 55% WP showed complete mycelial inhibition. Whereas the contact fungicide copper oxychloride had a mycelial growth of 5.70 cm even at higher concentration (Table 19).

The nature of mycelial growth varied from fluffy, cottony to sparse on media amended with various fungicides at 10, 25 50 and 100 ppm concentrations as compared to the fluffy growth of C7 isolate of *C. gloeosporioides*

Table 18: *In vitro* evaluation of new generation fungicides (50 ppm) against *C. gloeosporioides* causing anthracnose of black pepper (Poisoned food technique)

Treatments (Fungicides)	Mycelial growth in cm (50 ppm)*			Nature of mycelial growth
	5th day	7th day	10th day	
Azoxystrobin 23% SC	3.43 (2.12 ±0.008)^{c}	4.00 (2.24±0.013)^{c}	6.73 (2.78±0.016)^{c}	Fluffy
Kresoxim methyl 44.3% SC	1.30 (1.52 ±0.019)^{d}	2.00 (1.73±0.017)^{d}	2.5 (1.87±0.015)^{d}	Cottony
Hexaconazole 5% EC	1.20 (1.48 ±0.039)^{d}	1.80 (1.67±0.017)^{e}	2.40 (1.84±0.016)^{d}	Fluffy
Tebuconazole 25% EC	0.43 (1.18 ± 0.098)^{e}	0.13 (1.06±0.016)^{h}	0.40 (1.18±0.024)^{g}	No growth
Azoxystrobin11% + Tebucona-zole18.3% SC	0.37 (1.16 ± 0.110)^{e}	0.30 (1.14±0.025)^{g}	0.43 (1.19±0.037)^{g}	Small
Trifloxystrobin 25%+Tebuconazole55%WP	0.23 (1.11 ± 0.015)^{ef}	0.53 (1.23±0.013)^{f}	1.07 (1.44±0.012)^{f}	Feeble
Captan 70% + Hexaconazole5% WP	1.17 (1.47 ± 0.011)^{d}	1.90 (1.70±0.017)^{de}	2.10 (1.76±0.016)^{e}	Fluffy
Carbendazim 12 % + Mancozeb 63 %WP	0.00 (1.00 ± 0.000)^{f}	0.00 (1.00±0.000)^{i}	0.00 (1.00±0.00)^{h}	No growth
Copper oxy chloride 50% WP	4.37 (2.32 ±0.019)^{b}	5.50 (2.55±0.011)^{b}	7.23 (2.87±0.006)^{b}	Fluffy
Control	7.14 (2.85± 0.039)^{a}	8.89 (3.1± 0.008)^{a}	9 (3.16±0.000)^{a}	Fluffy
CD (0.05)	0.395	0.047	0.056	
SEm±	0.132	0.016	0.019	

*Mean ± SD of three replications; Values in parenthesis are square root transformed values

Table 19: *In vitro* evaluation of new generation fungicides (100ppm) against *C. gloeosporioides* causing anthracnose of black pepper (Poisoned food technique)

Treatments (Fungicides)	Mycelial growth in cm (100 ppm)*			Nature of mycelial growth
	5th day	7th day	10th day	
Azoxystrobin 23% SC	2.20 c (1.78±0.016)	3.73 c (2.17±0.028)	5.13 c (2.47±0.018)	Fluffy
Kresoxim methyl 44.3% SC	1.13 d (1.46±0.030)	1.77 d (1.66±0.010)	2.37 d (1.84±0.009)	Cottony
Hexaconazole 5% EC	0.40 e (1.18±0.024)	0.86 f (1.36±0.012)	1.30 f (1.52±0.019)	No growth
Tebuconazole 25% EC	0.00 e (1.00±0.000)	0.00 f (1.00±0.012)	0.00 g (1.00±0.000)	No growth
Azoxystrobin11% +Tebuconazole 18.3% SC	0.00 f (1.00±0.000)	0.00 g (1.00±0.00)	0.00 g (1.00±0.000)	No growth
Trifloxystrobin 25%+Tebuconazole 55%WP	0.00 f (1.00±0.000)	0.00 g (1.00±0.00)	0.00 g (1.00±0.000)	No growth
Captan 70% + Hexaconazole5%WP	1.07 e (1.44±0.031)	1.50 e (1.58±0.018)	2.17 g (1.77±0.019)	Fluffy
Carbendazim 12 % + Mancozeb 63 % WP	0.00 f (1.00±0.000)	0.00 g (1.00±0.00)	0.00 f (1.00±0.000)	No growth
Copper oxy chloride 50% WP	3.23 b (2.06±0.021)	4.66 b (2.38±0.014)	5.70 b (2.58±0.011)	Fluffy
Control	7.14 (2.85± 0.039) a	8.89 a (3.1± 0.008)	9 a (3.16±0.000)	Fluffy
CD (0.05)	0.056	0.039	0.035	
SEm±	0.019	0.013	0.012	

*Mean ± SD of three replications; Values in parenthesis are square root transformed values.

Regarding percentage growth inhibition of *C. gloeosporoides* at lower concentrations (10 ppm) among strobilurin fungicides, the maximum percentage inhibition was recorded by kresoxim methyl 44.3 SC (75.92 %) and among triazoles tebuconazole 25% EC (92.59 %). Under combination fungicides tested, maximum percentage inhibition at 10 ppm was exhibited by carbendazim 12% + mancozeb 63% WP (98.14) followed by azoxystrobin 11% + tebuconazole 18.3% SC (91.11 %) and trifloxystrobin 25% + tebuconazole55% WP (83.33%). At 100 ppm fungicides *viz*., carbendazim 12% + mancozeb 63% WP, tebuconazole 25% EC, azoxystrobin 11% + tebuconazole 18.3% SC and trifloxystrobin 25% + tebuconazole 55% WP completely suppressed the mycelial growth. The contact fungicide copper oxychloride was ineffective even at 100 ppm against the pathogen (Table 20) (Plate 19 to Plate 26)

Table 20: Percentage mycelial inhibition of *C. gloeosporioides* causing anthracnose of black pepper by new generation fungicides

Treatments (Fungicides)		Mycelial inhibition (%)*			
		10ppm	25ppm	50 ppm	100 ppm
T1	Azoxystrobin 23%SC	27.40 (31.56)^{f}	32.96 (35.04)^{f}	55.55 (48.19)^{e}	58.51 (49.90)^{e}
T2	Kresoxim methyl 44.3%SC	75.92 (60.61)^{d}	77.03 (61.37)^{d}	77.77 (61.87)^{d}	80.37 (63.70)^{d}
T3	Hexaconazole 5% EC	64.44 (53.44)^{e}	68.51 (55.90)^{e}	81.11 (64.28)^{d}	90.37 (71.93)^{b}
T4	Tebuconazole 25% EC	92.59 (74.21)^{b}	95.92 (78.37)^{b}	99.63 (87.34)^{ab}	100.00 (89.05)^{a}
T5	Azoxystrobin 11% +Tebuconazole18.3% SC	91.11 (72.67)^{b}	94.81 (76.86)^{b}	99.25 (85.63)^{b}	100.00 (89.05)^{a}
T6	Trifloxystrobin 25% + Tebuconazole55%WP	83.33 (65.91)^{c}	87.03 (68.89)^{c}	93.70 (75.48)^{c}	100.00 (89.05)^{a}
T7	Captan 70% + Hexaconazole 5% WP	72.96 (58.66)^{d}	75.92 (60.614)^{d}	78.88 (62.65)^{d}	83.33 (65.91)^{c}
T8	Carbendazim12% + Mancozeb 63%WP	98.14 (82.44)^{a}	100.00 (89.05)^{a}	100.00 (89.05)^{a}	100.00 (89.05)^{a}
T9	Copper oxy chloride 50% WP	25.92 (30.59)^{f}	35.55 (36.59)^{f}	38.88 (38.58)^{f}	48.14 (43.93)^{f}
T10	Control	0.00 (0.95)^{g}	0.00 (0.95)^{g}	0.00 (0.95)^{g}	0.00 (0.95)^{g}
	CD(0.05)	2.912	2.138	3.082	1.043
	SEm±	0.980	0.720	1.037	0.351

*Mean ± SD of three replications; Values in parenthesis are arc sine transformed values.

