STUDIES ON ANTHRACNOSE OF COWPEA CAUSED BY *Colletotrichum lindemuthianum* (Sacc. & Magnus) Brivosi & Cavara

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B.Sc. (Agriculture)

MASTER OF SCIENCE

IN

AGRICULTURE

(PLANT PATHOLOGY)

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE, PARBHANI

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PARBHANI – 431 402 (M.S.) INDIA

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BY

LOKHANDE AJIT DILIP

B.Sc. (Agriculture)

A thesis submitted to

Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani

in partial fulfillment of the

requirements for the Degree of

MASTER OF SCIENCE

IN

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DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled, **"STUDIES ON ANTHRACNOSE OF COWPEA CAUSED BY *Colletotrichum lindemuthianum* (Sacc. & Magnus) Brivosi & Cavara"**, submitted by me is based on the actual work carried out by me under the guidance and supervision of **Mahesh Sakharampant Dadke**. The extent of information derived from the existing literature have been duly cited and referenced. The existing research work or its any part is not submitted anywhere else for the award of any degree or diploma.

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Place: Parbhani (Lokhande Ajit Dilip)

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ABBREVIATIONS

%	=	Per cent
/	=	Per
@	=	At the rate of
a.i	=	Active ingredient
µ	=	Microns
C.D.	=	Critical Difference
Cm	=	Centimeter (s)
CRD	=	Completely Randomized Design
cv.	=	Cultivars
DAS	=	Days After Sowing
Dia.	=	Diameter
e.g.	=	Exempli gratia (for Example)
et al.	=	and others
etc.	=	Etcetera
Fig.	=	Figure (s)
G	=	Gram
hrs.	=	Hours
i.e.	=	That is
Kg	=	Kilogram (s)
L	=	Litre
LF	=	Laminar Flow Chamber
In vitro	=	Laboratory condition
M	=	Meter (s)
ml	=	Millilitre
mm	=	Millimetre
No.	=	Number (s)
^{o}C	=	Degree Celsius
PDA	=	Potato Dextrose Agar

psi	=	Pounds per square inch
SEm	=	Standard error of mean
sp.	=	Species
Sr.	=	Serial
T	=	Treatment
viz.,	=	Videlicet (namely)
WG	=	Water Dispersible Granule
WP	=	Wettable Powder
WS	=	Water Soluble
v/v	=	Volume/ volume
w/v	=	Weight/volume
w/w	=	Weight/weight
EC	=	Emulsifiable Concentrate
WG	=	Wettable Granules
WP	=	Wettable Powder
ppm	=	Parts per million
ha	=	hactare
MT	=	Megatonnes
µg	=	Microgram
lbs	=	Pound per Square inch

THESIS ABSTRACT

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1. Title of the thesis	:	Studies on Anthracnose of Cowpea Caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Brivosi & Cavara
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ABSTRACT

Cowpea [*vigna unguiculata* (L.)] also known as lobia, chowla, southern pea or black-eyed pea belong to the family leguminaceae. The anthracnose caused by *Colletotrichum lindemuthianum* is an important fungal disease of cowpea causing economic losses hence the present studies were carried out with the objectives like isolation and proving pathogenicity, study the efficacy of botanicals, bioagents and fungicides against *C. lindemuthianum* and screen the available cowpea cultivars against the anthracnose disease.

The fungus was isolated on PDA and the pathogenicity was proved in screen house by inoculating spore culture on local variety of cowpea. Extracts of seven locally available plants is prepared in water as well as in ethanol and evaluated at 10 and 20 % concentration. Garlic and Ginger extracts showed cent per cent mycelial inhibition followed by Turmeric. The seven bioagents evaluated by dual culture

technique showed that *Trichoderma harzianum* showed highest mycelial inhibition followed by *T. asperellum* and *T. virens.* Seven systemic fungicides were evaluated by poisoned food technique at 500,1000 and 1500 ppm and four combi products and three contact fungicides were evaluated at 1000,2000 and 2500 ppm against *C. lindemuthianum.* Among the systemic fungicides Propiconazole, Tebuconazole and Carbendazim showed cent per cent mycelial inhibition followed by Difenoconazole, Hexaconazole and Azoxystrobin. Among the combi products and non-systemic fungicides, Captan + Hexaconazole and Metalaxyl + Mancozeb showed cent per cent mycelial inhibition followed by Carbendazim + Mancozeb, Chlorothalonil and Copper oxychloride. Twelve cowpea varieties were screened in screen house by sowing in pots and artificial inoculation. Eight varieties showed absolute resistant reaction and four varieties (Maharani, Ankur VU-5, Ardhaveli and Cowpea Safal-311) showed moderately resistant reaction.

Key words: Anthracnose, Cowpea, *Colletotrichum*, Bioagents, Fungicides.

CHAPTER - I

INTRODUCTION

CHAPTER - I

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.], is an important multi utility crop locally known as lobiya, chowla (chowli), southern pea or black eye pea, that is adopted to warm condition and cultivated in the tropics and sub-tropics for dry grains, green edible pods for vegetable as well as fodder (Gupta *et al*., 2017a).

Cowpea, *Vigna unguiculata*, is cultivated around the world primarily for its seeds but also as a vegetable, a cover crop and for fodder (Claudius-Cole *et al*., 2014). The seeds are important in diets for the provision of protein to rural as well as the urban dwellers as a substitute for the animal protein. (Wakili, 2013) The crop's haulms are also a valuable source of livestock protein. (Owolade *et al*., 2006). Especially for the lower-income group population, the cowpea is a better alternative source of protein, minerals, ash, etc., as compared to other legumes. Dry cowpea seed is rich in protein (23-33%), carbohydrates (56-68%) and folic acid. In humid forests of South-western Nigeria, the cakes made from crushed and fried cowpea seeds are very popular and sold as convenience street food in roadsides (Ogu and Owoeye, 2013).

In India, during 2019-2020 area under beans was 221 thousand ha. with annual production of 2226 thousand MT (Anon., 2021). During 2017-18, the area and production of beans in Maharashtra was 5.50 thousand ha. with annual production of 55.48 thousand MT (Anon., 2018). Cowpea is an important staple food for millions of people in the arid and semi-arid tropics (Asiwe, 2009).

Anthracnose is one of the devastating worldwide fungal diseases that affect the above ground parts. This disease is induced by hemi biotroph deuteromycetous (fungi imperfecti) fungus called *Colletotrichum* (seed borne and soil borne) (Maya and Seal, 2015). The fungus *Colletotrichum lindemuthianum* is the most destructive disease of cowpea. Field-type cowpeas show various levels of resistance, whereas pole-type vegetable cowpeas are highly susceptible (Pradhan *et al*., 2017).

The term Anthracnose' literally means 'like coal' and was first used by Fabre and Dunal to describe a disease of grapes in which blackening of tissue was a characteristic feature. The fungus overwinters in the previous crop debris, and can also be seed-borne as dormant mycelia within the seed coat or as spores between the cotyledons; from where it initiates infection of hypocotyls and young leaves in the field. The management of plant diseases generally include strategies such as physical and cultural control, resistant cultivars, chemical and biological control. The integration of different management practices has the potential to provide an effective strategy for the control of Cowpea anthracnose. Some bio-control agents have been reported as a promising disease management tool. (Modi and Tiwari 2020).

Colletotrichum species are reported to cause anthracnose in more than 121 plant genera from 45 different families, including gymnosperms, angiosperms, ornamentals, fruit plants, vegetables, field crops or even grasses (Farr *et al*., 2016). Most crops grown throughout the world are susceptible to one or multiple species of *Colletotrichum* (Weir *et al*., 2012). Many species of *Colletotrichum* may be found on a single host or single *Colletotrichum* species may be able to infect different hosts (Freeman *et al*., 2013). Out of 900 *Colletotrichum* species, over 100 species cause anthracnose disease (Cannon *et al*., 2012).

Considering these issues, present investigations was be undertaken on cowpea anthracnose with following objectives:

OBJECTIVES:

1. To isolate and prove the pathogenicity of *Colletotrichum lindemuthianum.*
2. To study the efficacy of botanicals, bioagents and fungicides against

   C. lindemuthianum.
3. To screen the available cowpea cultivars against anthracnose disease (Pot Culture).

CHAPTER - II
REVIEW OF LITERATURE

CHAPTER - II

REVIEW OF LITERATURE

The literature available on *Colletotrichum* in general and *C. lindemuthianum* causing anthracnose of cowpea in particular in respect of its occurrence distribution, symptomatology, isolation, pathogenicity and the disease management strategies has been reviewed here under in the following paragraphs.

2.1 Occurrence and distribution

Anthracnose of cowpea is caused by the fungus *Colletotrichum lindemuthianum*. The fungus *C. lindemuthianum* was discovered by Lindemuth in 1875 (Tiffany and Gilman, 1954) and first described by Saccardo, (1878).

Pastor-Corrales *et al.* (1994) reported that the anthracnose, was caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams. -Scrib. is the most destructive disease of common bean (*Phaseolus vulgaris* L.) in the tropics and subtropics. Although widely distributed, anthracnose causes particularly severe economic losses in cool and humid areas such as the highlands of Latin America and Central and Eastern Africa.

Waller and Bridge (2000) reported that *Colletotrichum* is well represented in the warm moist environment of the humid and sub-humid tropics where they occur as saprobes, endophytes or pathogens on leaves, stems, flowers and sometimes fruits of both field and perennial susceptible crops.

Mohammed (2013) concluded that the anthracnose was first described from plant specimens obtained in Germany in 1875. Since then, the disease has become one of the most important and widely distributed throughout the world. It has been reported in USA, European countries, Canada, Latin America. In Brazil, more than 25 different *C. lindemuthianum* races have been identified.

Fitsum *et al.* (2014a) reported that the bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara is one of the most devastating seed borne diseases of common bean (*Phaseolus vulgaris* L.) in Ethiopia.

Sharma *et al.* (2014) reported that the disease of bean anthracnose caused by *C. lindemuthianum* was more prevalent in hilly regions that experiences frequent rains and cold climate during the *Kharif* season.

Chavan and Dhutraj (2017) reported that the anthracnose and pod blight of soybean was of common occurrence and distributed throughout all eight districts of the Marathwada region.

Badgujar *et al.* (2019) reported that the disease of chilli anthracnose was found to be predominant in all the eight districts of Marathwada region.

Bhatt *et al.* (2021) reported that the anthracnose disease of soybean is distributed throughout eleven districts out of twelve districts surveyed of Uttarakhand.

2.2 Yield losses:

The major fungal infection of bean anthracnose caused by *Colletotrichum lindemuthianum*. Infection of a susceptible cultivar in favourable conditions leading to an epidemic may result in 100% yield loss. (Fernandez *et al.*, 2000).

Conner *et al*. (2004) reported that, in the bean anthracnose plot, the seed born infection by *C*. *lindemuthianum* resulted in 15.2% to 32.1% yield reduction.

Sharma *et al.* (2007) reported that due to the bean anthracnose caused by *Colletotrichum lindemuthianum,* yield loss under cool and humid environments was up to 80-100%.

Sharma *et al.* (2008) reported that, the seed borne infection of common bean anthracnose caused more yield losses than background contamination. The pod infection had direct effect on seed yield as severity on pods ranging from 3-9 (0-9 scale) caused 10.52-57.76 per cent reduction in number of seeds per pod and 21.93-68.77 per cent reduction in seed weight per pod.

Bean crop suffers due to a number of fungal and bacterial diseases. Among these the bean anthracnose caused by *Colletotrichum lindemuthianum* is considered as an important disease for causing huge yield loss year after year. The mean incidence

and intensity of anthracnose in Kashmir during 2011 cropping season varied from 28.47-40.18 per cent and 10.69-16.42 per cent, respectively. (Junaid *et al.*, 2014).

Enyiukwu *et al.* (2014) reported 50% yield reduction in anthracnose affected cowpea plant.

Peeran *et al*. (2014) reported that, the anthracnose has caused serious reductions in the yield of beans in many parts of the world, resulting in yield losses as high as 95%.

Chavan and Dhutraj (2017) reported that the anthracnose and pod blight caused by (*C. truncatum* (Schw.) Andrus and Moore) is an important disease of soybean, causing yield losses up to 16-100 per cent.

Kulkarni and Raja (2019a) reported that the average grain yield loss of 39.98 per cent and stalk yield loss of 47.19 per cent were noticed due to anthracnose of green gram.

2.3 Symptomatology:

Adegbite and Amusa (2010) reported the symptoms of cowpea anthracnose. The disease includes purplish brown discolouration on pods, which may also extend to petioles, leaf veins and peduncles.

Mohammad (2013) described the symptoms of bean anthracnose caused by *C. lindemuthianum*. He reported that the anthracnose lesions are most common on leaf petiole and on the lower surfaces of leaves and leaf veins. Although infection may occur on both sides of the leaf and on the petiole, early signs of infection usually appear on the lower leaf surface along the veins, which show brick red to purplish red discoloration. Later, such discoloration also appears on the upper leaf surface. At the same time, brown lesions of various sizes, with black, brown, or purplish red margins, develop around small veins, eventually conidiophores rupture through the host cuticle and form acervuli on the plant surface.

Cowpea anthracnose characterized by sunken, black lesion is one of the major fungal diseases of cowpea which constrain its economic production. In affected cowpea plant up to 50% yield reduction occurs. (Enyiukwu *et al.,* 2014).

Falade (2016) described the symptom of cowpea anthracnose. The symptoms appear on leaves as small, dark brown to black lesions. The infected tissues show minute rust- coloured specks. The specks gradually enlarge and forms sunken lesion or eye spot.

Saxena *et al*. (2016) reported the symptoms of chilli anthracnose. The disease includes lesions on stems and leaves. Symptoms first appear as small sunken grayish brown spots with dark margins, further on which development of acervuli in concentric rings could be easily seen.

Gupta *et al*. (2017a) described the symptoms of chilli anthracnose caused by *C. capsici*. They reported that, the typical fruit symptoms are circular or angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses. Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scattered or in concentric rings on the lesions.

The anthracnose disease affects all the growth stages of the cowpea plant, but more often in the reproductive stage. Its symptoms include round brownish or purple specks which become darker and enlarge into lesions of about 2 cm in diameter. The symptoms are most visible on leaves and ripe fruits, but the disease also produce cankers on petioles and stems, thereby causing defoliation and rotting of the fruits. Infected fruits have water-soaked and sunken circular spots. (Falade *et al.*, 2018b).

Agam *et al*. (2019) described the symptoms of soybean anthracnose caused by *C. truncatum*. They reported that, the most prominent symptoms occurred on foliage were brown coloured patches with grey coloured centre on upper surface and scorched appearance on the lower surface. In advance stage necrosis of leaf vein, leaf rolling, petiole canker, and defoliation occurred. Typical symptoms observed on the pods were reddish brown spots which later turns black. Acervuli on infected pods resembled small pinkish coloured patches surrounded by the minute blackish brown setae. Infected pods finally dried out prematurely with shriveled and moldy seeds.

Conner *et al*. (2019) reported the symptoms of bean anthracnose. The Disease symptoms can appear on all above ground bean tissues. Typically, symptoms on leaves occur as brown to black lesions that run along the veins. Brown, sunken, ovoid

lesions form on the stems and pods. Pod infection usually results in discoloration, and sometimes shriveling of the seed.

Ahmad *et al*. (2020) reported the symptoms of mung bean anthracnose caused by *C. lindemuthianum*. They reported that the spots appeared first on the lower surface of leaf later on they appeared on leaf petiole, stem and also on pods. Circular, black, sunken spots with dark centre and bright red orange margins on leaves and pods. In severe infections, the affected parts wither off. Seedlings get blighted due to infection soon after germination of seed.

Kumar *et al*. (2020) reported that the black gram anthracnose disease symptoms are circular, black, sunken spots with a dark centre and bright red-orange margins on leaves.

Bhatt *et al*. (2021) described the symptoms of soybean anthracnose. The symptoms were characterized by irregular brown necrotic lesions and in later stages, the middle necrotic portion withered away and giving a shot hole type of symptoms. The symptoms on petiole were also evident in the form of sunken necrotic spot.

2.4 Isolation, purification, identification and pathogenicity

Adebanjo and Bankole (2003) isolated the *Colletotrichum lindemuthianum* from infected cowpea stems showing anthracnose lesions by plating surface sterilized segments (1% NaOCl for 2 minutes) on to Potato Dextrose Agar (PDA) complemented with 60 µg/ml chloramphenicol as a bacteriostatic.