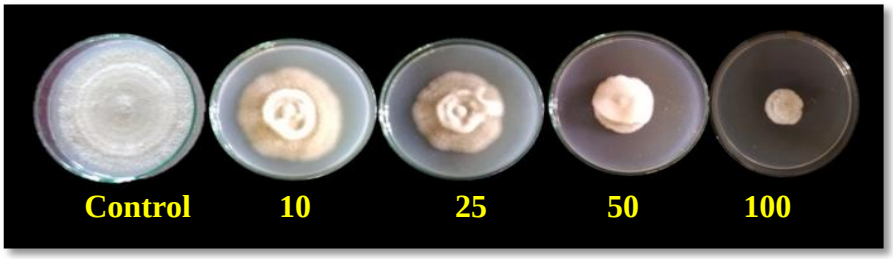


Plate 19: Effect of different concentrations of azoxystrobin (ppm) on mycelial growth of *C. gleosporioides*

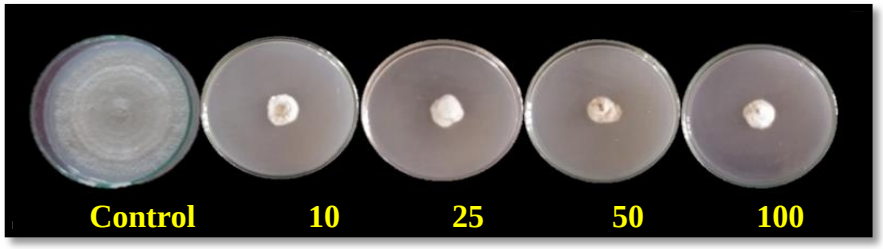


Plate20: Effect of different concentrations of kresoxim methyl (ppm) on mycelial growth of *C. gleosporioides*

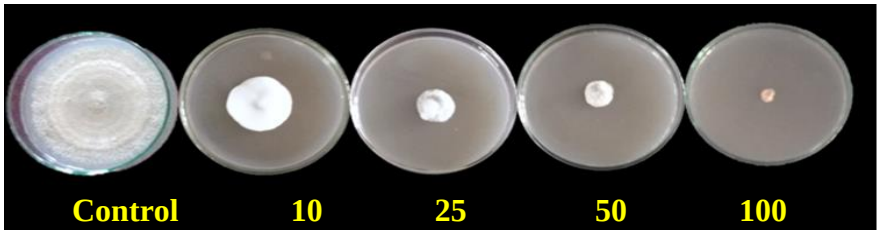


Plate 21: Effect of different concentrations of hexaconazole (ppm) on mycelial growth of *C. gleosporioides*

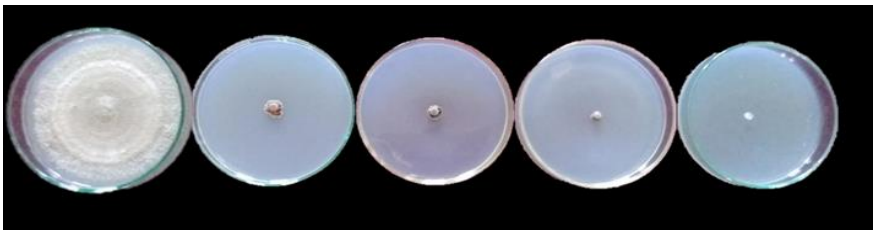


Plate 22: Effect of different concentrations of tebuconazole (ppm) on mycelial growth of *C. gleosporioides*

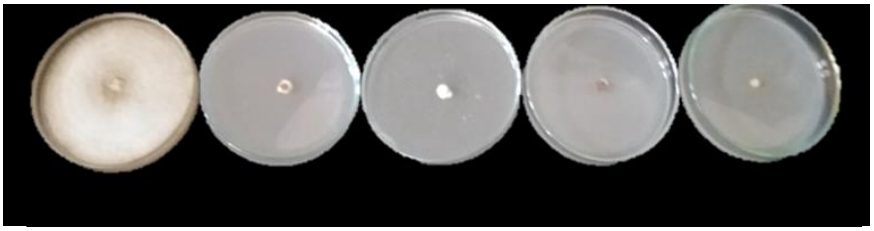


Plate 23: Effect of different concentrations of azoxystrobin + tebuconazole (ppm) on mycelial growth of *C. gleosporioides*

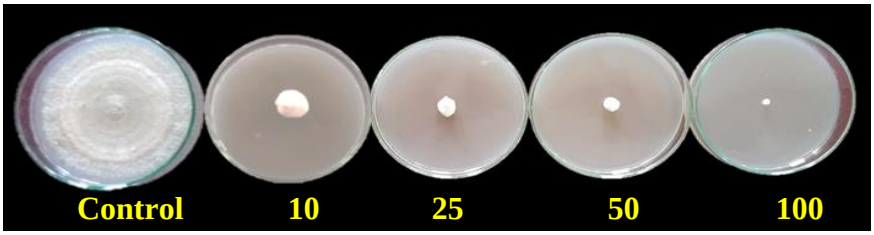


Plate 24: Effect of different concentrations of trfloxystrobin+ tebuconazole(ppm) on mycelial growth of *C. gleosporioides*

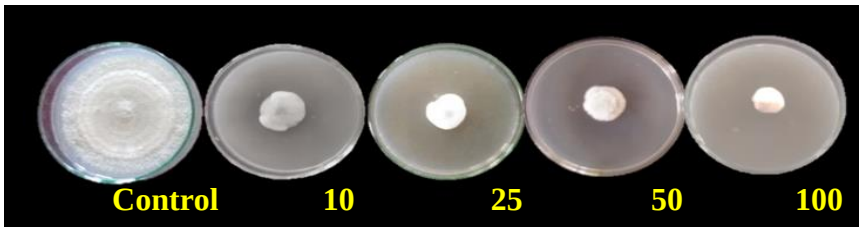


Plate 25: Effect of different concentrations of captan+ hexaconazole (ppm) on mycelial growth of *C. gleosporioides*

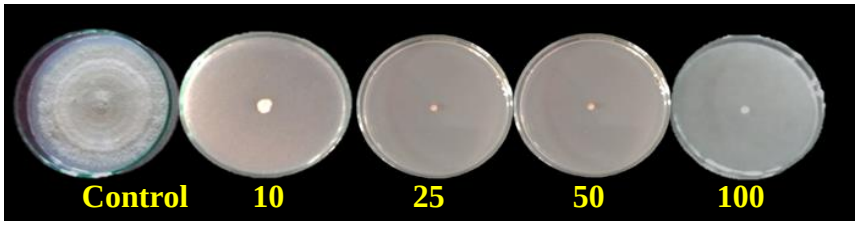


Plate 26: Effect of different concentrations of carbendazim+ mancozeb (ppm) on mycelial growth of *C. gleosporioides*

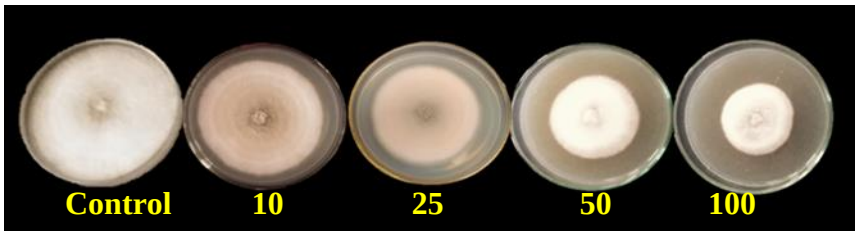


Plate 27: Effect of different concentrations of copper oxy chloride (ppm) on mycelial growth of *C. gleosporioides*

Discussion

5. DISCUSSION

Black pepper, the 'black gold' is one of the most important spice crops cultivated in Kerala. Incidence of diseases has become a serious threat limiting the black pepper cultivation. Among the fungal diseases, anthracnose is an economically important disease which affects leaves, spikes and berries; and gradually reduces the vigour of the plant. Growing resistant varieties is said to be the most economical and easy method to minimize crop loss due to diseases but may not be a complete successful approach due to the presence of diverse species and races of fungal pathogen. In the modern intensified agriculture, the most reliable means of plant diseases management is the use of biocontrol agents and need based application of fungicides. Taking into consideration the emergence of resistance to many of the conventionally used fungicides such as dithiocarbamate and copper fungicides, management strategies solely depending on such fungicides may not be sufficient to manage the disease. So there is a need to extend the choices by introducing new generation fungicides which are expected to provide better disease control owing to its target specific action, systemic nature, curativeness and less quantity needed in disease management.

In this context, the following study entitled "Varietal screening and management of anthracnose of black pepper using new generation fungicides" was undertaken with the objectives to screen KAU varieties and most popular local cultivar Karimunda for resistance against anthracnose of black pepper caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and evolve management strategy using new generation fungicides.

5.1. SURVEY AND COLLECTION OF DISEASED SAMPLES, SYMPTOMATOLOGY, ISOLATION OF PATHOGEN, PROVING PATHOGENICITY OF *C. gloeosporioides* CAUSING ANTHRACNOSE DISEASE OF BLACK PEPPER

5.1.1. Survey and diseased sample collection

A survey was conducted from June 2019 to October 2019 in Thiruvananthapuram (Vellayani and Kowdiar), Wayanad (Ambalavayal and

Meenangadi) and Idukki (Myladumpara, Pampadumpara, Kattappana and Kambalikandam) districts of Kerala. The survey revealed that the disease incidence and severity of anthracnose of black pepper varied with locations and were 10 – 60 per cent and 16.8 - 50.28 per cent respectively (Fig.1). The variation is attributed to the prevailing climatic conditions, black pepper varieties / cultivars grown and virulence of the pathogen at different surveyed locations.

The highest disease incidence and severity were recorded in Myladumpara location of Idukki district where a minimum temperature of 12.4°C and maximum of 22.1°C with 87 per cent relative humidity and 178.6 mm rain fall were prevailing during October 2019. The lowest disease incidence and severity were noticed in Kowdiar region of Thiruvanthapuram district where maximum temperature was 31.9 °C with a relative humidity of 90 per cent and lowest rainfall of 10.7 mm during June 2019. Hence from the current study it was found that weather parameters *viz.*, low temperature, high relative humidity and heavy rainfall favoured the incidence of anthracnose. These findings were in accordance with the study conducted by Biju *et al*. (2013) on anthracnose of black pepper, who reported that there was a positive correlation with disease incidence, rain fall, and minimum temperature; and a negative correlation with maximum temperature. The disease outbreak was found to be higher during the months of August and September with disease incidence of 37.9 - 39.1 per cent; during which the maximum temperature ranged from 23.7 to 24.3°C, minimum temperature from 17.6 to 18.7°C and monthly rainfall of 306.6-584.7 mm prevailed.

Similarly, a survey was conducted by Nisha (2018) to study the incidence of anthracnose in betel vine during December 2016 - April 2017 from three Southern districts of Kerala *viz*., Thiruvananthapuram, Kollam and Alappuzha. The disease incidence and severity ranged from 20 - 80 per cent and 5.70 - 20.00 per cent respectively. The highest disease severity and incidence were noticed in Cherthala region of Alappuzha where congenial climatic conditions *viz*., minimum temperature of 27°C and 84 per cent relative humidity prevailed during the month of April 2017.

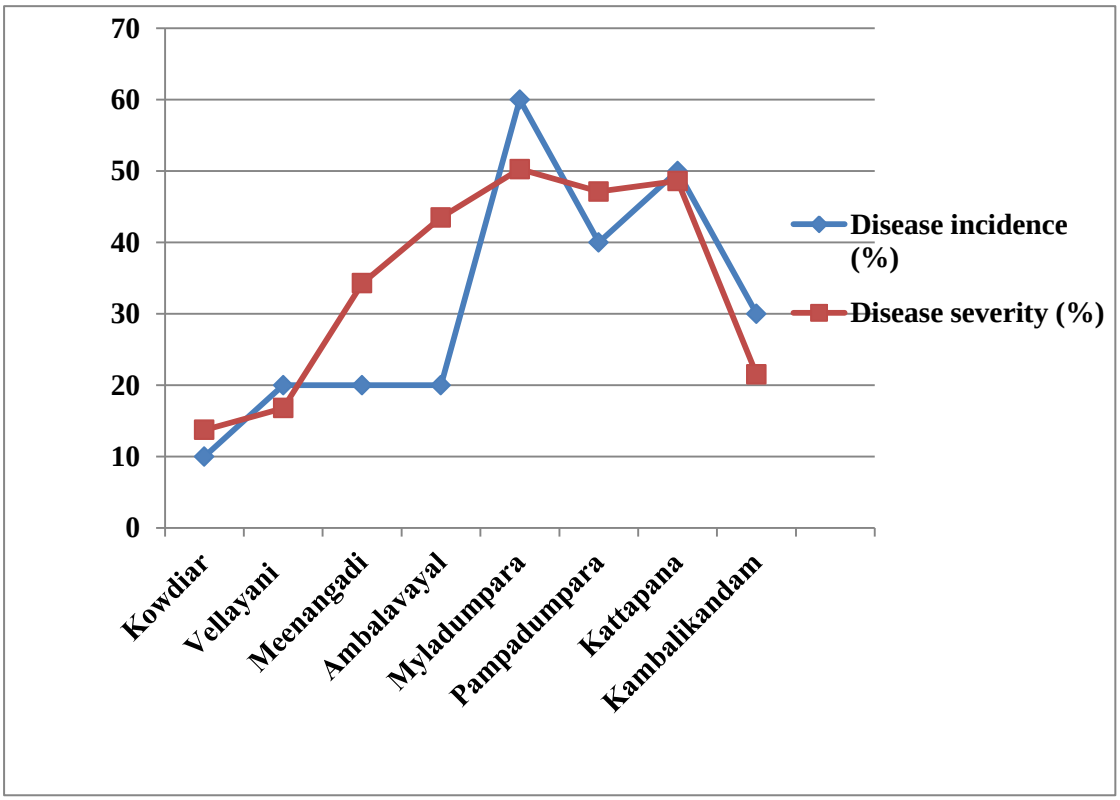


Fig. 1: Disease incidence and severity of black pepper anthracnose in surveyed locations

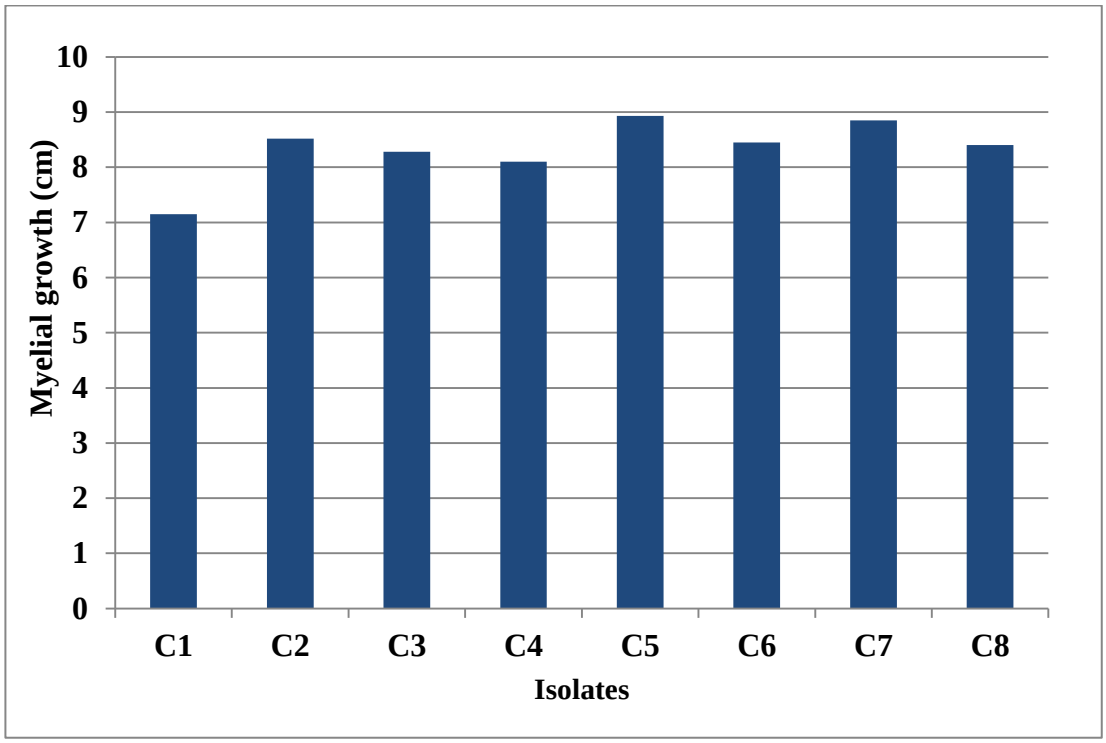


Fig. 2: Mycelial growth of isolates of *Colletotrichum* sp. in PDA medium (7DAI)