Lubbe *et al.* (2006) proved the pathogenicity of *Colletotrichum species* by inoculating plant parts, leaves or stems of Protea cultivars which were treated by either wounding or non-wounding. Wounds were made by pricking each leaf with a cork in which five insect mounting needles were inserted. Each plant was inoculated with 10 ml spore suspension of *Colletotrichum species*.

Sangdee *et al.* (2011) isolated *Colletotrichum capsici* using the tissue transplanting technique. The infected leaf samples of chilli were cut (5 x 5 mm) from the margins of infected tissue, surface sterilized by dipping in 1% sodium hypochlorite for 2 min. and rinsed several times with sterile distilled water before

being transferred onto the surface of water agar. The growing mycelium out of the plant tissue was sub-cultured to Potato Dextrose Agar and incubated at room temperature for 7 to 10 days.

Ogu and Owoeye (2013) isolated *Colletotrichum destructivum* from the infected leaves of cowpea. The infected cowpea pods were cut into small pieces and surface sterilized for one minute in 1.0% sodium hypochlorite (NaOCl) solution and rinsed in three changes of sterile distilled water. These pods were dried on sterilized filter paper and then plated on sterile Petri plates (9cm diameter) of Potato Dextrose Agar (PDA) and incubated at ambient temperature 28 ± 2°C for one week. After the emergence of mycelial growth, each of the fungal colonies were repeatedly sub-cultured to fresh Sterile PDA plates to obtain a pure culture of the pathogen.

Atghia *et al.* (2015) proved the pathogenicity test by spraying a conidial suspension (10^6 spores/ml) from seven days old culture of *Colletotrichum lindemuthianum* on leaves and stems of common bean and cowpea seedlings.

Chacko and Gokulapalan (2015) isolated *Colletotrichum capsici* from the infected fruit of chilli. The isolation was done by cutting small pieces from the advancing margin of lesions which were then immersed in 0.1% mercuric chloride for thirty seconds, washed three times in sterile distilled water, and blotted dry before being placed on PDA. The mycelium coming out of the tissues was sub-cultured to another Petri plate incubated at room temperature.

Maya and Seal (2015) isolated *Colletotrichum lindemuthianum* from the infected leaves of cowpea. The plant parts were cut into small bits and surface sterilize with 0.1% mercuric chloride ($HgC1_2$) solution for about one minute and washed repeatedly thrice in sterile distilled water before transferring them to sterile petri plates containing PDA media (5mm) under LF (Laminar Flow Chamber). Excess water was wiped off by sterile tissue paper.

Shivakumar *et al*. (2015) proved the pathogenicity of *Colletotrichum gloeosporioides* on mango by spray inoculation method. After 8 to 10 days of spraying. brown to black coloured sunken lesions were noticed on inoculated mango plants. Later those spots gradually start to increase in size to form brown necrotic areas.

Falade (2016) isolated *Colletotrichum lindemuthianum* from the infected leaves of cowpea. The leaves were cut into pieces of about 1-2cm and surface sterilized by immersing 0.2% NaOCl for two minutes. This was followed by two rinses in sterile distilled water and spraying with 70% isopropanol. The sterilized leaves were kept inside a laminar flow cabinet for 20-30 minutes to dry. Five sterilized leaf cuttings were appressed unto the surface of Potato Dextrose Agar (PDA) (Sigma-Aldrich) containing 0.05% chloramphenicol inside 9 cm Petri dishes and removed. For the isolation of the anthracnose pathogen, three of the surface sterilized leaf cuttings were placed on PDA-modified with chloramphenicol. The plates were sealed with parafilm and incubated separately at ambient temperature for 5-6 days.

Choudhary *et al*. (2017) isolated *Colletotrichum lindemuthianum* from the infected leaves of green gram. The section of 4-5 mm was cut from the margin of the infected lesions and sterilized for one minute in 0.1% mercuric chloride solution and rinsed with several changes of sterile distilled water. The sterile pieces were blotted on sterilized Petri plates containing solidified Potato Dextrose Agar (PDA) in aseptic conditions. The plates were incubated at ambient temperature (30+2°C) for 7 days after inoculation.

Gupta *et al*. (2017b) isolated *Colletotrichum capsici* from the infected leaves of chilli. The small pieces of infected tissue (2-3mm in length) were cut at the junction of diseased and healthy portion with the help of disinfected blade after surface sterilizing the sample with alcohol. These bits were surface sterilized in 0.1 per cent mercuric chloride solution ($HgCl_2$) for 30 seconds followed by three washing with sterilized distilled water in Petri-plates under aseptic conditions using laminar air flow. These bits were air dried by placing on sterilized blotting paper. Five bits were transferred aseptically to the Petri-plates containing sterile Potato Dextrose Agar (PDA) medium.

Birari *et al*. (2018) proved the pathogenicity test of *Colletotrichum capsici.* The inoculation was made by placing a fungal disc (5 mm) taken from a young fungal colony of *C. capsici* on the wound. The fruits inoculated by placing only a potato dextrose agar disc on the wound served as control. The inoculated fruits were kept in

moist chamber at room temperature (25-28 °C) for ten days. The pathogenicity test was closely monitored for symptom development.

Falade *et al.* (2018a) isolated *Colletotrichum lindemuthianum* from the infected leaves of cowpea. The leaves were cut into small pieces and surface sterilized by immersion in 0.2 % NaOCl for two minutes and followed by two rinses in sterile distilled water in a laminar flow cabinet. Three leaf cuttings per plate were placed on Potato Dextrose Agar (PDA). The plates were sealed with parafilm and incubated at 28°C for 5-6 days. Single spore of developing colonies was isolated and sub-cultured to obtain pure cultures.

Ganiyu *et al.* (2018) isolated *Colletotrichum lindemuthianum* from the cowpea leaves which were washed with tap water, surface-sterilized with 0.5% NaOCl, rinsed in three changes of sterile distilled water and air dried at, room temperature. Portion of leaves with fungal infections were cut (1-2 mm^2) at the border of the infection. Three leaf cuttings were placed on Potato Dextrose Agar (PDA) inside Petri plates. The plates were incubated at 28±2°C for 7 days. Single spore of developing colonies was isolated and sub-cultured-for purification. The isolate was stored on PDA slants for further use.

Lokhande *et al*. (2019) isolated *Colletotrichum capsici* from the infected leaves of chilli. The anthracnose infected parts were cut into small pieces by sterilized stainless steel blade and surface sterilized with 0.1% mercuric chloride for one minute followed by three washing with sterilized water. Anthracnose infected pieces were placed in petri plates containing 20 ml of solidified Potato Dextrose Agar (PDA) medium mixed with streptomycin sulphate to avoid bacterial contamination. Plates were kept for incubation at 28±2°C in an incubator. Fungal colonies appeared within 5-7 days, they were sub cultured in PDA slants and purified.

Bagade *et al.* (2020) isolated *Colletotrichum lindemuthianum* from the infected leaves of beans. The infected leaf sample was cut along with healthy leaf and surface sterilized with 0.1% sodium hypochlorite solution for one minute and washing with three times by sterilized distilled water. The bits were placed in petri plates containing PDA medium. All the above operations were carried out in sterilized condition (under laminar air flow unit). Then plates were incubated at 27±2°C for 10

days. The fungal growth, which developed around each bit, was then transferred to PDA medium slant for sub culturing.

Enyiukwu *et al*. (2020) isolated *Colletotrichum destructivum* from the infected pod of cowpea. The pods were cut in bits using surgical blade, sterilized in 70% ethanol, and finally washed in several changes of 200 ml of sterile distilled water. The tissues were plated in Petri dishes containing moistened Whatman No I. filter paper, and incubated for 7 days at 27°C. Then 39.50 g of dehydrated Potato Dextrose Agar (PDA) (Oxoid® Thermo Scientific Product, England, UK) was dissolved in 1000 ml of sterile distilled water in a 2L flask, stirred thoroughly with a glass stirrer, then stoppered and autoclaved at 15 Psi for 30 minutes. The mycelial growth from the plated tissues was sub-cultured repeatedly to obtain pure culture of the organism which was maintained on PDA.

Rao *et al.* (2020b) isolated *Colletotrichum spp*. from Dolichos bean and inoculated to other beans and vice versa for cross infectivity potential of each isolates.

Sushmitha and Zacharia (2021) isolated *Colletotrichum lindemuthianum* from the infected leaves of black gram. The section of 4-5 mm was cut from the margin of the infected lesions and sterilized for one minute in 0.1% mercuric chloride solution and rinsed with several changes of sterile distilled water. The sterile pieces were blotted on sterilized Petri plates containing solidified Potato Dextrose Agar (PDA) in aseptic conditions. The plates were incubated at ambient temperature (30±2°C) for 7 days after inoculation.

2.5 Disease management strategies

2.5.1 *In vitro* efficacy of botanicals

Amadioha (2003) evaluated the alcohol and water extracts of *Piper nigrum*, *Ocimum sanctum* and *Citrus limon* against *Colletotrichum lindemuthianum* in culture and in field experiments by checking the incidence and spread of the disease. He reported that the plant extracts of *Piper nigrum* were the best in reducing the growth of the pathogen in culture and in checking the spread of the anthracnose disease of cowpea in the field.

Shovan *et al.* (2008) studied the effect of plant extracts of Garlic, Ginger, Onion and Neem at three different concentrations (5, 10 and 20%) against the radial growth and mycelial dry weight of *Colletotrichum dematium*. Among the four plant extracts, Garlic extract at 20% concentration was appeared to be best in inhibiting the radial growth and mycelial dry weight of the test pathogen followed by Onion, Ginger and Neem extracts and each extract was significantly different from each other.

Gawade and Suryawanshi (2009) reported that Neem (72.56%) was found highly effective against *C. truncatum* causing soybean anthracnose followed by Parthenium (61.3%), Mehandi (46.03%) and Bougainvillea (28.98%). Leaf extract of Eucalyptus was found least effective in reducing per cent mycelial growth of *C. truncatum* with 9.99% inhibition.

Khan and Nasreen (2010) studied the Methanol extracts of *Lawsonia inermis*, *Withania somnifera*, *Datura metel*, *D. stramonium* and *Bauhinia racemose* against *C. lindemuthianum*. They reported that, among all extracts, *Lawsonia inermis* showed greatest percent inhibition of mycelial growth of *C. lindemuthianum* (76.47-87.77%) followed by *Withania somnifera* (54.44-78.88%).

Marinus Ngullie *et al.* (2010) reported that plant extracts of *Allium sativum* (10%) and *Azadirachta indica* (10%) demonstrated the highest inhibition of mycelial growth of the *Colletotrichum gloeosporioides* causing fruit rot of chilli.

Masangwa *et al.* (2012) reported that the *Carica* leaf water extracts at 5.0 mg/ml performed well and was comparable to Celest XL, by completely inhibiting the growth of *C. lindemuthianum* of common bean and cowpea.

Jagtap *et al.* (2012a and 2012b) tested the nine aqueous leaf extracts (@ 10 and 15%) against *Colletotrichum truncatum* causing anthracnose/ pod blight of soybean and reported that Garlic recorded least mean colony diameter (16.35 mm) and highest mean mycelial growth inhibition (81.82%). This was followed by Tulsi, Onion, Ginger, Neem, Parthenium and Eucalyptus. Mehandi was found least effective and caused minimum inhibition (40.36%) of the test pathogen.

Obi and Vargas (2013) evaluated four botanicals against *Colletotrichum destructivum* causing anthracnose of cowpea *viz*., *Azadirachta indica*, *Cymbopogon*

citratus, *Ocimum gratissimum* and *Xylopia aethiopica.* The extracts of *Xylopia aethiopica* and *Azadirachta indica* more effectively reduced both the growth of the pathogen *in vitro* and the spread of the disease *in vivo*.

Ogu and Owoeye (2013) studied the effect *Cyanthula prostrata* and *Diodia scandens* aqueous leaf extracts for inhibiting the mycelial growth and sporulation of *Colletotrichum destructivum*, causal agent of anthracnose disease of cowpea and showed that aqueous leaf extracts of *C. prostrata* and *D. scandens* at concentrations of 60-100% could significantly inhibit the sporulation and mycelial growth of *C. destructivum* in *in vitro.*

Aggarwal *et al*. (2015) reported that *Azadirachtin* caused maximum inhibition of linear growth (78.07 %) of MVL isolate followed by 73.03 and 69.14 per cent inhibition of linear growth of UDR and FN isolates, respectively of *C. lindemuthianum.* The neem oil was found to be next effective which caused 77.83 per cent inhibition of linear growth of FN isolate followed by 65.16 and 50.78 per cent inhibition of linear growth of MVL and UDR isolates, respectively.

Wagh *et al*. (2015) evaluated *in vitro* eight botanicals aqueous extract (each 5% and 10%) against *Colletotrichum capsici* in *in vitro*. They reported that among them, average inhibition of mycelial growth was recorded with *Allium sativum* (57.96%) followed by *Vetex negunda* (51.85%) and *Ocimum sanctum* (36.39%).

Falade (2016) evaluated the effects of hot water extracts of six indigenous plants *viz*., *Tridax procumbens*, *Jatropha gossypifolia*, *Sida acuta*, *Blighia sapida*, *Ricinus communis* and *Datura stramonium* on the growth of *Colletotrichum lindemuthianum* in the laboratory and subsequent control of the disease on the field. Result from the experiment showed that all the plants extract reduced the growth rate of *C. lindemuthianum*. *D. stramonium* was the most effective followed by *R. communis* and *J. gossypifolia* while *B. sapida* caused the least inhibition of *C. lindemuthianum* growth.

Rana *et al*. (2016) studied the effect of five plant extracts *viz*., Garlic, Onion, *Bhang*, Tulsi and *Aloe vera* at three different concentrations (5, 10 and 20%) against anthracnose of soybean. They reported that, among these, maximum growth inhibition was recorded at 20% concentration in Garlic followed by Onion and *Bhang*. *Aloe vera*

and Tulsi were not found effective at all in checking the mycelial growth of *Colletotrichum dematium var. truncate*.

Badgujar *et al*. (2017) evaluated eight botanicals in *in vitro* (each @10 and 15%) against *C. truncatum* and they reported that the highest average mycelial growth inhibition was recorded with Turmeric (57.16%), followed by Ghaneri (55.51%), Garlic (53.16%), Datura (51.85%), Tulsi (45.87%), Neem (38.71%), NSKE (27.35%) and Eucalyptus (18.27%) respectively.

Choudhary *et al*. (2017) studied the effect of plant extracts of Garlic, Neem, Ginger, Datura and Mehandi against *Colletotrichum lindemuthianum.* Among the treatments Garlic bulb extract was found most effective and recorded significantly highest growth inhibition (80.56%) of *C. lindemuthianum.*

Hingole *et al*. (2017) evaluated plant extracts against anthracnose/ pod blight disease of soybean caused by *Colletotrichum truncatum* and reported that, garlic (*Allium sativum*) recorded 27.85 and 33.61 per cent disease intensity and pod infection, respectively with 30.49 and 19.22 per cent reduction over unsprayed control in disease intensity and pod infection, respectively whereas onion (*A. cepa*) recorded 28.47 and 35.37 per cent disease intensity and pod infection, respectively with 27.81 and 14.22 per cent reduction over unsprayed control in disease intensity and pod infection, respectively.

Ganiyu *et al.* (2018) reported that the extracts of *Azadirachta indica*, *Acalypha wilkisiana* and *Carica papaya*, singly or in combination, reduced the incidence and severity of anthracnose disease of cowpea which translated to increase yield.

Kulkarni and Raja (2019b) tested ten plant extracts against *Colletotrichum truncatum* causing green gram anthracnose in *in vitro* and reported that, Azadirachtin at 10 per cent concentration was found to be best in inhibiting the mycelial growth of *C. truncatum* (63.34%) and found significantly superior over all the other extracts, followed by Eucalyptus oil (60.62%), Garlic (59.44%) and Neem Seed Kernel Extract (56.63%) at 10 per cent. Least inhibition of mycelial growth of *C. truncatum* was recorded in Bellary jali (35.55%) at 5 per cent concentration.

Lokhande *et al*. (2019) reported that the Eucalyptus oil at 5% concentration inhibited the mycelial growth of *C. capsici* by 100%, Neem oil by 61.33%, Garlic bulb extract by 55.12%, Datura leaf extract by 51.22% and Tulsi leaves extract by 46.66%.

Rahaman *et al.* (2019) studied the efficacy of seven plant extracts against *C. capsici* and reported that Ginger extract showed the lowest colony diameter (24.06 mm) and the highest mycelial growth inhibition (72.66 %) at 15 % concentration which was followed by Turmeric, Mahogany, Mehandi, Garlic and Tulsi. Neem extract was least effective and caused minimum growth inhibition (36.86 %) of *C. capsici* at the highest concentration (15%) of extract.