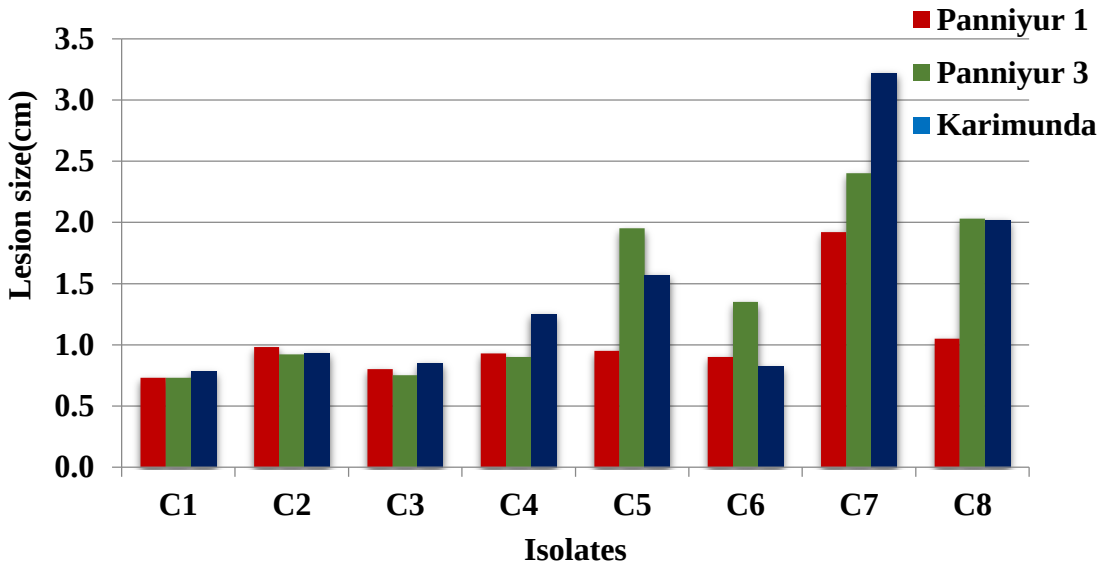


Fig. 3: Lesion size produced by *C. gloeosporioides* isolates on detached leaves of Panniyur 1, Panniyur 3 and Karimunda

5.1.2 Symptomatology

Symptom variations were noticed at surveyed locations *viz*., necrotic spots with a yellow halo, leaf blight and spike infection. Anthracnose symptoms were observed both in the nursery as well as in the main field. Symptoms initially appeared as circular necrotic spots surrounded by a yellow halo, mostly confined to the leaf lamina. Several lesions coalesce together resulted in leaf blight and drying of the margin from tip and finally lead to defoliation. Apart from the typical symptom, pin head sized acervuli were also seen at the centre of blighted areas. At high ranges of Idukki district along with the typical anthracnose symptoms such as necrotic spots on the foliage surrounded by yellow halo and leaf blight, spike infection leading to hollow (pollu) berries were also observed. Panniyur 1, Panniyur 2, Panniyur 5 and Karimunda were the most widely cultivated varieties in surveyed regions.

Sankar (2002) observed anthracnose symptoms both in the nursery as well as in the main field. The foliar infection caused by *Colletotrichum* sp. on black pepper varied from minute brown or black specks to large blighted area resulting in severe defoliation. On blighted areas, pin head sized acervuli of the fungus were also seen. Later, blighted areas became papery white or greyish white centre which shred off resulting in short hole symptom. The pathogen also affects spikes and berries. On the berries, sunken areas were observed which later resulted in hollow berries. Damage on spikes resulted in cent per cent yield loss. Chandrakant (2005) observed anthracnose symptoms on black pepper as initial chlorotic specks on lower leaves and later turned to small circular spots which were surrounded by a yellow halo. Gradually necrotic area appeared which were ashy white to papery and started to break resulting in short hole.

Biju *et al*. (2020) noticed diverse array of foliar symptoms of anthracnose during the survey conducted in black pepper growing areas of Kerala and Karnataka. They reported symptoms *viz.,* brown spot surrounded by yellow halo on the newly emerged leaves while spots with or without yellow halo on older leaves. Leaf blight symptoms were also noticed. In nurseries, pin-head size dark brown necrotic spots later surrounded by yellow halo were observed as common symptom. Besides these typical symptoms, an unusual symptom characterized by greyish necrotic lesion with

brown-blackish margins and randomly distributed pin–head sized structures was found particularly on older leaves.

5.1.3 Isolation of pathogen and proving pathogenicity

The pathogen associated with anthracnose of black pepper was isolated from infected samples from eight locations by tissue isolation method. The culture was purified using single spore isolation technique as described by (Dhingra and Sinclair, 1985).

The pathogenicity of eight isolates of *Colletotrichum* sp. was proved by following Koch's postulates. The pathogen when artificially inoculated on to the leaves of Panniyur 1, produced symptom within 2 to 3 days of inoculation. The fungus was re-isolated from the artificially inoculated leaves and was compared to original isolates to prove their similarity. The same result was observed by Chandrakant (2005) who proved pathogenicity of *C. gloeosporioides* of black pepper by following Koch's postulates. The pathogen took 5-6 days for symptom expression on artificial inoculation to healthy pepper leaves. Sreeja (2014) reported that *C. gloeosporioides* isolates of cowpea took 4-8 days for symptom expression**.**

5.2 MORPHOLOGICAL, CULTURAL AND PATHOGENIC VARIABILITY OF *Colletotrichum* ISOLATES AND IDENTIFICATION OF VIRULENT ISOLATE

Variability of *Colletotrichum* sp. was assessed by culture and morphological studies. In the current investigation, the eight isolates varied in their colony and morphological characters. The isolated cultures of *C. gloeosporioides* produced white to greyish mycelium with yellowish orange centre to light pink on the front view whereas on the rear view it appeared as whitish with orange centre to light pink, greyish green to dark grey coloured colony and had fluffy, cottony to sparse mycelial growth with regular margins. The isolates showed orange to pinkish colouration due to spore production. The above result was in agreement with observation made by Sankar (2002), who reported that *C. gloeosporioides* from black pepper had colony colour ranging from white, light grey to dark grey with a fluffy, cottony to sparse growth pattern. Salmon pink to pale orangish pink colouration was seen on rear view confirmed the presence of spore mass. Udhayakumar (2019) conducted a study for

assessing the morphology and culture characters of twenty *C. gloeosporioides* isolates causing mango anthracnose and found that all the isolates showed variability with respect to colony characters. Most of the isolates produced greyish white on the PDA medium in Petri dishes. The pigmentations like whitish, greyish brown, greenish grey, pinkish and pinkish brown were predominant in isolates.

Regarding the mycelial growth, the isolate C5 produced the maximum mycelial growth of 8.93 cm (7 DAI) which was on par with C7 isolate (8.85cm). The isolate C1 had the lowest mycelial growth of 7.15 cm. Days taken to grow the entire Petri dish ranged from 7.25 to 9.75 days (Fig. 2). Variation in mycelial growth was also observed by Papade *et al*. (2019); the isolates obtained from *Mangifera indica*, *Citrus aurantifolia*, *Catharanthus roseus*, *Psydium guavajava*, *Punica granatum*, *Carica papaya*, and *Glycine max* were labeled as Cg1 to Cg7. The study revealed that there was variation with respect to mycelial growth. Maximum radial mycelial growth was attained by Cg6 isolate (8.9 cm) at 8^{th} DAI and minimum radial mycelial growth was in Cg2 (7.2 cm). The present study revealed that among the isolates, C5 was comparatively a fast grower with an average growth of 1.28 cm day^{-}1 and took 7.25 days to complete (9 cm) its growth in Petri dish followed by C7 which had an average growth rate of 1.25 cm day^{-1} and took 7.75 days to complete the growth in Petri dish. The isolate C1 was a slow grower which took 9.75 days to complete growth in Petri dish and had the lowest growth rate of 1.02 cm day^{-1}. Similar results were obtained by Nisha (2018) where an average growth rate of *C. gloeosporioides* isolates from betel vine was observed as 1.04 - 1.28 cm day^{-1} and took 7 - 9 days to complete growth in Petri dish. Sreeja (2014) reported that an average growth rate of *C. gloeosporioides* from cowpea ranged from 0.80 to 1.38 cm day^{-1} and took 6 - 10 days for completion of growth. The variability in mycelial growth could be attributed to the virulence of the pathogen as suggested by Sangeetha and Rawal (2010). They reported that the isolates of *C. gloeosporioides* with faster mycelial growth were more virulent.

The mycelium of eight Colletotrichum isolates was hyaline and septate, and its width ranged from 2.21 - 3.45 µm and septal distance between 8.50 - 21.23 µm. Nisha (2018) had made similar observations on the mycelium of *C. gloeosporioides* from betelvine which was hyaline, septate, branched and had a width of 2.65 µm - 3.45 µm

and septal distance varied from 9.80 - 25.56 µm. Balaso (2014) and Aswani *et al*. (2016) reported the mycelial width of *C. gloeosporioides* from pomegranate and snake gourd as 1.31- 4.1 µm and 2.22 - 3.8 µm respectively.