Bagade *et al*. (2020) studied the efficacy of six aqueous plant extracts against *Colletotrichum lindemuthianum* and reported that highest mycelial growth inhibition was observed in Heena (88.55%) followed by Ginger (65.55%) and Karanj (56.00%). The lowest mycelial growth inhibition was observed in Neem (37.04%) followed by Nilgiri (32.22%) and Tulsi (26.33%).

Rao *et al*. (2020a) studied the effect of two plant extracts, *Lantana camera* and *Azadirachta indica* at three different concentrations (5, 7.5 and 10%) against *C. lindemuthianum* causing anthracnose of field bean. Among them, *Lantana camara* at 10% concentration, inhibited the spore germination by 60.38%, which was statistically at par with 7.5% concentration of *L. camara* (57.39%). The highest spore germination (46.60%) was recorded at 5.0 per cent concentration of *A. indica*.

Shova *et al*. (2020) studied the efficacy of five plant extracts against *C. lindemuthianum* and reported that, among the five plant extracts, *Lippia alba* showed complete growth inhibition of *C. lindemuthianum* at 20% concentration which was followed by *Azadirachta indica* (47.59%), *Thuja occidentalis* (46.05%), *Heliotropium indicum* (24.67%) and *Michelia champaca* (23.89%).

Vasuki *et al*. (2020) tested the effect of 12 plant extracts at 5 and 10% concentration against black gram anthracnose caused by *C. lindemuthianum* under *in vitro* conditions and reported that among the plant extracts tested, *Anisomeles malabarica* recorded lowest mycelial growth of 3.2 cm and 1.0 cm and highest mycelial inhibition of 64.4% and 88.8% followed by *Allium sativum* which recorded

the mycelial growth of 3.5 cm and 3.0 cm and mycelial inhibition of 61.1% and 66.6% against control @ 5% and 10% concentration, respectively.

Sushmitha and Zacharia (2021) evaluated the efficacy of bio agents and two botanicals against *Colletotrichum lindemuthianum* in *in vitro* and reported that among the plant extracts, Neem leaf extract @5% showed 20.84 % inhibition followed by Datura leaf extract @ 5% (10.22 %).

Gurjar *et al*. (2021) evaluated the efficacy of plant extracts against *Colletotrichum lindemuthianum* in *in vitro* and *in vivo* causing anthracnose of soybean and reported that, Garlic bulb extract inhibited maximum mycelial growth of 78.33% and 70.28% at 15% and 10% concentrations, respectively followed by Neem leaf extract 69.41% at 15% concentration.

2.5.2 *In vitro* efficacy of Bioagents:

Padder and Sharma (2009) evaluated the antagonistic activity of fungal bioagents against *C. lindemuthianum*. They reported that all the four antagonists caused significant inhibition of mycelial growth of the test fungus. Maximum inhibition of mycelial growth was obtained with *T. viride* (59.48%) followed by *T. harzianum* (55.87%) and *G. virens* (48.88%). Least inhibition of growth was found with *T. hamatum* (47.42%). All the bioagents overgrew the pathogen except *G. virens*, which colonized the plates up to two-third of surface area.

Gawade and Suryawanshi (2009) evaluated various fungal bioagents at 10 and 15 % against *C. truncatum* in *in vitro*. They reported that *T. viride* was found most effective with 41.79 per cent inhibition, followed by *V. lecanii* (23.75%) at both the concentrations. They further concluded that higher concentration (15%) was better than lower one (10 %) in inhibition of mycelial growth.

Marinus Ngullie *et al.* (2010) tested nine plant species and 7 antagonists against *C. gloeosporioides* causing fruit rot in Naga king chilli and reported that *Trichoderma viride* and *Pseudomonas fluorescens* were very effective in inhibiting mycelial growth of the pathogen. Further they reported that the field evaluation revealed that spraying with *T. viride* (2%) showed a maximum disease reduction of 61.41% followed by 2 % *P. fluorescens* (58.10%).

Rajesha *et al.* (2010) evaluated various bioagents in *in vitro* against *C. lindemuthianum*. They reported that the maximum inhibition of mycelial growth (73.54%) was observed in *Trichoderma harzianum* followed by *T. viride* (50.90%).

Padder *et al.* (2010) evaluated three bioagents under *in vitro* and *in vivo* conditions against *C. lindemuthianum*. All the three antagonistic fungi caused significant inhibition of mycelial growth, maximum being with *Trichoderma viride* (69.21%) followed by *T. harzianum* (60.32%).

Jagtap *et al.* (2012a and 2012b) evaluated the antagonistic effects of the four bio agents against *C. truncatum*, causing anthracnose of soybean. They reported that *T. viride* was found most effective and recorded 18.53 mm mean colony diameter and 79.40 per cent inhibition of the test pathogen. Mean colony diameter and per cent mycelial inhibition of test pathogen recorded by *T. harzianum, T. hamatum* and *P. fluorescens* were 31.87mm, 23.63mm, 27.60 mm and 64.58 %, 73.74% and 69.33 %, respectively.

Fitsum *et al.* (2014a) evaluated the antagonistic effects of the three bio agents against *C. lindemuthianum* causing common bean anthracnose. They reported that the highest Per cent Inhibition of Mycelial Growth (PIMG) (80.39%) was obtained from *T. viride*, followed by *T. harzianum* (75.49%) and *Pseudomonas fluorescens* (40.2%). Similarly, highly significant ($P<0.01$) differences were observed in the mycelial growth of *C. lindemuthianum*. The highest growth of mycelia (3.4 cm) was measured from the control, whereas the least (0.67 cm) was from the dual culture containing *T. viride*.

Fitsum *et al.* (2014b) evaluated some bioagents against *C. lindemuthianum* causing common bean anthracnose under field conditions and reported that, among them, plots treated with *T. viride* and *P. fluorescens* showed the least disease incidence (38.5%) at the initial date (39 DAS) of disease assessment. *P. fluorescens* showed the least disease incidence at 39, 53, 67 and 81 DAS, respectively.

Rekha and Dubey (2014) reported that *Trichoderma harzianum* showed highest (44.61%) inhibition in radial growth of *Colletotrichum dematium var Truncate* causing anthracnose of soybean.

Chacko and Gokulapalan (2015) studied effect of biocontrol agents against *Colletotrichum capsici* causing anthracnose of chilli in *in vitro* and reported that, in dual culture 90% inhibition of mycelial growth was found in *P. fluorescence* followed by *T. viride* (55.5%).

Chavan and Rewale (2016) evaluated bioagents against *Colletotrichum truncatum* causing soybean pod blight and reported that all the bioagents exhibited fungitoxic activity against *C. truncatum* and significantly inhibited mycelial growth over untreated control. Out of the six fungal antagonists tested, *T. viride* was found most effective and recorded least linear mycelial growth (22.66 mm) with highest mycelial inhibition (74.80%) of the test pathogen over untreated control (90.00 mm and 00.00%), followed by *T. hamatum* and *T. longibrachiatum*. The fungal antagonist *T. virens* was found least effective (col. dia. 32.33 mm and inhibition 64.06%). The bacterial antagonists *Pseudomonas fluorescens* was also found fungistatic and recorded 32.16 mm linear mycelial growth and 64.25 per cent inhibition.

Badgujar *et al*. (2017) evaluated some fungal and bacterial antagonists against *Colletotrichum truncatum* causing soybean anthracnose in *in vitro*. They reported that *T. viride* recorded significantly highest inhibition of mycelial growth (78.85%), followed by *T. harzianum* (71.04%), *T. longibrachiatum* (66.33%), *T. koningii* (60.93%), *Aspergillus flavus* (59.00%), *Aspergillus niger* (53.11%), *Pseudomonas fluorescens* (48.71%) and *Bacillus subtilis* (45.44%), respectively.

Hingole *et al*. (2017) tested two bioagents against *C. truncatum* causing soybean pod blight under field conditions and reported that, among them, *T. viride* recorded 29.33 and 35.28 per cent disease intensity and pod infection, respectively with 26.77 and 15.33 per cent reduction over unsprayed control in disease intensity and pod infection respectively. Whereas, *P. fluorescens* recorded 29.34 and 36.44 per cent disease intensity and pod infection, respectively with 26.09 and 12.44 per cent reduction over unsprayed control in disease intensity and pod infection, respectively. Both the bio-agents were found at par with each other.

Ahamad *et al*. (2018) evaluated three bioagents under *in vitro* conditions against *C. truncatum* causing anthracnose of soybean. They reported that *T.*

harzianum was found to be most effective and recorded 67 per cent inhibition of *C. truncatum* followed by *T. viride* (58%) and *T. asperellum* (50%).

Birari *et al.* (2018) reported that *Trichoderma viride* inhibited the mycelial growth of *Colletotrichum capsici* causing anthracnose of chilli by 80.11% and *Pseudomonas fluorescens* by 65.44%, respectively.

Rawat *et al*. (2018) evaluated the antagonistic effects of the three bio agents *viz*., *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma asperellum* against *C. capsici* in *in-vitro* conditions by using dual culture techniques. They reported that among the three antagonists, *T. viride* showed 56.34% inhibition followed by *T. harzianum* (50.67%). The least inhibition (34.0%) was recorded in *T. asperellum*.

Kulkarni and Raja (2019b) evaluated six bioagents against *C. truncatum* causing anthracnose of green gram in *in vitro*. They reported that the *Trichoderma harzianum* gave highest growth inhibition (64.38%) followed by *Gliocladium virens* (58.47%), *T. koningii* (54.37%) and *T. viride* (50.46%). The least growth inhibition of the fungus was observed in *Bacillus subtilis* (35.44%) and *Pseudomonas fluorescens* (26.56%).

Lokhande *et al*. (2019) reported that *Trichoderma viride* and *Pseudomonas fluorescens* inhibited the mycelial growth of *C. capsici* causing chilli anthracnose by 74.22 and 57.66 per cent, respectively.

Bagade *et al*. (2020) evaluated two fungal and two bacterial bio-agents against *C. lindemuthianum* in *in vitro*. They reported that, the maximum inhibition of mycelial growth was recorded with *Trichoderma reesei* (77.77 %) followed by *Bacillus subtilis* (59.44%), *Pseudomonas fluorescens* (56.77%), and *Trichoderma asperellum* (48.61%).

Rajashree *et al*. (2020) tested ten strains of fungal bioagents (*Trichoderma viride* and *T. harzianum*) and nine strains of bacterial bioagents (*Bacillus subtilis* and *Pseudomonas fluorescens*) against *Colletotrichum truncatum* under *in vitro* by dual culture technique. They reported that among the ten fungal bioagents screened against *C. truncatum*, the highest mycelial inhibition was found in the *T. viride* strain (Tv-29)

followed by strain Tv- 1 and Tv- 10. While among the bacterial bioagents, *B. subtilis* strain (Bs - 21) and *P. fluorescens* strain (Pf-26) showed highest mycelial inhibition.

Rao *et al*. (2020a) evaluated three bioagents against *C. lindemuthianum,* causing anthracnose of field bean in *in vitro*. They reported that out of three bioagents, *Trichoderma viride* showed highest inhibition of spore germination (53.51%) followed by *Bacillus subtilis* (51.16%), while least inhibition of spore germination was by *Pseudomonas fluorescens* (40.51%).

Sharma *et al*. (2021) tested antagonistic potential of six bio control agents against *Colletotrichum lindemuthianum* causing anthracnose of bean in *in vitro* and reported that the highest mycelial growth inhibition of 53.69 per cent was observed with *T. koningii* (DMA-8) which was statistically at par with *T. harzianum* (TH-11) with 49.14 per cent inhibition, P. *fluorescens* (47.99 %) and *T. viride* (45.45 %). However, *T. harzianum* (TH-5) and *T. koningii* (JMA-11) were less effective with minimum inhibition of 35.63 and 39.43 per cent, respectively.

Sushmitha and Zacharia (2021) evaluated some bio-agents against *Colletotrichum lindemuthianum* in *in vitro* and reported that, among them, *Trichoderma harzianum* was found most effective and recorded 5.33 mm mean colony diameter and recorded significantly highest growth inhibition (93.59 %) of *C. lindemuthianum* as compared to carbendazim (treated check) and untreated control which was followed by *Pseudomonas fluorescens* with 61.92 per cent growth inhibition.

2.5.3 *In vitro* efficacy of fungicides

Sartori and Maringoni (2008) evaluated the efficacy of five fungicides of different active ingredients and some blending (Carbendazim, Chlorothalonil, Thiophanate-Methyl, Chlorothalonil + Thiophanate-methyl, Trifloxystrobin, Propiconazole and Trifloxystrobin + Propiconazole), at concentrations of 0, 1, 10, 100 and 1000 µg/ml, in a Potato Dextrose Agar culture medium against *C. lindemuthianum* infecting common bean. They reported that, seven isolates with low sensitivity to Carbendazim and Thiophanate-Methyl (ED interval greater than 1000 µg/ml) thus suggesting cross-resistance.

Shovan *et al.* (2008) evaluated five fungicides against *Colletotrichum dematium*. They reported that among these five fungicides, Tilt-250 EC completely inhibited the mycelial growth of *C. dematium* 100, 200 and 400 ppm concentrations. Vitavax-200 and Rovral 50WP gave 68.20-77.41 % and 55.26-68.20 % inhibition of the radial colony growth of the test pathogen at 100, 200 and 400 ppm, respectively. Dithane M-45 and Cupravit were appeared to be significantly inferior in comparison to other fungicides in inhibiting the colony growth. Complete inhibition of mycelial dry weight of *C. dematium* was achieved with 100, 200 and 400 ppm of Tilt-250 EC. Mycelial dry weight was reduced by 83.45% and 73.52% with the highest concentrations of Vitavax 200 and Rovral 50% WP, respectively, where Vitavax 200 was significantly superior to Rovral 50% WP but significantly inferior to Tilt-250EC. Dithane M 45 at 400 ppm inhibited only 28.61% mycelial dry weight and statistically inferior to Rovral 50% WP but superior to Cupravit, which was appeared to be significantly inferior in comparison to all other fungicides.

Gawade and Suryawanshi (2009) evaluated fungicides against *Colletotrichum truncatum* under laboratory conditions and reported that, Carbendazim with maximum inhibition (90.59%) of mycelial growth was found highly effective against the pathogen, followed by Propiconazole (87.95%), Hexaconazole (86.15%) and Difenoconazole (84.81%). Chlorothalonil with 70.23% inhibition was found comparatively less effective against the pathogen.

Rajesha *et al.* (2010) evaluated various fungicides against *Colletotrichum lindemuthianum* causing anthracnose of Dolichos bean under laboratory conditions. They reported that, among the different contact fungicides tested, Mancozeb was found to be more effective and inhibited cent per cent (100%) mycelial growth of *C. lindemuthianum*, followed by Propineb (48.32%) and Chlorothalonil (37.39%) at a concentration of 800 ppm. Among different systemic fungicides tested, Carbendazim inhibited cent per cent (100%) mycelial growth followed by Propiconazole (100%) and Difenoconazole (84.87%) at a concentration of 400 ppm respectively.

Jagtap *et al.* (2012b) evaluated various fungicides against *Colletotrichum truncatum*, causing anthracnose of soybean under laboratory conditions. They reported that, Carbendazim recorded least mean colony diameter (7.52 mm) and highest inhibition (91.63%) of mycelial growth of *C. truncatum* over untreated

control. This was followed by, Mancozeb which recorded mean colony diameter of 10.38 mm and mean mycelial growth inhibition of 88.45 per cent, followed by Carbendazim + Mancozeb, Propiconazole, Hexaconazole, Difenoconazole, Propineb and Fosetyl AL. Fungicide Chlorothalonil was found comparatively least effective and recorded 53.98 mm mean colony diameter and 40.01 per cent mean mycelial growth inhibition, respectively.

Mohammed *et al.* (2013) reported that the combined effect of Mancozeb seed treatment with Carbendazim spray at 10 days interval and Carbendazim seed treatment and spray at 10-day interval has reduced the bean anthracnose severity by 46.5% and 41% respectively.

Chavan and Suryawanshi (2014) evaluated *in vivo* bio efficacy of five effective fungicides against *Colletotrichum truncatum* causing anthracnose of soybean, during *Kharif* 2012 and *Kharif* 2013. They reported that, Carbendazim (@ 0.1%) was found most effective with highest average reduction in the disease intensity and pod infection to the tune of 51.37 and 76.89 per cent (*Kharif* 2012) and 47.92 and 72.05 per cent (*Kharif* 2013), respectively.

Fitsum *et al.* (2014a) studied *in-vitro* efficacies of the three fungicides, Mancozeb, Folpan and Mancolaxyl against *Colletotrichum lindemuthianum*. They reported that Mancozeb showed least mycelial growth with 2.4 cm at 100 ppm and 2.2 cm at 250 ppm concentration, whereas the growth of mycelia was not allowed at both 500 and 1000 ppm. Mancolaxyl showed comparatively better inhibition at 100, 250 and 500 ppm fungicidal concentrations than Folpan.

Aggarwal *et al*. (2015) evaluated various fungicides against *Colletotrichum lindemuthianum*. All the tested fungicides significantly inhibited the mycelial growth of *C. lindemuthianum* at all concentrations of 250, 500, 1000 and 2000 ppm. Tebuconazole inhibited 100 per cent linear growth of all the three isolates (MVL, UDR, and FN) at 250, 500, 1000 and 2000 ppm concentrations. SAAF at 250 ppm concentration inhibited 81.18 per cent of linear growth of isolate FN and this was followed by 73.21 and 73.17 per cent of isolate UDR and MVL, respectively. The next best fungicide was Carbendazim which inhibited 75.67 per cent of linear growth of isolate FN. Similarly, Mancozeb at 2000 ppm concentration inhibited 74.33

per cent linear growth of isolate FN. SAAF at 500 ppm concentration inhibited 88.78 per cent linear growth of isolate EN.

Chacko and Gokulapalan (2015) tested nine fungicides against *C. capsici*. They reported that among the fungicides, Propiconazole @ 0.05% and Difenoconazole @ 0.1% completely inhibited the mycelial growth (100%) of *C. capsici* followed by Captan + Hexaconazole 0.1% with 86.66 per cent growth inhibition over control. The fungicide Carbendazim @ 0.05% recorded 75% inhibition. This was followed by Mancozeb 0.2% which recorded 70% inhibition. Azoxystrobin @ 0.1% recorded 67.50% inhibition. The least inhibition (63.77%) was recorded in Chlorothalonil @ 0.1%.

Souza Filho *et al*. (2015) reported that the treatments with Pyraclostrobin and the mixture of Metiram + Pyraclostrobin were most effective in inhibiting the colony growth and conidial germination of *C. lindemuthianum* at concentration of 1, 10, 100 and 1000 µg/ml concentration.

Wagh *et al*. (2015) evaluated *in vitro* seven fungicides (each 500, 1000 and 1500 ppm) against *Colletotrichum capsici* causing chilli anthracnose. They reported that the highest mycelial growth inhibition was recorded with Propiconazole (100%) followed by Difenoconazole (89.75%) and Copper hydroxide (82.71%).

Sreeja *et al.* (2016) evaluated fungicides against *Colletotrichum gloeosporioides*, inciting anthracnose of cowpea in *in vitro*. They reported that Propiconazole (0.1%), Flusilazole (0.1%), Tebuconazole (0.1%) and Carboxin + Thiram (0.4%) caused cent per cent (100%) inhibition of the mycelial growth of the pathogen. The *in vivo* evaluation showed that three rounds of foliar application of Flusilazole (0.1%) at 30, 45 and 60 days after seed emergence recorded the lowest disease index (5.60) followed by Tebuconazole at 0.1% (8.30). The field evaluation revealed that foliar application of Flusilazole or Tebuconazole at 0.1% recorded the lowest anthracnose (0.93) index followed by treatment with Copper oxychloride (0.2%).

Badgujar *et al*. (2017) evaluated various systemic fungicides against *Colletotrichum truncatum* causing anthracnose of soybean under laboratory conditions and reported that, among them highest mycelial inhibition was recorded

with the fungicide Propiconazole which was highly fungistatic and recorded significantly highest average mycelial inhibition (100%). This was followed by fungicide, *viz*., Carbendazim (94.71%) and Hexconazole (95.38%). Fungicide Penconazole was found less effective (88.81%) in inhibiting mycelial growth of *Colletotrichum truncatum.*

Hingole *et al*. (2017) evaluated various fungicides against *Colletotrichum truncatum*, under field conditions. They reported that, Carbendazim (@ 0.1%) recorded least mean disease intensity (22.44%) and mean pod infection (12.08%). It also recorded highest reductions in the disease intensity (47.14%) and pod infection (70.98%) over unsprayed control. The second best fungicide found was Mancozeb (@0.2%) which recorded the minimum mean disease intensity (23.81%) and mean pod infection (13.54%) and there by caused 41.73 and 67.46 per cent reductions in the disease intensity and pod infection, respectively over unsprayed control. This was followed by the fungicides, Carbendazim 12% + Mancozeb 63% (@0.1%), Propiconazole (@0.1%) and Propineb (@ 0,2%) which recorded the mean disease intensity of 26.42, 26.97 and 27.87 per cent, respectively and mean pod infection of 19.65, 24.75 and 32.43 per cent, respectively.

Ahamad *et al*. (2018) evaluated various fungicides against *Colletotrichum truncatum*, causing anthracnose of soybean under laboratory conditions and reported that, Carbendazim recorded highest inhibition (98%) of mycelial growth of the test pathogen which was at par with Hexaconazole (97%), whereas Mancozeb (42.67%), and Chlorothalonil (33.67%) were not effective. Fungicide Chlorothalonil was found comparatively least effective than other fungicides.

Agam *et al*. (2019) evaluated various fungicides against *Colletotrichum truncatum*, causing anthracnose of soybean under laboratory conditions and reported that, Carbendazim, Tebuconazole and Propiconazole (@0.1%) were found significantly superior over rest of the treatments.

Asalkar *et al.* (2019) evaluated thirteen fungicides at different concentrations against *Colletotrichum gloeosporioides*, causing aonla anthracnose in *in vitro*. They reported that, all the treatments significantly inhibited mycelial growth of the test pathogen over untreated control. The pathogen was most sensitive to systemic

fungicides. Carbendazim + Mancozeb (100 % inhibition) followed by Azoxystrobin (99.50 %) and Propiconazole (97.51%). This was followed by Carbendazim (96.41 %).

Katediya *et al*. (2019) tested four systemic, three non-systemic and four combined fungicides at different concentrations *in vitro* against *C. capsici* through poisoned food technique. They reported that among the systemic fungicides, the complete growth inhibition of the fungus was recorded in Difenoconazole and Propiconazole at 500 and 1000 ppm concentrations. The next best systemic fungicide was Pyraclostrobin which at 1000 ppm inhibited 100 per cent mycelial growth. Among the non-systemic fungicides, the complete growth inhibition of the fungus was recorded in Copper oxychloride at 1000, 1500, 2000 and 2500 ppm and Mancozeb at 2500 ppm concentrations. The least effective fungicide was Chlorothalonil at 1000, 1500, 2000 and 2500 ppm concentrations. Among the combined fungicides, the complete growth inhibition of the fungus was recorded in Captan 70% + Hexaconazole 5% and Carbendazim 12% + Mancozeb 64% at all the concentrations. The next best combined fungicides were Pyraclostrobin 85g/L + Epoxiconazole 62.5g/L and Zineb 68% + Hexaconazole 4% which completely inhibited per cent mycelial growth at 500 and 1000 ppm concentration.

Bagade *et al*. (2020) evaluated various fungicides against *Colletotrichum lindemuthianum* under laboratory conditions. They reported that, Carbendazim + Mancozeb @ 0.25% and Carboxin + Thiram @ 0.3% were the most effective for arresting 100% mycelial growth followed by Pyraclostrobin @ 0.1% (91.48%), Copper oxychloride @ 0.25% (88.55%) and Propineb @ 0.3% (72.22%). Least mycelial growth inhibition observed in Azoxystrobin @ 0.1% (54.44%).

Kumar *et al*. (2020) tested nine fungicides against anthracnose disease of black gram under field conditions. They reported that among them, seed treatment with Carbendazim @ 3 g/kg seed and foliar spray of mixture of Azole and Strobilurin group T3 Natio@ 0.05% (Tebuconazole + Trifloxystrobin) and T7-Amistar top @ 0.1% (Azoxystrobin + Difenoconazole) were found significantly (at 0.05%) superior over rest of the treatments. Both showed minimum (6%) disease severity with maximum and (78.51%) disease control. Significant increase in yield (47.5%) also observed. The treatment T1 (Folicur @ 0.1%), T2 (Tilt @0.1%), T4 (Saaf@ 0.2%),

T5 (Bavistin @ 0.2%), T8 (Kavach @ 0.2%) and T9 (Blitox @ 0.2%) spray also showed good results in reducing the black gram anthracnose disease severity per cent (64.29, 57.14, 64.28, 57.14, 71.42 and 64.28% and also increased the per cent yield 37.5, 35, 42.5, 36.25, 45 and 45% respectively.

Rao *et al*. (2020a) evaluated some fungicides at three different concentrations (0.05, 0.1 and 0.15%) against *Colletotrichum lindemuthianum* causing anthracnose of field bean under laboratory conditions. They reported that, Propiconazole recorded lowest spore germination (12.93%) corresponding highest inhibition (85.44%) at 0.15%, while highest spore germination (42.30%) corresponding minimum inhibition (52.57%) was noticed at 0.05% of Azoxystrobin.

Shova *et al.* (2020) evaluated five fungicides against *Colletotrichum lindemuthianum*. They reported that, among the five fungicides, complete inhibition of the radial growth of *Colletotrichum lindemuthianum* was observed with Nativo 75 WG at 100 ppm concentration, out of the rest four fungicides, highest growth inhibition of *C. lindemuthianum* was observed with CM 75 WP (68.84%) which was followed by Knowin 50 WP (63.08%), Rovral 50 WP (60.25%) and Dithane M 45 (59.10%) at 500 ppm concentration.

Rajashree *et al*. (2020) evaluated seven systemic, seven contact and seven combi fungicides against *C. truncatum* under *in vitro* by poison food technique. They reported that, among systemic fungicides evaluated, Thiophanate methyl and Triazole group of fungicides like Propiconazole 25% EC, Difenoconazole 25% EC and Tebuconazole 25.9% EC recorded highest mycelial inhibition of *C. truncatum* at all the three concentrations (0.05, 0.1 and 0.15%) and Azoxystrobin showed least mean mycelial inhibition (75.55%). Among contact fungicides evaluated, Propineb 70% WP recorded highest mean mycelial inhibition (82.71%) followed by Copper oxychloride 50% WP (78.51%). Among the combi fungicides evaluated, cent per cent inhibition was recorded in Tricyclazole 18% + Mancozeb 62% WP and Carbendazim 12% + Mancozeb 63% WP at 0.3 per cent concentration.

Gurjar *et al*. (2021) evaluated various fungicides against *Colletotrichum truncatum* causing anthracnose of soybean under laboratory conditions and reported that, Tebuconazole was found most effective with complete inhibition of *C.*

truncatum mycelial growth at 500 and 1000 ppm concentrations followed by Tebuconazole at 250 ppm (94.44%), Propiconazole 1000 ppm (93.33%) and Hexaconazole 1000 ppm (91.48%). Chlorothalonil was found least effective with 56.29, 61.85, 65.92 and 70.00% inhibition at 100, 250, 500 and 1000 ppm, respectively.

2.6 Screening of cowpea varieties

Amusa *et al.* (1994) conducted bioassays of culture filtrates of the two pathogens (anthracnose and brown blotch) using 14 cultivars each of cowpea and soybean, and reported that host plants reacted differentially by producing different sizes of lesions. Cultivars were grouped as susceptible, moderately resistant and resistant, and groupings were similar to those of soybean cultivars.

Hossain *et al*. (2001) screened 105 soybean exotic and national origin germplasm against *C. truncatum* causing anthracnose of soybean under field condition. They reported that 36 germplasm were found to be free from infection of *C. truncatum* (highly resistant) while 19, 37, 3, 5 and 5 germplasms were graded as resistant, moderately resistant, moderately susceptible. susceptible and highly susceptible, respectively.

Sharma *et al.* (2012) screened some varieties of common bean germplasm against *Colletotrichum lindemuthianum*, causing bean anthracnose. They reported that among the indigenous collections 47, 34, 32 and 18 accessions showed resistance to race 3, 515, 598 and 529, respectively, whereas among exotic accessions 16, 22, 18 and 11 exhibited resistance to these races. Out of sixty-five accessions, 7 indigenous collections *viz.*, IC328537, IC-328538, IC-448888, IC-313294, IC278723, IC-339645, IC-341862 and 3 exotic accessions *viz.*, EC-169813, EC-398530 and EC-500226 showed resistance to various races, whereas 13 accessions were susceptible to all the races.

Haq *et al*. (2013) screened some germplasm against *C. capsici*, causing chilli anthracnose by two methods *viz*, pin prick and spray method. They reported that, completely immune or resistant germplasm was not found from both of the inoculation methods Maximum per cent disease incidence was recorded in Gola Peshawari and was ranked as highly susceptible followed by Longi which was also

found highly susceptible from both of the inoculation methods. Among the available germplasm, Talhar, Sanam, C-33, C-19 and C-72 were found moderately resistant.

Sajeesh *et al*. (2014) Screened 11 soybean genotypes against anthracnose disease. They reported that 64 per cent genotypes were showed moderately resistant reaction. 27 per cent and nine per cent genotypes were showed moderately susceptible and resistant reaction respectively. Genotype DSb 12 showed the resistant reaction.

Udaya Sankar *et al*. (2015) screened 21 horse gram germplasm against *Colletotrichum dematium* in *in vitro.* Based on per cent disease index (PDI), they identified one accession, IC470275 as immune and the remaining accessions as either moderately resistant or susceptible.

Arunakumara and Satyanarayana (2016) screened 20 chilli genotypes during *Kharif* 2012 and 2013 against *C. capsici* under field conditions. They reported that out of 20 genotypes evaluated, two genotypes *viz*, Arka Meghana and Byadagi Kaddi showed resistant reaction. Whereas, four genotypes *viz*, Musalwadi, Ujwala, Arka Lohit and Sankeshwara showed moderately resistant reaction and rest of them showed moderately susceptible and susceptible reaction.

Marak *et al.* (2017) screened 41 green gram genotypes against *Colletotrichum truncatum* under field conditions. They reported that 4 genotypes CZMK-1, PM-D5, Sukumar and TARM- 18 showed resistant reaction, 34 genotypes showed moderately resistant reaction, 3 genotypes PM-4, Pusa-1174 and Sonali showed moderately susceptible reaction.

Awori *et al.* (2018) conducted field screening of bean at Kachwekano Zonal Agricultural Research and Development Institute, Uganda. They reported that cultivars G2333, TU, AB136, K10, K13, BRS Cometa, Kaboon and SEL 1308 showed resistant reaction to *Colletotrichum lindemuthianum*.

Chavan *et al.* (2018) screened 40 soybean genotypes against anthracnose disease under field conditions. They reported that none of the genotypes showed immune and resistant reaction, 16 genotypes showed moderately resistant reaction and 21 genotypes showed susceptible reaction.

Sunilkumar *et al*. (2018) screened under field condition 235 soybean genotypes for pod blight disease complex at MARS, Dharwad, during *Kharif* 2016 and 2017, respectively. They reported that none of them were immune or absolutely resistant. One genotype *i.e.* MACS 1505, showed resistant reaction to pod blight, most of the entries like RKS 18, DSb 28-3, DSb 23-2 and DSb 30-2 were moderately resistant and SL 1104, DSb 32, RSC 10-70, KDS 921, NRC 125, RSC 10-71, DS 3106. RSC 10-52. JS 20 116 are moderately susceptible in reaction. Genotypes like JS 335, KDS 726 and KDS 780 were showed susceptible reaction and three genotypes JS 93-05. Punjab 1 and Bragg were highly susceptible to disease.

Nataraj *et al.* (2020) observed that among 225 soybean germplasms evaluated against anthracnose disease, only five germplasms *viz*., AKSS 67, EC 538828, EC 34372, EC 457254 and Karune showed highly resistant reaction.

Prajapati *et al*. (2020) screened ten chilli genotype against anthracnose disease caused by *C. capsici* under lab condition. They reported that among ten genotype, six genotypes of chilli including LUCKNOW-1, CHIHYB-11, CHIVAR -7, CHIHYB-8, CHIHYB-13 and Pant C-1 were moderately resistant having mean lesion length of 1.94 and 1.02 cm with PDI of 5.54 and 13.49 whereas the genotypes CHIHYB-12 and CHIVAR -2 showed absolutely susceptible reaction having mean lesion length of 2.61 and 2.13 cm with PDI of 27.72 and 31.81%, respectively. The genotypes CHIHYB-9 and NISHANT exhibited highly susceptible reaction having mean lesion length of 3.80 and 3.95 cm with PDI of 51.05 and 58.27 per cent respectively. Pant C-1 variety showed least mean lesion length (1.02 cm) with minimum PDI (5.54%) and found to be much better than other varieties. None of the arieties were found resistant to anthracnose disease.