Conidia, the asexual spores were single celled with an oil globule at the centre and were either cylindrical, oblong or dumbbell. The conidial size varied from 9.4 - 12.1 µm x 3.6 - 4.6 µm. The findings were in consonance with observation made by Jagatap *et al*. (2015), who reported that conidia of *C. gloeosporioides* obtained from anthracnose infected pomegranate samples were oblong or dumbbell shaped with one or two oil globules measuring an average size of 15.74 µm - 5.43 µm. Similarly, Poornima (2007) conducted study on morphological variability of *C. gloeosporioides* isolated from black pepper, betel vine and thippali and found that average conidial size varied from 12.67 x 4.7 µm, 14.63 x 4.7 µm and 15.52 x 4.7 µm for thippali, black pepper and betel vine isolates respectively. Appressorial size also varied among the isolates from 8.5 – 11.2 x 3.5 – 4.3 µm. Parashar (2013) found comparatively large sized appressoria during the studies on cultural and morphological features of *C. gloeosporioides* from chilli and its size ranged from 11.06 - 12.95 µm x 10.08 - 12.10 µm.

Morphological studies on appearance, colour, rate of growth of mycelia and microscopic observations on conidial and appressorial characters showed significant variation among the isolates suggesting the variability among *C. gloeosporioides* isolates causing black pepper anthracnose in Kerala. *Colletotrichum* sp. are highly variable with regards to colony morphology, conidial shape and size, appressoria, pigmentation, fungicide sensitivity, pathogenicity and other traits (Katan, 2000; Martinez *et al*., 2008)

5.2.3. Screening for pathogenic variability of different isolates of *C. gloeosporioides*

The pathogenic variability of the eight *C. gloeosporioides* isolates were assessed on three black pepper varieties *viz.*, Panniyur 1, Panniyur 3 and Karimunda by detached leaf assay. Virulence rating was assessed based on the days taken for symptom development, size of lesion developed and rate of lesion development.

The isolate C7 was identified as the most virulent isolate which produced lesion size of 1.92 cm, 2.40 cm, and 3.22 cm on Panniyur 1, Panniyur 3 and Karimunda respectively at 5 DAI. The isolate C7 produced symptoms within two days after artificial inoculation in the three varieties tested with a higher rate of lesion development of 0.40 (Panniyur 1), 0.49 (Panniyur 3) and 0.66 (Karimunda) cm day^{-1} (Fig. 3). Higher rate of lesion development on Karimunda variety showed its susceptibility to the virulent pathogen. Sankar (2002) conducted pathogenic variability study by leaf inoculation method. The different isolates of *C. gloeosporioides* from black pepper produced lesion size between 0.9 cm - 1.6 cm at 8 DAI. The isolate which produced larger lesion size (C6) was identified as most virulent which is in confirmation with the present study.

Comparative study on virulence of *C. gloeosporioides* isolates revealed that under similar conditions of incubation, the isolates produced varied lesion size on leaves confirming the pathogenic variability. Higher virulence of C7 isolate could be substantiated by its faster growth (8.85 cm on 7 th day of incubation in PDA medium) under lab conditions and produced salmon pink colouration on rear side of Petri dish indicating high sporulation. The C5 isolate showed faster rate of mycelial growth which was on par with C7 isolate, but failed to exhibit faster rate of lesion development in host. This variation could be attributed to the avirulent nature of C5 isolate under *in vitro* condition.

The pathogenic variability among *C. gloeosporioides* isolates could be an indication of different pathotypes or races in different locations of Kerala. The results of present study showed the existence of variation within the population from different regions of Kerala. Ploetz and Prakash (2000) pointed out that the differences in virulency level were probably due to the existence of more than one race or special form of *Colletotrichum* sp. Such behavior could be due to the frequent application of fungicides with a single mode of action (Verdecia, 1999), which could encourage the emergence of more aggressive races that are more difficult to control.

5.3 VARIETAL SCREENING

Rooted cuttings of Panniyur 1 to Panniyur 8 and local cultivar Karimunda with three replications were maintained for varietal screening. Spore suspension (10^6 spores ml^{-1}) was prepared with the most virulent isolate of *C. gloeosporioides* (C7) and was sprayed using an atomizer on abberated leaves. The varietal screening was done on the basis of days taken for symptom appearance, lesion size developed and per cent disease index. In this study, Panniyur 4 was found to be highly susceptible with a PDI of 51.43 and showed a rapid increase in PDI from 36.6 (5 DAI) to 51.43 (7 DAI). The lowest PDI was recorded in Panniyur 2 (14.28) followed by Panniyur 8 (20.00) (Fig. 4).

Based on PDI, grading was done and varieties were categorized. Panniyur 2 and Panniyur 8 were categorized under resistant varieties. Panniyur 2 is a vigorously growing clone from the open-pollinated progeny of the cultivar Balankotta and has the highest piperine content (6.6 %) compared to other varieties. Balankotta has high oil content, and is reportedly tolerant to moisture stress and almost tolerant to foot rot. Panniyur-8 is a hybrid variety (Panniyur 6 x Panniyur 5) which is field tolerant to Phytophthora foot rot and drought. It has piperin content of 5.7 per cent and comes second position in piperine content after Panniyur 2. Panniyur 1 (Uthiran Kotta x Cheriyakaniyakadan), Panniyur 3 (Uthirankotta x Cheriyakaniyakkadan), Panniyur 5 (Open pollinated seeding of Perumkodi), Panniyur 7 (selection from open pollinated Kaluvally) and Karimunda were found to be susceptible to anthracnose pathogen when artificially inoculated under *in vitro* conditions. Panniyur 4, clonal selection from Kuthiravally was categorized as highly susceptible variety against anthracnose pathogen.

The tolerance in Panniyur 2 and Panniyur 8 varieties against anthracnose pathogen *C. gloeosporioides* could be attributed to the piperine content in leaves. Piperine is a natural amide compound extracted from *Piper nigrum*. As an important natural alkaloid, piperine exhibited a wide spectrum of biological and pharmacological activities (Yasir *et al*., 2018). Wang *et al*. (2020) and tested efficacy of high-potential fungicides derived from piperine against six species of plant pathogen fungi, including *Rhizoctonia solani*, *Fusarium graminearum*, *Phomopsis*

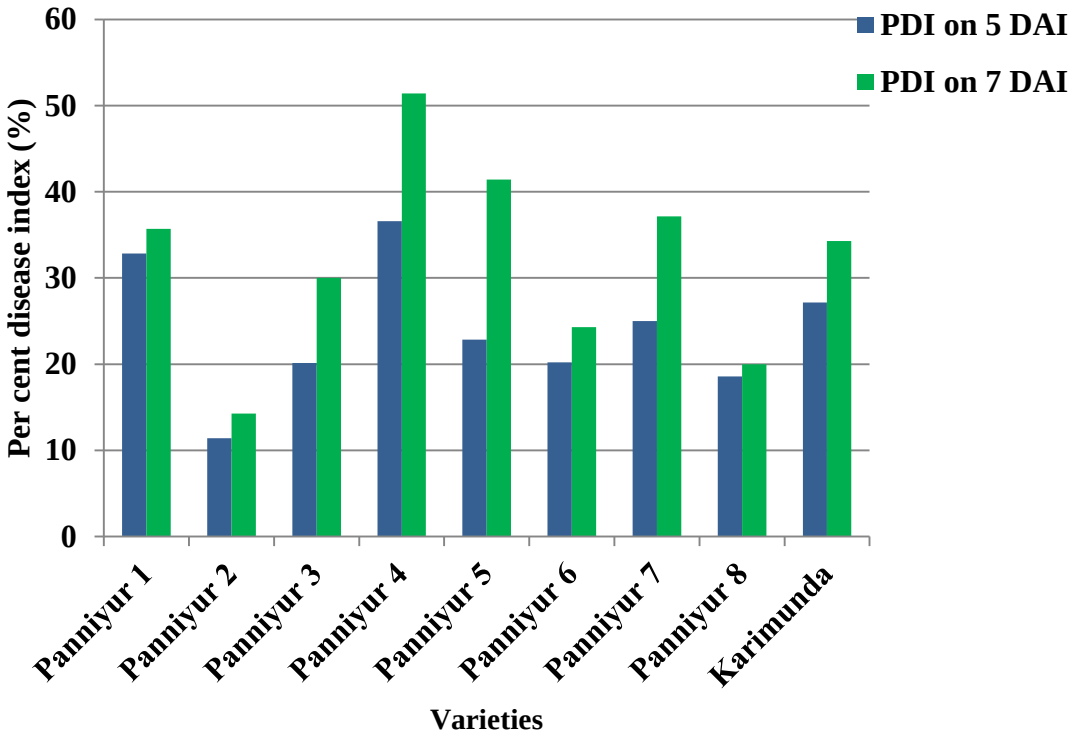


Fig. 4: Disease severity of anthracnose in black pepper varieties

adianticola, *Alternaria tenuis*, *Phytophthora capsici* and *Gloeosporium theae-sinensis*. They concluded that some compounds of the essential oil derivatives based on piperine exhibited good inhibitory activities against fungal strains, and also found that some of the target compounds have better inhibitory activities than carbendazim at the concentration of 100 μg ml^{-1}.