Sharath *et al*. (2020) screened 31 chilli accessions against anthracnose disease caused by *C. capsici* under *in vivo* and *in vitro* condition. They reported that under field condition out of 31 accessions eight were found resistant, remaining 23 accessions were found moderately resistant at 90 days after transplanting. The reaction during harvest stages varied from that of 90 days after harvest which recorded, two resistant accessions, eight moderately resistant accessions, 20 moderately susceptible accessions and Byadagi Dabbi as susceptible. During *in vitro* screening 18 accessions were found resistant, eight were moderately resistant, three

were moderately susceptible and two were susceptible to the pathogen during green stage of the fruit. The reaction of chilli fruits during turning red stage recorded, 18 accessions as moderately resistant, eight as moderately susceptible and one (DCA-298) was susceptible. The reaction varied in red stage of fruit development wherein, five accessions were resistant, 18 accessions were moderately resistant, six were moderately susceptible and two were susceptible to the pathogen. The accessions DCA-67, 69, 189, 302 and 305 were reacted resistant to the *C. capsici* in field and artificial screening.

Gupta *et al*. (2022) screened 87 common bean genotypes against *Colletotrichum lindemuthianum* under epiphytotic conditions. They reported that among them 22 genotypes were highly resistant. 34 genotypes were moderately resistant. 27 genotypes were moderately susceptible and four genotypes were found susceptible.

CHAPTER - III

MATERIALS AND METHODS

CHAPTER - III

MATERIAL AND METHODS

During the studies on Anthracnose of cowpea caused by *Colletotrichum lindemuthianum*. The experiments were conducted *in vitro* at Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani during 2020-2021, to fulfill the objectives defined. The details of the material used and methods followed for various studies during the research are described here in the following paragraphs;

3.1 Experimental Materials:

The various kind of materials i.e., seed, fungicides, botanicals, bioagent, chemicals, glassware, culture media and other miscellaneous materials required for conducting present studies were obtained from Department of Plant Pathology, College of Agriculture, Parbhani.

3.1.1 Experimental site:

All experiments were planned and conducted in laboratory and screenhouse, Department of Plant Pathology, College of Agriculture, Parbhani.

3.1.2 Disease samples:

Leaves exhibiting typical symptoms of cowpea anthracnose (*Colletotrichum lindemuthianum*) disease were collected in the paper bags from cowpea fields, in the Parbhani district and subjected to the isolation on Potato Dextrose Agar medium (Plate-I).

3.1.3 Culture media:

Potato dextrose agar (PDA) as basal culture medium was used for isolation, purification, multiplication and maintenance of the cultures of *Colletotrichum lindemuthianum*.

3.1.4 Cowpea seeds collection:

Cowpea seeds of various varieties were collected from the local market of Parbhani and from the Safal Seeds & Biotech Ltd. Jalna.

3.1.5 Chemicals:

Standard chemicals, reagents, fungicides, fertilizers etc. required to conduct the experiments were obtained from the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

3.1.6 Glass-ware:

The commonly used glass-ware (Borosil and Corning Make) such as Petri dishes, conical flasks, volumetric flasks, test tubes, measuring cylinder, glass rods, beakers, pipettes, funnel etc. were obtained from the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

3.1.7 Equipment:

The laboratory equipment such as Autoclave, Hot air oven, Laminar airflow Cabinet, BOD incubator, Refrigerator, Binocular Research Microscope, Electronic weighing balance, pH meter, pestle mortar -cum-grinder etc, available at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani, were utilized, as and when required.

3.1.8 Miscellaneous:

Polythene bags (20 × 302 cm), plant protection appliances inoculation needle, forceps, blotter paper, paper bags, spirit lamp, sodium hypochlorite, labels, scales, Nylon rope, sand, soil, FYM, Screen house etc, available at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani, were used during the course of present investigation.

3.1.9 Botanicals:

Plant species reported to exhibit antifungal and therapeutic properties against fungal pathogens and available locally were collected from the field of College of

PLATE – I

A) Naturally infected cowpea field showing anthracnose symptoms

B) Symptoms on leaves

C) Symptoms on stem

PLATE- I: Naturally infected cowpea plant showing anthracnose symptoms

Agriculture, VNMKV, Parbhani and adjoining fields. Following locally available plant species / botanicals were used for *in vitro* studies. (Table-3.1)

Table 3.1 List of botanicals used against *C. lindemuthianum*

Sr. No.	Botanical Name	Local Name	Plant Part Used
1	*Zingiber officinale*	Ginger	Rhizome
2	*Azardirachta indica*	Neem	Leaves
3	*Allium sativum*	Garlic	Bulb
4	*Ocimum sanctum*	Tulsi	Leaves
5	*Allium cepa*	Onion	Bulb
6	*Annona squamosa*	Custard apple	Leaves
7	*Curcuma longa*	Turmeric	Rhizome

3.2.1 Bioagents:

Pure cultures of biocontrol agents were obtained from Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani. maintained and multiplied on appropriate culture media and used for various studies. (Table-3.2)

Table 3.2 List of bioagents used against *C. lindemuthianum*

Sr. No.	Biocontrol agents
1	*Trichoderma asperellum*
2	*T. harzianum*
3	*T. virens*
4	*Aspergillus niger*
5	*Metarhizhium anisopliae*
6	*Verticillium lecanii*
7	*Paecilomyces lilacinus*

3.2.2 Fungicides:

Following fourteen fungicides (Systemic, non-systemic and combi products) available at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani were used in various *in vitro* studies. (Table-3.3)

Table 3.3 List of fungicides used against C. *lindemuthianum*

Sr. No.	Fungicides and Al	Trade name	Manufacturing company
Systemic fungicides			
1	Propiconazole 25% EC	Tilt	Syngenta India Ltd., Mumbai
2	Tebuconazole 29.9% EC	Folicur	Bayer crop science Ltd., Mumbai
3	Hexaconazole 5% EC	Contaf	Greencrop Internt. Pvt. Ltd., Pune
4	Carbendazim 50% WP	Bavistin	BASF India Ltd., Mumbai
5	Pyraclostrobin 20% WG	Headline	BASF India Ltd., Mumbai
6	Difenoconazole 25% EC	Score	Syngenta India Ltd., Mumbai
7	Azoxystrobin 25% EC	Amistar	Syngenta India Ltd., Mumbai
Combi-products and non-Systemic fungicides			
8	Mancozeb 75% WP	Dithane M-45	Indofil industries Ltd., Mumbai
9	Chlorothalonil 75% WP	Kavach	Syngenta India Ltd., Mumbai
10	Copper oxychloride 50% WP	Blitox	Syngenta India Ltd., Mumbai
11	Propineb 70% WP	Antracol	Bayer crop science Ltd., Mumbai
12	Captan 70% + Hexaconazole 5% (75 WP)	Taqat	Rallis India Ltd., Mumbai
13	Carbendazim 12 % + Mancozeb 63 % (75 % WP)	SAAF	Greencrop Internt. Pvt. Ltd., Pune
14	Metalaxyl 8 % + Mancozeb 64 % (72 % WP)	Master	Syngenta India Ltd., Mumbai

3.2.3 Methodology

Details of methodologies adopted for various experiments conducted during present investigations are detailed as below.

3.2.4 Sterilization or disinfection

The glass wares were sterilized in Hot air oven at 180°C for 60 to 90 min. Solid as well as liquid media were sterilized in autoclave at 121°C and 15 lbs pressure for 20 min. Plant tissues were surface disinfected using 2 - 3 per cent sodium hypochlorite ($NaOCl_2$) solution for 2-3 min. and then washed in three sequential changes with sterile distilled water. The isolation or inoculation was performed under aseptic conditions of laminar air-flow cabinet. The working hands and working bench were also disinfected with denatured spirit or 70 % ethanol. Minor metallic tools (blades, inoculation needles, cork borer, forceps, etc.) were sterilized / disinfected by direct heating on flame of the spirit lamp.

3.2.5 Isolation of the pathogen

Fresh samples of diseased leaves, showing typical symptoms of anthracnose were collected from the fields, brought to the laboratory, washed thoroughly with distilled water, blot dried and cut with sharp sterilized blade into small bits (5mm), keeping half healthy and half diseased portion intact. These pieces were surface sterilized with 0.01 per cent sodium hypochlorite for two minutes and then washed by giving three changes with sterile distilled water to remove traces of Sodium hypochlorite and blot dried. This surface sterilized and blot dried leaf bits were then inoculated on the autoclaved, solidified and cooled PDA (Potato Dextrose Agar) medium in Petri plates under aseptic conditions of Laminar–air-flow cabinet.

Inoculated plates were then incubated in BOD incubator at 27 ± 1^{0}C temperature. For obtaining the pure culture of test pathogen from the plant sample inoculated Petri plates, hyphal tip technique was used. The pure culture so obtained was sub cultured aseptically multiplied and stored in refrigerator for further studies

3.2.6 Purification and Maintenance:

Purification of the fungal pathogen was done by single spore isolation method or single hyphal-tip technique. Following single hyphal-tip technique, grown the colony of the fungus on the PDA medium and selected the single growing hyphal tip cut the selected portion with the help of cork borer and transferred on the PDA slant medium and incubated at the temperature 27±1^{0}C and observed the pure growth of the culture. Through frequent sub-culturing, the pathogen was purified and its pure culture was maintained on PDA slants in test tubes and stored in refrigerator for further studies.

3.2.7 Pathogenicity of the pathogen

Seeds of cowpea local cultivar susceptible to anthracnose were surface sterilized with 0.01% NaOCl and sown (@ 3 seeds/Pot) in the earthen pots (25 cm dia.) filled with steam sterilized potting mixture of soil: sand: FYM (2:1:1). Three healthy growing cowpea seedlings per pot were maintained, watered regularly and kept in the screen house for further development. The test pathogen (*Colletotrichum lindemuthianum*) was mass multiplied on the basal culture medium PDA in Petri dishes. Spore-cum mycelial suspension of the test pathogen was prepared from 7-8 days old culture in plates by flooding with 5-10 ml sterile distilled water. The resultant spore cum-mycelial suspension was filtered through double-layered muslin cloth and filtrate obtained was suitably diluted with sterile distilled water to get inoculum concentration of 3-5×10^{6} spores/ml. Thirty to thirty-five days old potted seedlings of cowpea were spray inoculated with spore-cum-mycelial suspension of the test pathogen, using hand automizer and covered with polythene bags during evening, kept as such overnight. The cowpea seedlings sprayed with distilled water were maintained as untreated control. Both treated and untreated cowpea seedlings were maintained in screenhouse for further development of the symptoms.

3.2.9 *In vitro* evaluation of botanicals

An experiment was carried out to evaluate locally available plant species (each @ 10% and 20%) for their fungitoxicity if any, against anthracnose of cowpea. The details are given below.

Details of the experiment:

Design: CRD

Treatments: Eight

Replication: Three

Tr. No. Treatment details

T_1 : *Zingiber officinale*

T_2 : *Azardirachta indica*

T_3 : *Allium sativum*

T_4 : *Ocimum sanctum*

T_5 : *Allium cepa*

T_6 : *Annona squamosa*

T_7 : *Curcuma longa*

T_0 : Control (untreated)

Fresh samples were washed in tap water and finally washed thrice using sterilized distilled water. They were crushed in a sterilized pestle and mortar by adding a little quantity of sterile distilled water just enough to crush the sample easily. The extract was collected by filtering through the two layers of muslin cloth. Finally filtrate thus obtained from the leaves and roots were used as stock solution. To study the antifungal mechanism of plant extract poisoned food technique was followed as suggested by Nene and Thapliyal (1993). 10 and 20 ml of stock solution were taken separately and mixed with 90 and 80 ml sterilized molten Potato Dextrose Agar medium respectively, so as to get 10 and 20 per cent concentrations. The medium was shaken thoroughly for uniform mixing of plant extract.

About 20 ml medium was poured into each of the 90 ml sterilized Petri plates. Three replications were maintained for each treatment. Suitable control plates were maintained. Each plate was seeded with 5 mm mycelial discs aseptically taken from the periphery of 7 days old culture and incubated at 27±1°C till the growth of the colony touched the periphery in control plate. Mean colony diameter in each case was recorded. The efficacy of botanicals were expressed as per cent inhibition of mycelial

growth over control which was calculated by using the formula given by Vincent (1927).

$$\text{Per cent Inhibition} = \frac{C - T}{C} \times 100$$

Where, C = Growth of the test fungus in untreated control plates.

T = Growth of the test fungus in treated plates

3.3.1 *In vitro* efficacy of bioagents

A total of seven fungal bioagents as detailed under treatments were evaluated in *in vitro* against *C. lindemuthianum*, applying Dual culture technique (Dennis and Webster, 1971). Seven days old pure cultures of the test pathogen and test bioagents grown on PDA medium were used for the study. Two 5 mm culture discs, one each of the test pathogen and the test bioagent were cut out with sterilized cork borer and inoculated at equidistance and exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates and plates were incubated at 27±1°C. PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogen were maintained as control.

Details of the experiment:

Design: CRD

Treatments: Eight

Replication: Three

Tr. No.	**Treatment details**
T_1	: *Trichoderma asperellum*
T_2	: *Trichoderma harzianum*
T_3	: *Trichoderma virens*
T_4	: *Aspergillus niger*
T_5	: *Metarhizhium anisopliae*
T_6	: *Verticillium lecanii*
T_7	: *Paecilomyces lilacinus*
T_0	: Control (untreated)

Observations on radial mycelial growth colony diameter of *C. lindemuthianum* was recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition of the pathogen with the bioagents, over untreated control was calculated by using formula suggested by (Arora and Upadhyay, 1978).

$$\text{Per cent growth inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

3.3.2 *In vitro* efficacy of fungicides

3.3.3. Systemic fungicides

Efficacy of seven systemic (each @ 500, 1000 and 1500 ppm) was evaluated *in vitro* against *C. lindemuthianum* by applying Poisoned Food Technique (Nene and Thapliyal,1993). Three Petri plates / treatment / replication were maintained and also untreated control plates were maintained

Details of the experiment:

Design: CRD

Treatments: Eight

Replication: Three

Tr. No.	**Treatment details**
T_1	: Propiconazole 25% EC
T_2	: Tebuconazole 29.9% WG
T_3	: Hexaconazole 5% EC
T_4	: Carbendazim 50% WP
T_5	: Pyraclostrobin 20% WG
T_6	: Difenoconazole 25% EC
T_7	: Azoxystrobin 25% EC
T_8	: Control (untreated)

3.3.4 Combi-products and non-systemic fungicides

Efficacy of four non-systemic and three combi-product fungicides was tested under laboratory at 1000, 2000 and 2500 ppm concentration against *C. lindemuthianum* by applying poison food technique (Nene and Thapliyal, 1993). Three petri plates/treatments/replication were maintained along with suitable control plates.

Details of the experiment:

Design: CRD

Treatments: Eight

Replication: Three

Tr. No. Treatment details

T_1 : Mancozeb 75% WP

T_2 : Chlorothalonil 75% WP

T_3 : Copper oxychloride 50% WP

T_4 : Propineb 70% WP

T_5 : Captan 70% + Hexaconazole 5% (75 WP)

T_6 : Carbendazim 12 % + Mancozeb 63 % (75 % WP)

T_7 : Metalaxyl 8 % + Mancozeb 64 % (72 % WP)

T_0 : Control (untreated)

Observations on radial mycelial growth / colony diameter of the *C. lindemuthianum* was recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition of the pathogen with the test fungicides over the untreated control were calculated by using the formula (Vincent, 1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Growth of the test fungus in untreated control plates.

T = Growth of the test fungus in treated plates

3.3.5 Screening of Cowpea varieties against Anthracnose

Twelve varieties of cowpea were evaluated for their reaction against *Colletotrichum lindemuthianum* (Table 3.4). The pots were filled with steam sterilized potting mixture of soil: sand: FYM (2:1:1), watered regularly and maintained in screen house. After four weeks of sowing, these potted cowpea plants were spray inoculated with spore-cum-mycelial suspension of *C. lindemuthianum* and during evening hours, covered with transparent polythene bags, for overnight. After two weeks of inoculation, the plants begin to express the typical symptoms. Observation on first appearance of the disease symptoms, disease incidence and disease severity were recorded by applying 0-9-point disease rating scale given by Schoonhoven and Pastor- Corrales (1987). (Table-3.5)

Table 3.4 List of cowpea varieties screened against *C. lindemuthianum*:

Sr. No.	Name of varieties
1	Cowpea Safal-209
2	Cowpea Safal-311
3	Maharani
4	Ardhveli
5	Faguni uphar-31
6	Ankur VU-5
7	KSP-150
8	Savita
9	KSP-145
10	KSP-5335
11	KSP-178
12	KSP-170

Table 3.5 Details of disease rating scale of cowpea anthracnose (Schoonhoven and Pastor- Corrales, 1987)

Score	Symptoms
1	Absence of symptoms
2	Up to 1% of the leaf veins affected, visible only on the lower leaf surface.
3	Up to 3% of the leaf veins affected, visible only on the lower leaf surface.
4	Up to 1% of the leaf veins affected, visible on both surfaces of the leaves.
5	Up to 3% of the leaf veins affected, visible on both surfaces of the leaves.
6	Leaf veins affected, visible on both leaf surfaces and the presence of some lesions on stems, branches and petioles.
7	Necrotic spots on most of the leaf veins and in a large part of the adjacent mesophyll tissue, which ruptures, as well as the presence of abundant lesions on the stem, branches and petioles.
8	Necrotic spots on almost all the leaf veins and very abundant on stem, branches and petioles leading to ruptures, leaf shedding and reduction of plant growth.
9	Most of the plants are dead.

Based on numerical rating observed, per cent disease intensity (PDI) was worked out by applying formula given by Mc-Kinney (1923).

$$PDI = \frac{\text{Summation of numerical rating}}{\text{No. of leaves / plants observed x maximum rating}} \times 100$$

On the basis of PDI, cowpea germplasms lines/ cultivars were classified into different categories as per (Gupta *et al.*, 2022). (Table-3.6).

Table 3.6 Categories of cowpea cultivars according to their reaction to *C. lindemuthiannum* (Gupta *et al.*, 2022).

PDI %	Categories
0	Absolutely resistant (AR)
0.01-12.21	Highly resistant (HR)
12.22-33.33	Moderately resistant (MR)
33.34-55.55	Moderately susceptible (MS)
55.56-77.77	Susceptible (S)
77.78-100	Highly susceptible (HS)

3.4. Statistical analysis:

The data obtained in various experiments was analysed statistically by using OP-STAT statistical programme available at Central Computer Center, VNMKV Parbhani.

CHAPTER - IV

RESULTS AND DISCUSSION

CHAPTER - IV

RESULTS AND DISCUSSION

Present studies on *Colletotrichum lindemuthianum* were undertaken during 2021-22 on the aspects *viz*., symptomatology, isolation, identification, pathogenicity test, *in vitro* efficacy of botanicals, bioagents, fungicides and screening (pot culture). The results obtained on all these aspects are being presented in the following paragraphs:

4.1. Isolation and purification of the pathogen:

The isolation of *Colletotrichum lindemuthianum* was carried out on Potato Dextrose Agar media as detailed under material and methods. After five to seven days of incubation fungal colonies with profuse mycelial growth were emerged from the diseased bits of leaves. Hyphal tip method was found suitable for purification. The test pathogen was identified as *C. lindemuthianum* based on comparing culture growth obtained with earlier descriptions and the microscopy. The growth of the *C. lindemuthianum* on PDA slant and Petri plates were similar with the earlier descriptions.

4.1.1 Identification

Based on symptoms on leaves, cultural characteristics of fungus and microscopic observations test pathogen was identified as *Colletotrichum lindemuthianum* (Sacc. & Magnus) Brivosi & Cavara, the cause of the anthracnose. (Plate-II)

The isolated and purified test pathogen was identified by comparing the symptoms, colony growth characters and microscopic observations, with the findings of earlier reporters. Adebanjo and Bankole (2003), Falade, (2016), Gupta *et al*. (2017b), Ganiyu *et al*. (2018) and Sushmitha and Zacharia (2021) and they were found similar to the earlier findings hence confirmed as *Colletotrichum lindemuthianum.*

4.1.2 Symptomatology

During field study and pathogenicity test the typical symptoms induced by *Colletotrichum lindemuthianum* on cowpea plants were observed critically. Anthracnose infection occurred on both sides of the leaf and on the petiole. Early signs of infection usually appeared on the lower leaf surface along the veins, which showed brick red to purplish red discoloration. Later, such discoloration also appeared on the upper leaf surface. At the same time, brown lesions of various sizes, with black, brown, or purplish red margins, developed around small veins, eventually conidiophores ruptured through the host cuticle and formed acervuli on the plant surface.

Similar symptoms were earlier reported by Adegbite and Amusa (2010), Mohammad (2013) and Falade *et al*. (2018b).

4.1.3 Pathogenicity test:

The pathogenicity test of *Colletotrichum lindemuthianum* was conducted in screen house by inoculating spore culture on the 30-35 days old healthy plants of cowpea grown in earthen pots using local cowpea susceptible variety. After a week of incubation, the typical symptoms of cowpea anthracnose on leaves were observed similar to those observed on naturally infected cowpea with *Colletotrichum lindemuthianum* under field conditions. (Plate-III).

Similar pathogenicity of *Colletotrichum lindemuthianum* on leaves, stem, pods and seedling stage of cowpea were reported earlier by several research workers like Lubbe *et al*. (2006), Shivakumar *et al*. (2015) and Birari *et al*. (2018).

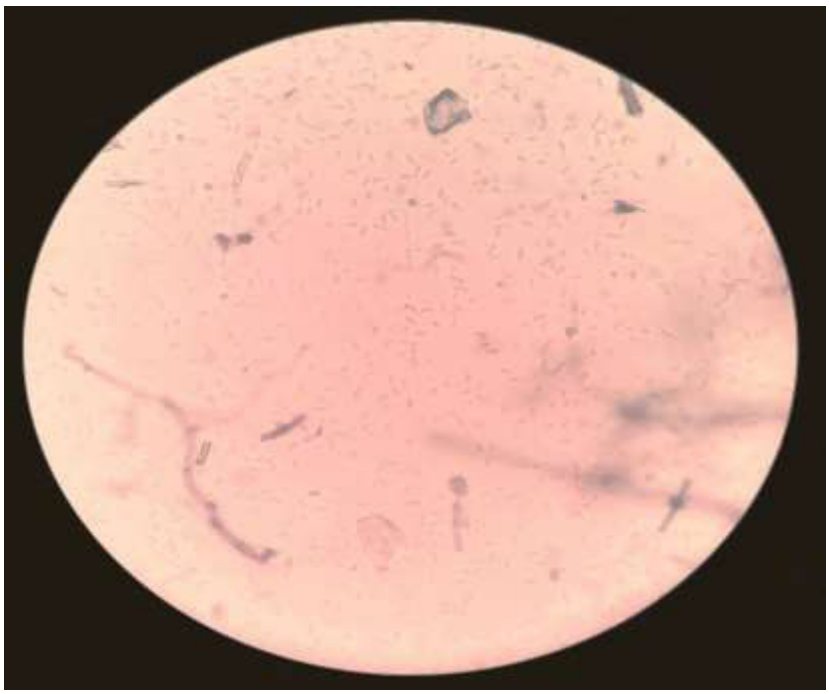
4.3. Disease management strategies

4.3.1 Efficacy of botanicals against *Colletotrichum lindemuthianum*

Water and ethanol-based extracts of seven botanicals *viz*., Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Tulsi (*Ocimum sanctum*), Turmeric (*Curcuma longa*), Custard apple (*Annona squamosa*), Ginger (*Zingiber officinale*) and Onion (*Allium cepa*) were evaluated against *C. lindemuthianum* at ten and twenty per cent concentrations by applying Poisoned Food Technique (Nene and Thapliyal, 1993) as

PLATE-II

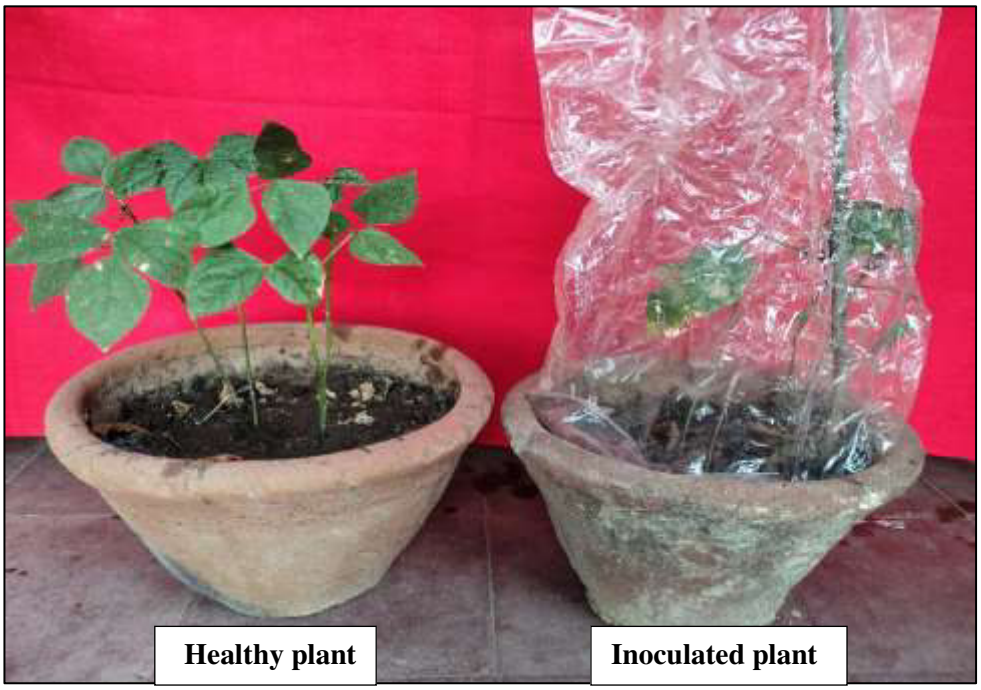
A) Pure culture of *Colletotrichum lindemuthianum*



B) Micro photograph showing conidia of *C. lindemuthianum* at 10X

PLATE II: Pure culture and microphotograph conidia of *C. lindemuthianum*

PLATE – III



Symptoms of *C. lindemuthianum* on cowpea plant

PLATE III: Pathogenicity of cowpea anthracnose caused by *Colletotrichum lindemuthianum*

explained under material and methods. Effects of these botanicals on radial mycelial growth and per cent inhibition of *C. lindemuthianum* was recorded and the results obtained are presented below.

4.3.1.1: Water based 10 % extracts of botanicals:

Radial mycelial growth:

Results (Table 4.1) revealed that the water based 10 % extracts of Turmeric (T_4) (09.33 mm), Garlic (T_2) (12.00 mm) and Ginger (T_6) (22.33 mm) were found effective in controlling mycelial growth of *C. lindemuthianum*. These treatments were statistically significant with each other and rest of the treatments including untreated control (T_0) which recorded full mycelial growth of *C. lindemuthianum* (90.00mm).

The water based 10 % extracts of Neem (T_1), Tulsi (T_3), Custard apple (T_5) and Onion (T_7) were not effective and allowed full mycelial growth of *C. lindemuthianum* (90.00 mm). These treatments were statistically at par with untreated control (T_0) (90.00mm).

Table 4.1: *In vitro* effects of water-based 10 % extracts of botanicals on *Colletotrichum lindemuthianum*

Tr. No	Botanicals	Radial mycelial growth(mm)	Per cent inhibition at concentration
T_1	Neem *(Azadirachta indica)*	90.00	00.00 (00.00)*
T_2	Garlic *(Allium sativum)*	12.00	86.66 (68.55)
T_3	Tulsi *(Ocimum sanctum)*	90.00	00.00 (00.00)
T_4	Turmeric *(Curcuma longa)*	09.33	89.63 (72.21)
T_5	Custard apple *(Annona squamosa)*	90.00	00.00 (00.00)
T_6	Ginger *(Zingiber officinale)*	22.33	75.18 (60.10)
T_7	Onion *(Allium cepa)*	90.00	00.00 (00.00)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E.(m) ±		**0.40**	**0.36**
C.D. at 1%		**1.22**	**1.11**

* Figure in parenthesis are angular transformed values.

Per cent inhibition of *C. lindemuthianum*:

The results (Figure 4.1 and plate IV) revealed that the water based 10 % extracts of *Curcuma longa* (T_4) (89.63 %), *Allium sativum* (T_2) (86.66 %) and *Zingiber officinale* (T_6) (75.18%) were found effective in inhibiting the mycelial growth of *C. lindemuthianum*. These treatments were statistically significant with each other and rest of the treatments including untreated control (T_0).

The water based 10 % extracts of *Azadirachta indica* (T_1), *Ocimum sanctum* (T_3), *Annona squamosa* (T_5) and *Allium cepa* (T_7) were failed to inhibit the mycelial growth of *C. lindemuthianum* (00.0 %). These treatments were statistically at par with untreated control (T_0).

4.3.1.2: Water based 20 % extracts of botanicals:

Radial mycelial growth:

Results (Table 4.2) revealed that the water based 20 % extracts of Garlic (T_2) and Ginger (T_6) were found most effective in controlling mycelial growth of *C. lindemuthianum*. These treatments not allowed the mycelial growth of *C. lindemuthianum* to develop in the base media (00.00 mm). These treatments were statistically at par with each other and significant over rest of the treatments including untreated control (T_0), which recorded full mycelial growth of *C. lindemuthianum* (90.00 mm). The next best botanical treatment found was Turmeric (T_4), which allowed only 9.83 mm mycelial growth of *C. lindemuthianum*, followed by Onion (T_7) and Tulsi (T_3), which recorded 50.67 mm and 77.16 mm mycelial growth of *C. lindemuthianum*, respectively.

The water based 20% extracts of Neem (T_1) and Custard apple (T_5) were not effective and allowed full mycelial growth of *C. lindemuthianum* (90.00 mm). These treatments were statistically at par with untreated control (T_0).