5.4. *IN VITRO* SCREENING OF FUNGICIDES AGAINST MOST VIRULENT ISOLATE OF *C. gloeosporioides*

The study was conducted to evaluate the efficacy of new generation fungicides in controlling the mycelial growth of *C. gloeosporioides in vitro*. Fungicides *viz.*, triazoles, strobilurins and their combinations and contact fungicides at four concentrations (10, 50, 100 and 250 ppm) were tested against the pathogen by poisoned food technique.

Percentage growth inhibition of *C. gloeosporioides* at lower concentrations (10 ppm) among strobilurin fungicides, the maximum percentage inhibition was recorded by kresoxim methyl 44.3 SC (75.92); and tebuconazole 25% EC (92.59) among triazoles. Among combination fungicides tested, maximum percentage inhibition at 10 ppm was exhibited by carbendazim 12% + mancozeb 63% WP (98.14) followed by azoxystrobin 11% + tebuconazole 18.3% SC (91.11) and trifloxystrobin 25% + tebuconazole 55% WP (83.33). At 100 ppm fungicides *viz*., carbendazim 12% + mancozeb 63% WP, tebuconazole 25% EC, azoxystrobin 11% + tebuconazole 18.3% SC and trifloxystrobin 25% + tebuconazole 55% WP completely suppressed the mycelial growth. The contact fungicide copper oxychloride was ineffective even at 100 ppm against the pathogen (Fig.5).

Parvathy and Girija (2016) observed *in vitro* suppression of *C. gloeosporioides* causing anthracnose of black pepper using tebuconazole (0.1 %) and the combination fungicide carbendazim + mancozeb (0.1%) with 100 per cent inhibition and the least inhibition of 2.42 per cent with copper hydroxide (0.25%). Kinjal *et al.* (2016) reported the effectiveness of carbendazim (12%) + mancozeb (63%) at 0.075 per cent and the combination fungicide trifloxystrobin + tebuconazole (0.075%) in managing anthracnose of mung bean under *in vitro* conditions. Similarly Ann and Mercer (2017) reported the efficacy of tebuconazole and trifloxystrobin against *C. gloeosporioides* infestation in black pepper under *in vitro* and *in vivo*. Trifloxystrobin 25% + tebuconazole 55% WP at 0.4 g/L completely inhibited the mycelial growth under *in vitro* condition. Dev and Narendrappa (2016) conducted *in vitro* evaluation of fungicides against *C. gloeosporioides* causing anthracnose of pomegranate. They also pointed out cent per cent inhibition of the fungus with the combination fungicide trifloxystrobin 25% + tebuconazole 55% WP even at 100 ppm. Similarly, tebuconazole also showed cent per cent mycelial inhibition at 500 ppm.

Kumar *et al*. (2007) collected six isolates of *C. gloeosporioide*s causing mango anthracnose and designated as Cg1 to Cg7. They studied fungicidal resistance / sensitivity of *C. gloeosporioides* using four systemic fungicides *viz*., carbendazim, thiophanate- methyl, propiconazole and hexaconazole. The results indicated that all isolates were resistant to copper oxychloride even at 1000 ppm.

The resistance development may be attributed to continuous and indiscriminate use of the same fungicide without rotation or alternating with other fungicides. High levels of resistance in most of the fungal species were attributed to amino acid substitution in -tubulin gene (Buhr and Dickman, 1993).

Adikshita (2017) reported *in vitro* efficacy of combination fungicides trifloxystrobin 25% + tebuconazole 55% WP in suppressing mycelial growth of *C. gloeosporioides* causing anthracnose in mango and it recorded 100 per cent mycelial growth inhibition even at 50 ppm concentration. Similarly Sharma *et al*. (2019) also reported the efficacy of combination fungicides *viz*., trifloxystrobin + tebuconazole, azoxystrobin + tebuconazole in complete mycelial inhibition of *C. gloeosporioides* even at 50 ppm.

The results from the *in vitro* experiment suggested that use of fungicides with different mode of action could help in the successful management of the disease in the field and also to prevent development of cross – resistance in the pathogen instead of using fungicides with same mode of action.

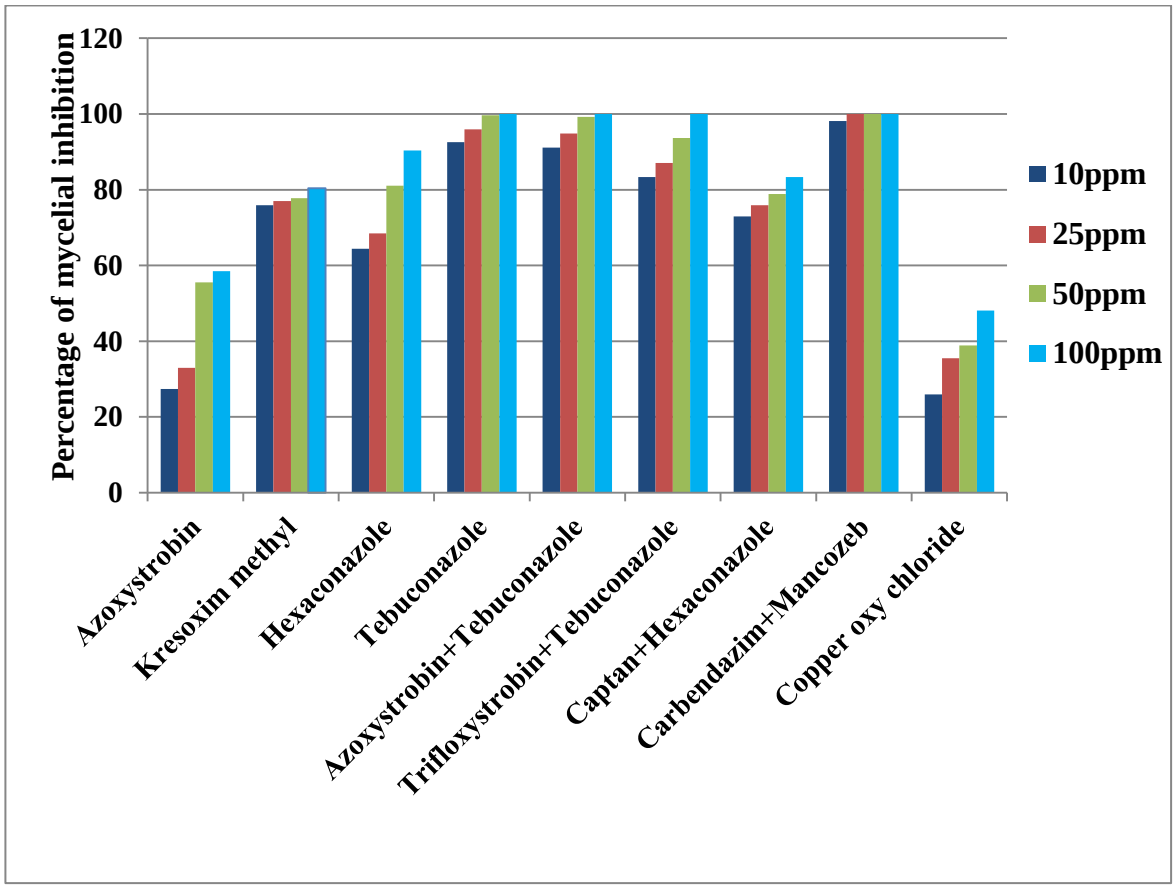


Fig. 5: Mycelial growth inhibition of *C. gloeosporioides* at different concentration of fungicides

Summary

6. SUMMARY

Black pepper is one of the most precious spice crops in the world. Incidence of pest and diseases has become a serious threat to black pepper cultivation. Anthracnose, an important fungal disease of black pepper infects leaves, stem, spikes and berries; and causes drastic reduction in yield due to severe spike shedding and formation of 'pollu' berries. The present research work entitled **"**Varietal screening and management of anthracnose of black pepper using new generation fungicides." was conducted at Department of Plant Pathology, College of Agriculture, Vellayani during 2018 - 2020 with the objective to screen KAU varieties and most popular local cultivar Karimunda for resistance against anthracnose of black pepper caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and evolve management strategy using new generation fungicides.