Per cent inhibition of *C. lindemuthianum*:

The results (Figure 4.1 and Plate-V) revealed that the water based 20 % extracts of *Allium sativum* (T_2) and *Zingiber officinale* (T_6) were found most effective

PLATE – IV

T1= *Azadirachtaindica*

T2= *Allium sativum*

T3=*Ocimum sanctum*

T4= *Curcuma longa*

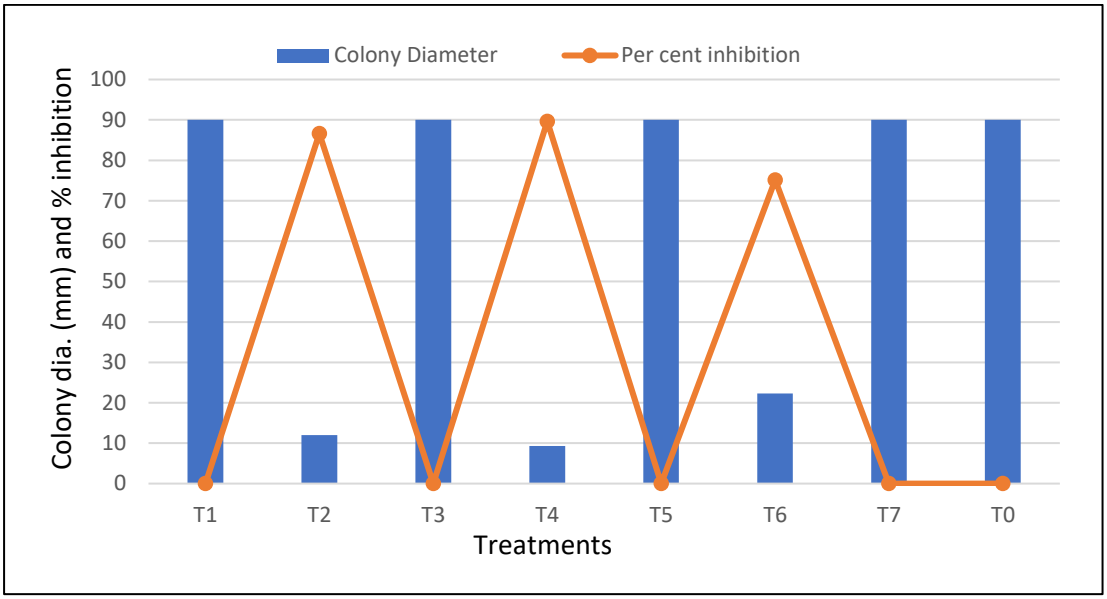
T5= *Annona squamosa*

T6= *Zingiber officinale*

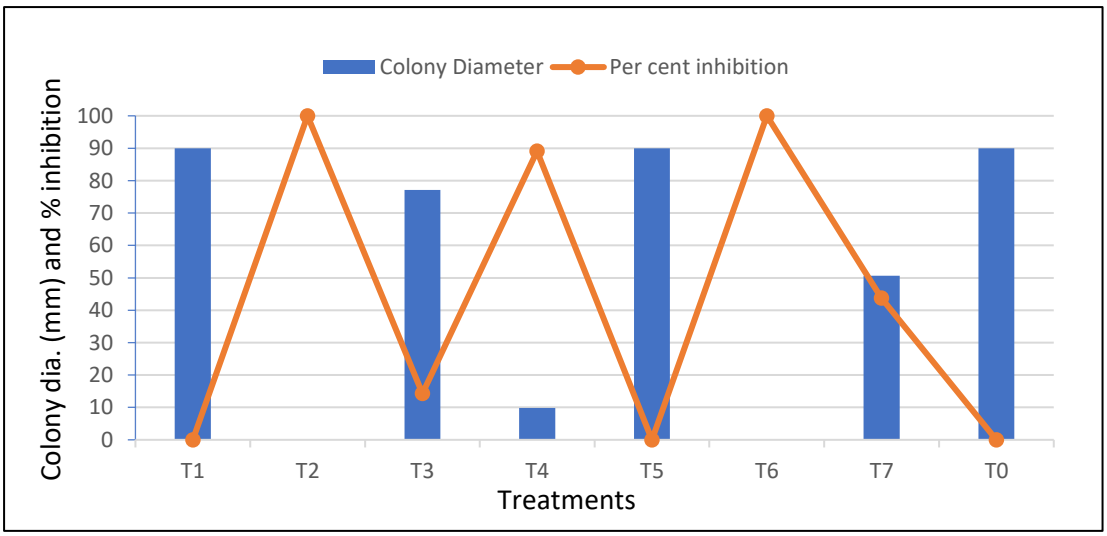
T7= *Allium cepa*

T0= Control

PLATE- IV: Effects of 10% water-based extracts of botanicals on *Colletotrichum lindemuthianum*



@ 10 %



@ 20 %

T1:	Neem	T5:	Custard apple
T2:	Garlic	T6:	Ginger
T3:	Tulsi	T7:	Onion
T4:	Turmeric	T0:	Control

4.1: Effects of water-basedextracts of botanicals on *Colletotrichum lindemuthianum*.

PLATE – V

T1= *Azadirachtaindica*

T2= *Allium sativum*

T3=*Ocimum sanctum*

T4= *Curcuma longa*

T5= *Annona squamosa*

T6= *Zingiber officinale*

T7= *Allium cepa*

T0= Control

PLATE-V: Effects of 20% water-based extracts of botanicals on *Colletotrichum lindemuthianum*

in inhibiting the mycelial growth of *C. lindemuthianum*. These treatments showed cent per cent (100 %) inhibition of mycelial growth of *C. lindemuthianum*. These treatments were statistically at par with each other and followed by *Curcuma longa* (T_4) (89.07 %), *Allium cepa* (T_7) (43.70 %) and *Ocimum sanctum* (T_3) (14.26 %). Treatment T_4, T_7 and T_3 were statistically significant with each other and over rest of the treatments including untreated control (T_0).

The water based 20 % extracts of *Azadirachta indica* ($T_{1)}$ and *Annona squamosa* (T_5) were failed to inhibit the mycelial growth of *C. lindemuthianum* (00.0 %). These treatments were statistically at par with untreated control (T_0).

Table 4.2: *In vitro* effects of water-based 20 % plant extracts of botanicals on *Colletotrichum lindemuthianum*

Tr. No.	Botanicals	Radial mycelial growth(mm)	Per cent inhibition at concentration
T_1	Neem *(Azadirachta indica)*	90.00	00.00 (00.00)*
T_2	Garlic *(Allium sativum)*	00.00	100.00 (90.00)
T_3	Tulsi *(Ocimum sanctum)*	77.16	14.26 (21.82)
T_4	Turmeric *(Curcuma longa)*	09.83	89.07 (74.06)
T_5	Custard apple *(Annona squamosa)*	90.00	00.00 (00.00)
T_6	Ginger *(Zingiber officinale)*	00.00	100.00 (90.00)
T_7	Onion *(Allium cepa)*	50.67	43.70 (41.29)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E.(m) ±		**2.88**	**3.28**
C.D. at 1%		**8.73**	**9.92**

* Figure in parenthesis are angular transformed values.

The similar results were reported by earlier workers, Wagh *et al*. (2015) reported that among the tested aqueous plant extracts the highest mycelial growth inhibition of *C. capsici* was recorded with Garlic (57.96%). Badgujar *et al.* (2017) tested eight botanicals in *in vitro* (each 10 and 15%). They reported that among the plant extracts tested, highest inhibition of mycelial growth of *C. truncatum* recorded

with Turmeric (57.16%). Rana *et al.* (2016) studied the effect of five botanicals in *in vitro* (each 10 and 20%) and reported that, among the plant extract maximum mycelial growth inhibition of C. *dematium* recorded in Garlic followed by onion. Other researchers also reported similar finding such as, Choudhary *et al*. (2017), Bagade *et al*. (2020) and Gurjar *et al.* (2021).

4.3.2.1: Ethanol based 10 % extracts of botanicals:

Radial mycelial growth:

Results (Table 4.3) revealed that the ethanol based 10 % extract of Garlic (T_2), Turmeric (T_4) and Ginger (T_6) were found most effective in controlling mycelial growth of *C. lindemuthianum*. These botanicals did not allowed mycelial growth of *C.lindemuthianum* to develop in the basal media (00.00 mm). Treatments of ethanol based 10 % extracts of Garlic, Turmeric and Ginger were statistically at par with each other and significant over rest of the treatments including untreated control (T_0), which recorded full mycelial growth of *C. lindemuthianum* (90.00 mm). These treatments were statistically at par with each other and with extract of Onion (T_7) (12.33 mm). The ethanol based 10% extract of Tulsi (T_3) was next best treatment, which recorded 52.17 mm mycelial growth of *C. lindemuthianum*. This was statistically at par with Neem extract (T_1), which recorded 61.00 mm mycelial growth of *C. lindemuthianum*. The ethanol based 10 % extract of Custard apple (T_3) was not effective and allowed full mycelial growth (90.00 mm) of *C. lindemuthianum*, and was statistically at par with untreated control (T_0).

Per cent inhibition of *C. lindemuthianum*:

The results (Figure 4.2 and plate-VI) revealed that the ethanol based 10 % extract of *Allium sativum* (T_2), *Curcuma longa* (T_4) and *Zingiber officinale* (T_6) were found most effective in inhibiting the mycelial growth of *C. lindemuthianum*. These treatments showed cent per cent (100%) inhibition of mycelial growth of *C. lindemuthianum* and were statistically at par with each other. Next best ethanol based 10 % botanical extract found was *Allium cepa* (T_7) (86.30%) which was statistically significant over rest of the treatments and followed by *Ocimum sanctum*(T_3) (42.03%) and *Azadirachta indica* (T_1) (32.22%). These treatments were statistically at par with each other but significant over rest of the treatments including untreated control (T_0).

PLATE – VI

T1= *Azadirachta indica*

T2= *Allium sativum*

T3=*Ocimum sanctum*

T4= *Curcuma longa*

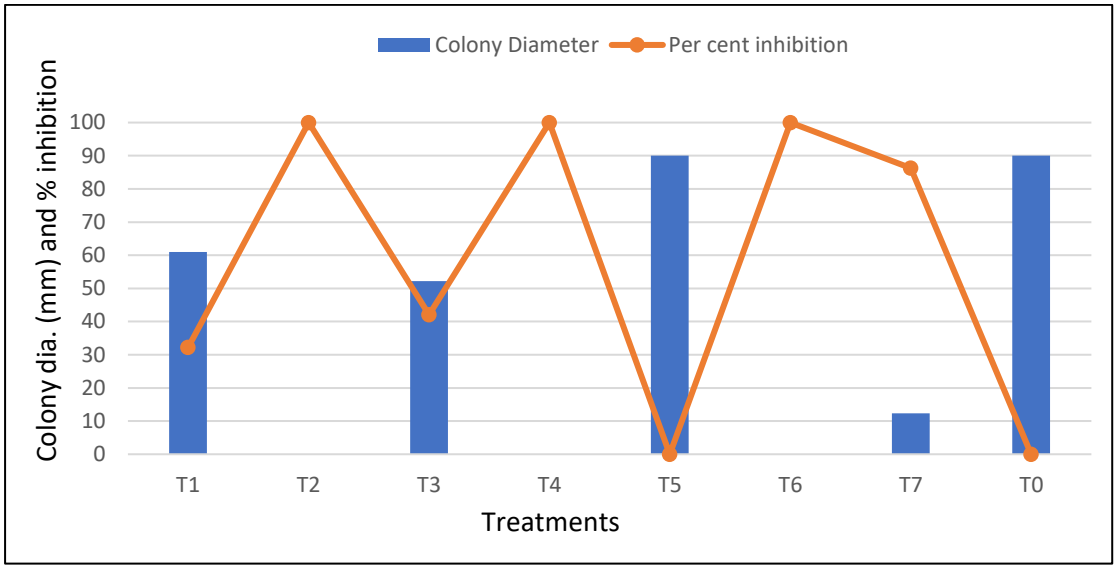
T5= *Annona squamosa*

T6= *Zingiber officinale*

T7= *Allium cepa*

T0= Control

PLATE- VI: Effects of 10% ethanol-based extracts of botanicals on *Colletotrichum lindemuthianum*



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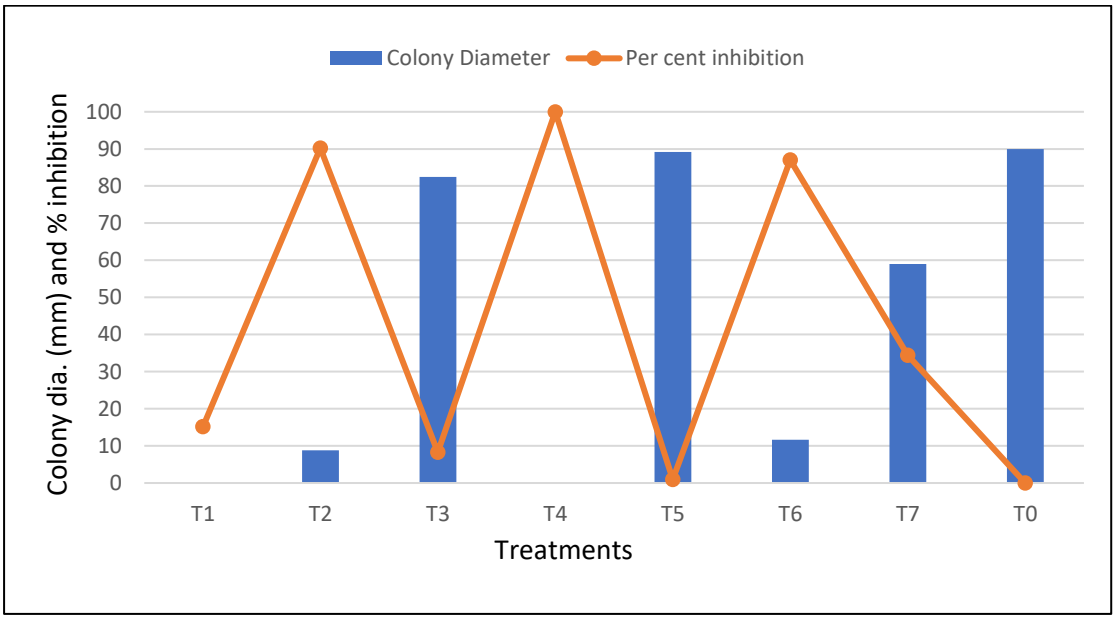


@ 20 %

T1:	Neem	T5:	Custard apple
T2:	Garlic	T6:	Ginger
T3:	Tulsi	T7:	Onion
T4:	Turmeric	T0:	Control

4.2: Effect of ethanol-based extracts of botanicals on *Colletotrichum lindemuthianum*

The ethanol based 10 % extract of *Annona squamosa* (T_5) was failed to inhibit the mycelial growth of *C. lindemuthianum* (00.00%) and was statistically at par with untreated control (T_0).

Table 4.3: *In vitro* effects of ethanol-based 10 % extracts of botanicals on *Colletotrichum lindemuthianum*

Tr. No.	Botanicals	Radial mycelial growth(mm)	Per cent inhibition at concentration
T_1	Neem *(Azadirachta indica)*	61.00	32.22 (34.10)*
T_2	Garlic *(Allium sativum)*	00.00	100.00 (90.00)
T_3	Tulsi *(Ocimum sanctum)*	52.17	42.03 (40.17)
T_4	Turmeric *(Curcuma longa)*	00.00	100.00 (90.00)
T_5	Custard apple *(Annona squamosa)*	90.00	00.00 (00.00)
T_6	Ginger *(Zingiber officinale)*	00.00	100.00 (90.00)
T_7	Onion *(Allium cepa)*	12.33	86.30 (68.26)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E. (m) ±		**5.85**	**3.95**
C.D. at 1%		**17.70**	**11.94**

* Figure in parenthesis are angular transformed values.

4.3.2.2: Ethanol based 20 % extracts of botanicals

Radial mycelial growth:

Results (Table 4.4) revealed that the ethanol based 20 % extract of Turmeric (T_4) was found most effective in controlling mycelial growth of *C. lindemuthianum* (00.00 mm). This treatment was statistically significant over rest of the treatments including untreated control (T_0) (90.00 mm). This was followed by Garlic (T_2) (08.83 mm) which was statistically at par with Ginger (T_6) (11.67 mm) and significant over rest of the botanical treatments. The next best treatment found was ethanol based 20 % extract of Onion (T_7) (59.00 mm) followed by Neem (T_1) (76.33 mm) which was statistically at par with Tulsi (T_3) which recorded 82.50 mm radial mycelial growth of

C. lindemuthianum. The botanical Custard apple (T_5) was not effective in controlling the mycelial growth of *C. lindemuthianum* and recorded 89.17 mm mycelial growth which was statistically at par with Tulsi (T_3) (82.50 mm) and untreated control (T_0).

Per cent inhibition of *C. lindemuthianum*:

The results (Figure 4.2 and plate- VII) revealed that the ethanol based 20 % extract of *curcuma longa* (T_4) was found most effective in inhibition of *C. lindemuthianum* (100 %). This treatment was statistically significant over rest of the treatments including untreated control (T_0) (00.00 %). This was followed by *Allium sativum* (T_2) (90.18 %) which was statistically at par with *Zingiber officinale* (T_6) (87.03 %) and significant over rest of the botanical treatments. The next best treatment found was ethanol based 20 % extract of *Allium cepa* (T_7) (34.44 %) followed by *Azadirachta indica*(T_1) (15.18 %) which was statistically at par with *Ocimum sanctum* (T_3) which recorded only 08.33 % inhibition of *C. lindemuthianum*. The botanical *Annona squamosa* (T_5) was not effective in controlling the mycelial growth of *C. lindemuthianum* and inhibited only 00.92 % growth, which was statistically at par with *Ocimum sanctum* (T_3) (08.33 %) and untreated control (T_0).

Table 4.4: *In vitro* effects of ethanol-based 20 % plant extracts of botanicals on *Colletotrichum lindemuthianum*

Tr. No.	Botanicals	Radial mycelial growth(mm)	Per cent inhibition at concentration
T_1	Neem *(Azadirachta indica)*	76.33	15.18 (22.74)*
T_2	Garlic *(Allium sativum)*	08.83	90.18 (72.13)
T_3	Tulsi *(Ocimum sanctum)*	82.50	08.33 (13.78)
T_4	Turmeric *(Curcuma longa)*	00.00	100.00 (90.00)
T_5	Custard apple *(Annona squamosa)*	89.17	00.92 (04.27)
T_6	Ginger *(Zingiber officinale)*	11.67	87.03 (69.36)
T_7	Onion *(Allium cepa)*	59.00	34.44 (35.80)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E. (m) ±		**2.79**	**3.35**
C.D. at 1%		**8.43**	**10.15**

* Figure in parenthesis are angular transformed values.