A survey was carried out in two locations in Thiruvananthapuram district (Kowdiar and Vellayani), two locations in Wayanad district (Ambalavayal and Meenangadi) and four locations in Idukki district (Myladumpara, Pampadumpara, Kattappana and Kambalikandam). The higher disease index (50.28 %) and incidence (60.0 %) were recorded from the Myladumpara of Idukki district. The weather parameters *viz.*, low temperature, high relative humidity and heavy rainfall favoured the incidence of anthracnose. Symptom variation was noticed at surveyed locations *viz*., necrotic spots with a yellow halo, leaf blight and spike infection. Anthracnose was observed both in nursery as well as in the main field. Symptoms initially appeared as circular necrotic spots surrounded by a yellow halo, mostly confined to the leaf lamina. Several lesions coalesce together resulted in leaf blight, drying of the margin from tip and finally led to defoliation. Apart from the typical symptom, pin head sized acervuli were also seen at the centre of blighted areas. At high ranges of Idukki district along with the typical anthracnose symptoms such as necrotic spots on the foliage surrounded by yellow halo and leaf blight, spike infection leading to hollow (pollu) berries were also observed. *Colletotrichum* cultures were isolated from the diseased samples and eight pure cultures of *Colletotrichum* sp. (C1 to C8) from different locations were obtained. The pathogenicity of the eight isolates of *Colletotrichum* sp. were proved by Koch postulates.

The morphological characters of the eight different isolates were studied in potato dextrose agar (PDA) medium. The mycelia of *Colletotrichum* sp. appeared whitish with yellowish orange centre, light pink, off white to greyish in the front view while it appeared as whitish with orange centre to light pink, greyish green colony on the rear side. The mycelial growth was fluffy, cottony or sparse with regular margins. Pinkish colouration may be attributed to the spore production. Days taken to grow the entire Petri dish ranged from 7.25 to 9.75 days. The mycelium of the fungus was hyaline and septate, and its width ranged from 2.21 - 3.45 µm. The septal distance of the different *Colletotrichum* isolates ranged between 8.50 - 21.23 µm. The conidia were single celled with an oil globule at the centre. The conidial shape was either cylindrical, oblong or dumbbell. The conidial and appressorial size varied from 9.4 - 12.1 µm x 3.6 - 4.6 µm and 8.5 - 11.2 µm x 3.5 - 4.3 µm respectively. The morphological and microscopic characters of the different isolates revealed that the pathogen isolated was *C. gloeosporioides*.

The pathogenic variability of the eight *C. gloeosporioides* isolates were assessed on three black pepper varieties *viz.*, Panniyur 1, Panniyur 3 and Karimunda by virulence rating. The isolate C7 was identified as the most virulent isolate which produced lesion size of 1.92 cm, 2.40 cm, and 3.22 cm on Panniyur 1, Panniyur 3 and Karimunda respectively at 5 days after inoculation (DAI). The isolate C7 produced symptoms within two days after inoculation in the varieties tested with a higher rate of lesion development of 0.40 (Panniyur 1), 0.49 (Panniyur 3) and 0.66 (Karimunda) cm day^{-1}.

Cultivation of tolerant varieties of black pepper could be the most economical, easy and reliable method to minimize crop loss due to anthracnose. In this study, KAU varieties (Panniyur 1 to 8) and local cultivar Karimunda were screened against the virulent isolate of *C. gloeosporioides* (C7). Among the varieties screened, Panniyur 4 was found to be highly susceptible with highest PDI of 51.43 (7 DAI); whereas Panniyur 2 had the lowest PDI of 14.28 (7 DAI) followed by Panniyur 8 with PDI 20.00 (7 DAI). Panniyur 2 and Panniyur 8 were categorized as resistant, whereas Panniyur 1, Panniyur 3, Panniyur 5, Panniyur 6, Panniyur 7 and Karimunda as susceptible and Panniyur 4 as highly susceptible. Hence, Panniyur 2 and Panniyur 8

could be cultivated in anthracnose prone areas after field evaluation. The pathogen produced symptoms in susceptible varieties within 2 DAI, whereas, the tolerant varieties took 3 - 4 days to initiate the infection.

New generation fungicides are gaining attention owing to its target specific action, systemic nature, curativeness and less quantity in disease control. *In vitro* evaluation was carried out to study the effect of nine fungicides *viz*., azoxystrobin 23 SC, kresoxim methyl 44.3 SC, hexaconazole 5 EC, tebuconazole 25EC, azoxystrobin 11% + tebuconazole 18.3% SC, trifloxystrobin 25% + tebuconazole 55% WP, captan 70% + hexaconazole 5% WP, carbendazim 12% + mancozeb 63% WP, copper oxy chloride 50% WP on the mycelial growth of *C. gloeosporioides* (C7) by poisoned food technique at four different concentration *viz.*, 10, 25, 50 and 100 ppm. Screening of fungicides revealed that the combination fungicide carbendazim 12% + mancozeb 63 % completely inhibited the mycelial growth even at 10 ppm. The combination fungicides azoxystrobin 11% + tebuconazole 18.3% SC at 25 ppm and trifloxystrobin 25% + tebuconazole 55% WP at 50 ppm were also effective against the pathogen. Kresoxim methyl of strobilurin and tebuconazole of triazole were effective in inhibiting mycelial growth of *C. gloeosporioides* (80.37 and cent per cent respectively at 100 ppm).The contact fungicide copper oxychloride was ineffective even at 100 ppm against the pathogen. Thus, the combination fungicides even at 10 to 25 ppm were most effective against *C. gloeosporioides* causing anthracnose of black pepper.

The present study revealed the use of tolerant varieties along with need based application of new generation fungicides to keep the destructive disease under control. The future line of work should include screening of more black pepper varieties under field condition to assess their reaction to anthracnose, elucidation of the factors governing resistance to the disease and the efficacy of new generation fungicides under field condition.

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Appendices

APPENDIX – I

COMPOSITION OF MEDIA USED

Potato Dextrose Agar (PDA) Medium

Potato	: 200 g
Dextrose	: 20 g
Agar	: 20 g
Distilled water	: 1 L

APPENDIX – II

COMPOSITION OF STAIN USED

Lactophenol Cotton Blue

Anhydrous lactophenol : 67 ml

Distilled water : 20 ml

Cotton blue : 0.1 g

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

Varietal screening and management of anthracnose of black pepper using new generation fungicides

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Abstract of the thesis
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ABSTRACT

The study entitled "Varietal screening and management of anthracnose of black pepper using new generation fungicides" was conducted at Department of Plant Pathology, College of Agriculture, Vellayani during 2018 - 2020 with the objective to screen KAU varieties and most popular local cultivar Karimunda for resistance against anthracnose of black pepper caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and evolve management strategy using new generation fungicides.

As a part of the study, diseased samples were collected from three black pepper growing tracts of Kerala *viz*., Thiruvananthapuram, Wayanad and Idukki. Sample collections were made from two locations from Thiruvananthapuram (Kowdiar and Vellayani), Wayanad (Meenangadi and Ambalavayal) and Idukki (Myladumpara, Pampadumpara, Kattapana and Kambilikandam). Disease incidence and severity were assessed from the surveyed locations. The highest percentage disease index was observed in Myladumpara (50.28%) followed by Kattapana (48.62%). Weather parameter *viz.*, temperature, relative humidity and rainfall were recorded during the survey period. The weather parameters *viz.*, low temperature, high relative humidity and heavy rainfall favoured the incidence of anthracnose. The symptoms of the anthracnose appeared as small necrotic spots with a yellow halo on the leaf lamina. Several lesions coalesce together resulted in leaf blight and defoliation. In Pampadumpara, spike infection was also observed along with leaf spot. *Colletotrichum* cultures were isolated from the diseased sample by tissue isolation technique and eight pure cultures of *Colletotrichum* sp. (C1 to C8) were obtained. The pathogenicity of the eight isolates of *Colletotrichum* sp. from different locations were proved by Koch postulates.

The morphological characters of the eight different isolates were studied in potato dextrose agar (PDA) medium. The isolated cultures of *Colletotrichum* sp. produced whitish with yellowish orange centre to light pink, off white to greyish coloured colony having fluffy, cottony to sparse mycelial growth with regular margins. Days taken to grow the entire petridish ranged from 7.25 to 9.75 days. The mycelium of the fungus was hyaline and septate, and its width ranged from 2.21 - 3.45 µm. The septal distance of the different Colletotrichum isolates ranged between 8.50 -

21.23 µm. The conidia were single celled with an oil globule at the centre. The conidial shape was either cylindrical, oblong or dumbbell. The conidial and appressorial size varied from 9.4 - 12.1 µm x 3.6 - 4.6 µm and 8.5 – 11.2 µm x 3.5 – 4.3 µm respectively. The isolates were identified as *Colletotrichum gloeosporioides.*

The pathogenic variability of the eight *C. gloeosporioides* isolates were assessed on three black pepper varieties *viz.*, Panniyur 1, Panniyur 3 and Karimunda by virulence rating. The isolate C7 was identified as the most virulent isolate which produced lesion size of 1.92 cm, 2.40 cm, and 3.22 cm on Panniyur 1, Panniyur 3 and Karimunda respectively at 5 days after inoculation (DAI). The isolate C7 produced symptoms within two days after artificial inoculation in the three varieties tested with a higher rate of lesion development of 0.40 (Panniyur 1), 0.49 (Panniyur 3) and 0.66 (Karimunda) cm day^{-1}.

KAU varieties (Panniyur 1 to 8) and local cultivar Karimunda were screened against the most virulent isolate of *C. gloeosporioides*. Among the varieties screened, Panniyur 4 was found to be highly susceptible with highest PDI of 51.43 (7 DAI), whereas Panniyur 2 had the lowest PDI of 14.28 (7DAI) followed by Panniyur 8 with PDI 20.00 % (7DAI) and were found to be tolerant to anthracnose infection. Panniyur 1, Panniyur 7 and Panniyur 5 were also found to be moderately susceptible. The pathogen produced symptoms in susceptible varieties within 2 DAI, whereas the tolerant varieties took 3-4 days to initiate the infection.

In vitro screening of new generation fungicides revealed that kresoxim methyl of strobilurin and tebuconazole of triazole were the most effective in inhibiting mycelial growth of *C. gloeosporioides* (80.37% and cent percent respectively). The combination fungicide carbendazim 12% + mancozeb 63 % completely inhibited the mycelial growth at 25, 50 and 100 ppm. The combination fungicides azoxystrobin 11% + tebuconazole 18.3% SC and trifloxystrobin 25% + tebuconazole 55% WP were also effective against the pathogen at 100 ppm. The contact fungicide copper oxychloride was ineffective against the pathogen @ 10, 25, 50 and 100 ppm.

The present study revealed the use of tolerant varieties along with need based application of new generation fungicides to keep the destructive disease under control. The future line of work should include screening of more black pepper varieties under

field condition to assess their reaction to anthracnose, elucidation of the factors governing resistance to the disease and the efficacy of new generation fungicides under field condition.

സംഗ്രഹം

"കുരുമുളകിലെ പൊള്ളുരോഗത്തിനെതിരെ വിവിധ ഇനങ്ങളുടെ രോഗപ്രതിരോധ ശേഷിയും നൂതന കുമിൾനാശിനികൾ ഉപയോഗിച്ചുള്ള നിയന്ത്രണമാർഗങ്ങളും" എന്ന വിഷയത്തിൽ വെള്ളായണി കാർഷിക കോളേജിലെ സസ്യരോഗ വിഭാഗത്തിൽ 2018 - 2020 കാലയളവിൽ നടത്തിയ ഗവേഷണത്തിന്റെ ഫലങ്ങൾ സംക്ഷി-പ്ത രൂപത്തിൽ ചേർക്കുന്നു .

പഠനത്തിൻറെ ഭാഗമായി പൊള്ളുരോഗത്തിൻറെ വ്യാപനവും തീവ്രതയും വിലയിരുത്താൻ കുരുമുളക് കൃഷി ചെയ്യുന്ന തിരുവനന്തപുരം, വയനാട്, ഇടുക്കി ജില്ലകളിൽ സർവേ നടത്തുകയുണ്ടായി. അതിനായി തിരുവനന്തപുരം (വെള്ളായണി, കവടിയാർ), വയനാട് (മീനങ്ങാടി, അമ്പലവയൽ) , ഇടുക്കി (മയിലാടുംപാറ , പാമ്പാടുംപാറ , കട്ടപ്പന, കമ്പളികണ്ടം) എന്നീ സ്ഥലങ്ങൾ സന്ദർശിച്ചു. പൊള്ളുരോഗലക്ഷണങ്ങളുള്ള ഇലകൾ ശേഖരിക്കുകയും അവയുടെ രോഗവ്യാപനവും തീവ്രതയും വിലയിരുത്തുകയുണ്ടായി. ഏറ്റവും കൂടുതൽ രോഗതീവ്രത ഇടുക്കിയിലെ മയിലാടുംപാറയിലും (50.28 %) കട്ടപ്പനയിലും (48.62 %) ഉള്ളതായി കണ്ടെത്തി. രോഗത്തിന് അനുയോജ്യമായ കുറഞ്ഞ അന്തരീക്ഷ ഊഷ്മാവും കൂടിയ ആർദ്രതയും ഇടുക്കി ജില്ലയിൽ രോഗതീവ്രത കൂട്ടുന്നതായി കണ്ടെത്തി. രോഗഹേതുവായ *കൊളറ്റോട്രിക്കം സ്പീഷിസ്* കുരുമുളകുവള്ളികളിൽ വൈവിധ്യമാർന്ന രോഗലക്ഷങ്ങൾ പ്രകടിപ്പിച്ചു. ഇല പൊട്ടുകളിൽ തുടങ്ങി ഇലകരിച്ചിലിലേക്കും തുടർന്ന് തിരിപൊഴിച്ചിലിലേക്കും രോഗം വ്യാപിക്കുന്നതായി കണ്ടെത്തി.

രോഗംബാധിച്ച കുരുമുളകുവള്ളികളിൽ നിന്നും രോഗഹേതുവായ *കൊളറ്റോട്രിക്കം ഗ്ലിയോസ്പോറിയോയിഡ്സ്* കുമിളിൻറെ 8 ഇനങ്ങളെ (ഐസൊലേറ്റ്സ്) വേർതിരിച്ചെടുത്തു പഠനങ്ങൾ നടത്തി. കുമിളിന്റെ 8 ഇനങ്ങളുടെ രോഗതീവ്രത ശേഷി പന്നിയൂർ ഇനങ്ങളിലും കരിമുണ്ടയിലും നടത്തി. ഏറ്റവും രോഗതീവ്രതശേഷിയുള്ള ഇനം സി 7 ആണെന്ന് കണ്ടെത്തി. സി 7 ഉപയോഗിച്ച് കുരുമുളക് ഇനങ്ങളിൽ ആന്ത്രാക് നോസിനെതിരെയുള്ള പ്രതിരോധശേഷിയെ നിർണയിച്ചു. ഇതിനായി കാർഷിക സർവകലാശാല പുറത്തിറക്കിയ പന്നിയൂർ ഇനങ്ങളും (പന്നിയൂർ 1 മുതൽ പന്നിയൂർ 8) കരിമുണ്ടയിലും പഠനം നടത്തി പന്നിയൂർ ഇനങ്ങളിൽ പന്നിയൂർ 4 ന് രോഗപ്രതിരോധ ശേഷി കുറവും, പന്നിയൂർ 2 നും പന്നിയൂർ 8 നും രോഗപ്രതിരോധശേഷി ഉള്ളതായും കണ്ടെത്തി. ഈ കുമിൾ, പ്രതിരോധശേഷി കുറഞ്ഞ ഇനങ്ങളിൽ 2 ദിവസത്തിനുള്ളിൽ രോഗലക്ഷണങ്ങൾ പ്രകടിപ്പിച്ചു തുടങ്ങി.

*കൊളറ്റോട്രിക്കം ഗ്ലിയോസ്പോറിയോയിഡ്സ്*നെതിരെ നൂതന കുമിൾനാശിനികളുടെ പ്രവർത്തനശേഷിയെകുറിച്ച് പരീക്ഷണശാലയിൽ പഠനം നടത്തി. പഠനത്തിന് വിധേയമാക്കിയ സ്പർശകുമിൾനാശിനി, അന്തർവ്യാപനശേഷിയുള്ള കുമിൾനാശിനി, ഇവയുടെ കോമ്പിനേഷൻ കുമിൾനാശിനി എന്നിവയിൽ കോമ്പിനേഷൻകുമിൾനാശിനിയായ കാർബണ്ടാസിം+ മാങ്കോസബ്, അസോക്സിസ്ട്രോബിൻ+ടെബുകോണസോൾ എന്നിവ 100പിപിഎം അളവിൽ ഈ കുമിളിനെ നിയന്ത്രിക്കുന്നതായി കണ്ടെത്തി.

പ്രസ്തുത പഠനത്തിൽനിന്ന് രോഗപ്രതിരോധശേഷിയുള്ള ഇനങ്ങൾ കൃഷിചെയ്യും കോമ്പിനേഷൻകുമിൾനാശിനികളായ കാർബണ്ടാസിം + മാങ്കോസബ്, അസോക്സിസ്ട്രോബിൻ+

ടെബുകോണസോൾ എന്നിവ യഥാക്രമം ഉപയോഗിച്ചും ഈ കുമിളിനെ നിയന്ത്രികാം.