PLATE – VII

T1= *Azadirachta indica*

T2= *Allium sativum*

T3=*Ocimum sanctum*

T4= *Curcuma longa*

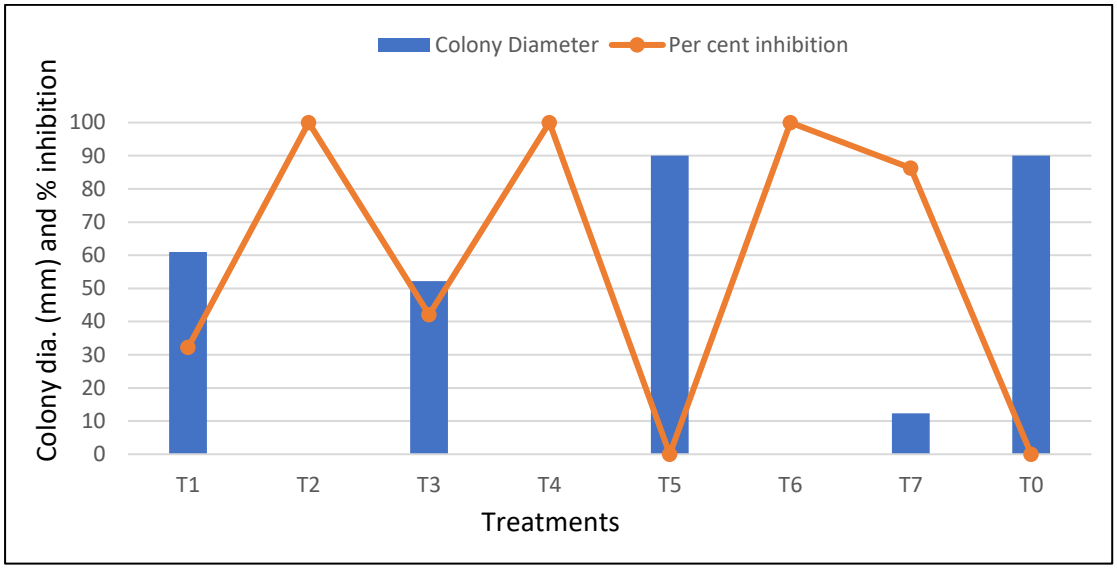
T5= *Annona squamosa*

T6= *Zingiber officinale*

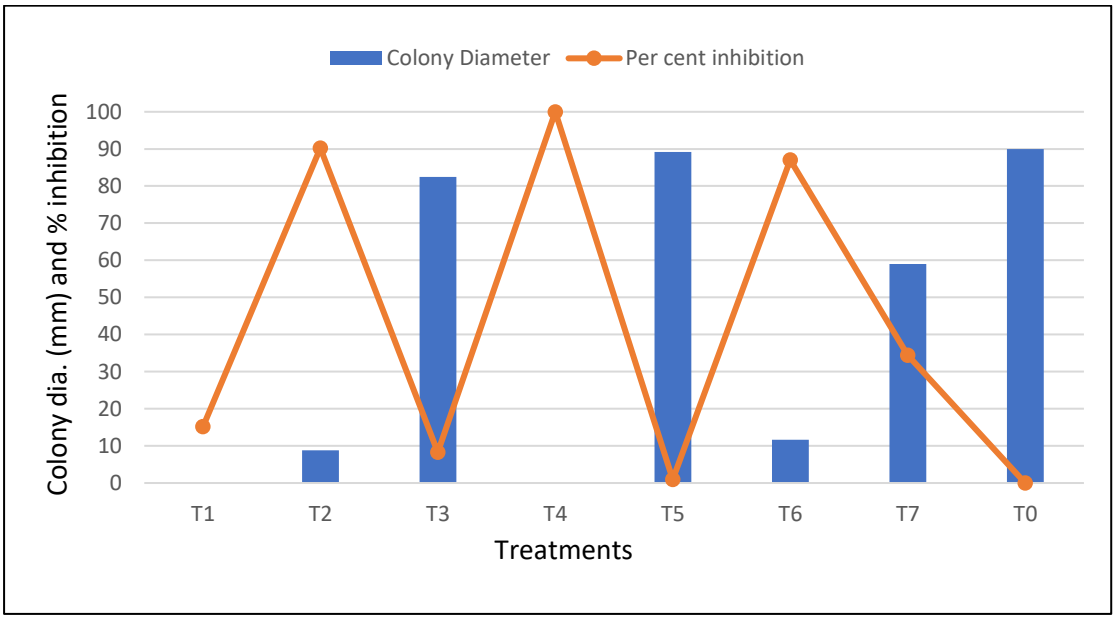
T7= *Allium cepa*

T0= Control

PLATE- VII: Effects of 20% ethanol-based extracts of botanicals on *Colletotrichum lindemuthianum*



@ 10 %



@ 20 %

T1:	Neem	T5:	Custard apple
T2:	Garlic	T6:	Ginger
T3:	Tulsi	T7:	Onion
T4:	Turmeric	T0:	Control

4.2: Effect of ethanol-based extracts of botanicals on *Colletotrichum lindemuthianum*

The similar results were reported by earlier workers, Amadioha, (2003) reported that, alcohol-based extract of *Piper nigrum* was best in inhibiting the mycelial growth of *C. lindemuthianum* followed by *Ocimum santum*. Shovan *et al.* (2008) reported that, Garlic extract at 20% concentration was found best in inhibiting radial mycelial growth of *C. dematium.* Other researchers also reported similar finding such as, Khan and Narseen (2010), Kulkarni and Raja (2019b) and Vasuki *et al.* (2020).

4.3.2 Efficacy of bioagents against *Colletotrichum lindemuthianum*:

In present studies, seven biocontrol agents were tested against *C. lindemuthianum* by applying dual culture technique as detailed under material and methods and the results obtained are presented below.

Radial mycelial growth:

Results (Table 4.5) revealed that out of the seven biocontrol agents tested, *Trichoderma harzianum* (T_2) was found most effective in controlling the mycelial growth of *C. lindemuthianum* (41.00 mm) which was statistically at par with *T. asperellum* (T_1) (46.33 mm) and significant over rest of the treatments including untreated control (T_0), which recorded 90.00 mm mycelial growth of *C. lindemuthianum*. The *T. asperellum* (T_1) was statistically at par with *T. virens* (T_3) (54.67 mm). The *T. virens* (T_3) was statistically significant over *T. harzianum* (T_2) and *Verticillium lecanii* (T_6) and statistically at par with *T. asperellum* (T_1) (46.33 mm), *Aspergillus niger* (T_4) (63.33 mm), *Metarhizium anisopliae* (T_5) (65.33 mm) and *Paecilomyces lilacinus* (T_7) (65.33 mm). The treatment of *Aspergillus niger* (T_4) was at par with the treatments of *Metarhizium anisopliae* (T_5) (65.33 mm), *Paecilomyces lilacinus* (T_7) (65.33 mm) and *T. virens* (T_3) (54.67 mm). The treatment of *Verticillium lecanii* (T_6) was not effective in controlling the mycelial growth of *C. lindemuthianum* (67.33 mm) which was statistically at par with *Aspergillus niger* (T_4) (63.33 mm), *Metarhizium anisopliae* (T_5) (65.33 mm) and *Paecilomyces lilacinus* (T_7) (65.33 mm). The untreated control recorded full mycelial growth of *C. lindemuthianum* (90.00 mm).

Per cent inhibition of *C. lindemuthianum*:

Results (Figure 4.3 and plate-VIII) revealed that out of the seven biocontrol agents tested, *Trichoderma harzianum* (T_2) was found most effective in inhibiting the growth of *C. lindemuthianum* (54.44 %) which was statistically at par with *T. asperellum* (T_1), which recorded 48.52 % inhibition of *C. lindemuthianum* over untreated control (T_0) (00.00 %). The *T. asperellum* (T_1) was statistically at par with *T. virens* (T_3) (39.25 %). The least mycelial inhibition (25.18 %) of *C. lindemuthianum* was recorded with *Verticillium lecanii* which was statistically at par with *Metarhizium anisopliae* (T_5) (27.41 %), *Paecilomyces lilacinus* (T_7) (27.41 %), *Aspergillus niger* (T_4) (29.63 %) and T. *virens* (T_3) (39.25 %).

Table 4. 5: Efficacy of bioagents against *C. lindemuthianum*

Tr. No.	Bioagents	Radial mycelial growth (mm)	Per cent inhibition
T_1	*Trichoderma asperellum*	46.33	48.52 (44.10)*
T_2	*Trichoderma harzianum*	41.00	54.44 (47.54)
T_3	*Trichoderma virens*	54.67	39.25 (38.67)
T_4	*Aspergillus niger*	63.33	29.63 (32.86)
T_5	*Metarhizium anisopliae*	65.33	27.41 (31.55)
T_6	*Verticillium lecanii*	67.33	25.18 (30.06)
T_7	*Paecilomyces lilacinus*	65.33	27.41 (31.55)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E. (m) ±		**3.64**	**2.40**
C.D. at 1%		**11.00**	**7.27**

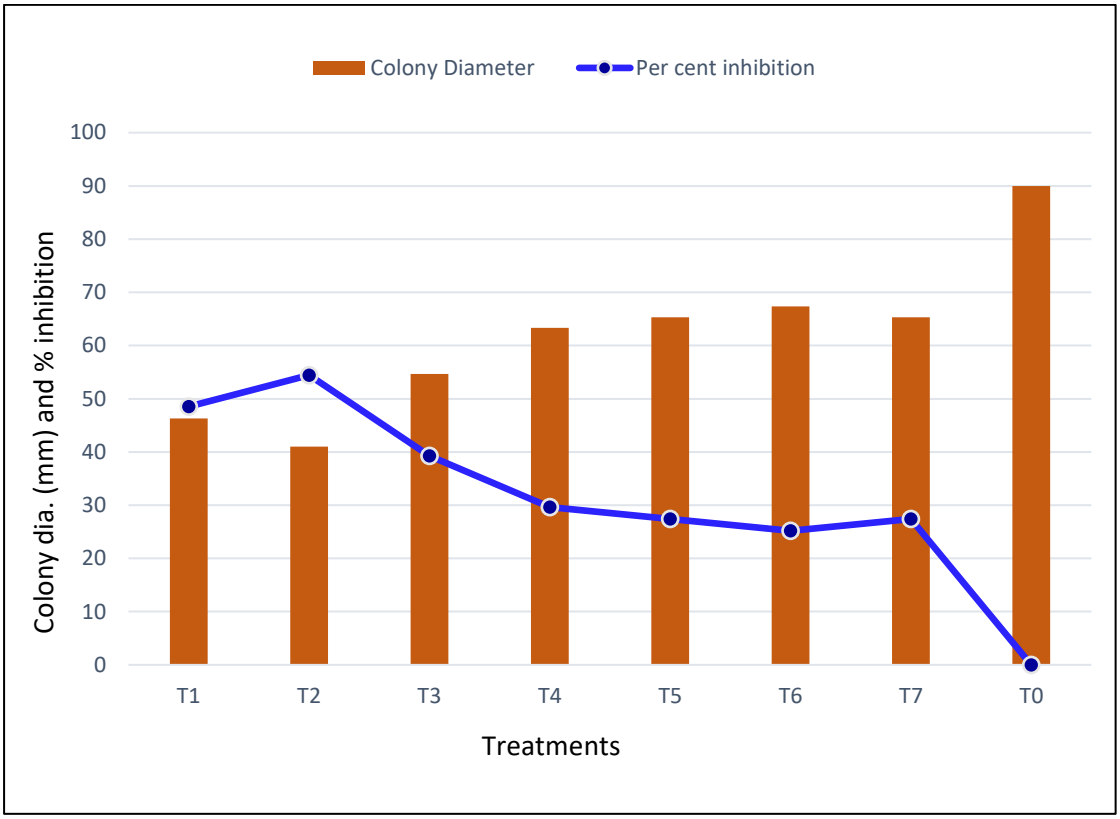
* Figure in parenthesis are angular transformed values.

The similar results were reported by earlier workers, Rajesha *et al.* (2010) reported that the highest mycelial growth inhibition of *C. lindemithianum* was recorded with *Trichoderma harzianum* (73.54%) followed by *T. viride* (50.90%) Padder *et al.* (2010) also reported that under *in vitro* and *in vivo* conditions, the highest mycelial growth inhibition of *C. lindemithianum* was recorded with

PLATE – VIII

T1= *Trichoderma asperellum*
T2= *Trichoderma harzianum*
T3= *Trichoderma virens*
T4= *Aspergillus niger*
T5= *Metarhizium anisopliae*
T6= *Verticillium lecanii*
T7= *Paecilomyces lilacinus*
T0= Control

PLATE- VIII: Efficacy of bioagents against *Colletotrichum lindemuthianum*



T1: *Trichoderma asperellum*
T2: *Trichoderma harzianum*
T3: *Trichoderma virens*
T4: *Aspergillus niger*
T5: *Metarhiziumanisopliae*
T6: *Verticillium lecanii*
T7: *Paecilomyceslilacinus*
T0: Control

4.3: Efficacy of bioagents against *Colletotrichum lindemuthianum*

Trichoderma viride (69.21%) followed by *harzianum* (60.32%). Some other researchers also reported similar findings such as Ahamad *et al*. (2018), Rawat *et al*. (2018), Sharma *et al*. (2021) and Sushmitha and Zacharia (2021).

4.3.3 Efficacy of fungicides against *Colletotrichum lindemuthianum*

In present studies seven systemic fungicides (@ 500, 1000 and 1500 ppm) and seven non-systemic and combi product fungicides (@1000, 1500 and 2000 ppm) were tested for their efficacy against *C. lindemuthianum* by applying Poisoned Food Technique (Nene and Thapliyal, 1993) using Potato Dextrose Agar (PDA). Effects of these fungicides on radial mycelial growth and per cent inhibition of *C. lindemuthianum* were recorded and are presented here under.

4.3.3.1: Systemic fungicides at 500 ppm:

Radial mycelial growth:

The results (Table 4.6) revealed that at 500 ppm the systemic fungicides Propiconazole (T_1), Tebuconazole (T_2) and Carbendazim (T_4) were found most effective in controlling the mycelial growth of *C. lindemuthianum*. These treatments did not allow *C. lindemuthianum* mycelium to grow (00.00 mm) and were statistically at par with each other. The next effective systemic fungicide found was Difenconazole (T_6), which recorded 10.67 mm mycelial growth of *C. lindemuthianum* and was statistically significant over rest of the treatments including untreated control (T_0).

The fungicide Pyraclostrobin (T_5) was least effective in controlling the mycelial growth of *C. lindemuthianum* at 500 ppm (53.17 mm). It was statistically significant over rest of the treatments and followed by Azoxystrobin (T_7) (18.00 mm) and Hexaconazole (T_5) (15.33 mm), which were statistically at par with each other and significant over rest of the treatments.

Per cent inhibition of *C. lindemuthianum*:

The results (Figure 4.4 Plate-IX) revealed that at 500 ppm concentration the systemic fungicides Propiconazole (T_1), Tebuconazole (T_2) and Carbendazim (T_4) completely inhibited the mycelial growth of *C. lindemuthianum* (100.00 %) and were

statistically at par with each other and significant over rest of the treatments. The next best systemic fungicide found was Difenoconazole (T_6), which recorded 88.14 % inhibition of radial mycelial growth of *C. lindemuthianum* over untreated control (T_0) (00.00%) and was statistically significant over rest of the treatments.

The fungicide Pyraclostrobin (T_5) (40.92%) was least effective in inhibition of radial mycelial growth of *C. lindemuthianum* at 500 ppm concentration and was statistically significant over rest of the treatments. It was followed by Azoxystrobin (T_7) (80.00%) and Hexaconazole (T_3) (82.96%), which were statistically at par with each other and significant over rest of the treatments.

Table 4.6: *In vitro* effects of 500 ppm systemic fungicides on *Colletotrichum lindemuthianum*

Tr. No.	Fungicides	Radial mycelial growth (mm)	Per cent inhibition of mycelial growth
T_1	Propiconazole 25% EC	00.00	100.00 (90.00)*
T_2	Tebuconazole 29.9% EC	00.00	100.00 (90.00)
T_3	Hexaconazole 5% EC	15.33	82.96 (65.59)
T_4	Carbendazim 50% WP	00.00	100.00 (90.00)
T_5	Pyraclostrobin 20% WG	53.17	40.92 (39.72)
T_6	Difenoconazole 25% EC	10.67	88.14 (69.84)
T_7	Azoxystrobin 25% EC	18.00	80.00 (63.41)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E. (m) ±		**1.25**	**0.84**
C.D. at 1%		**3.79**	**2.56**

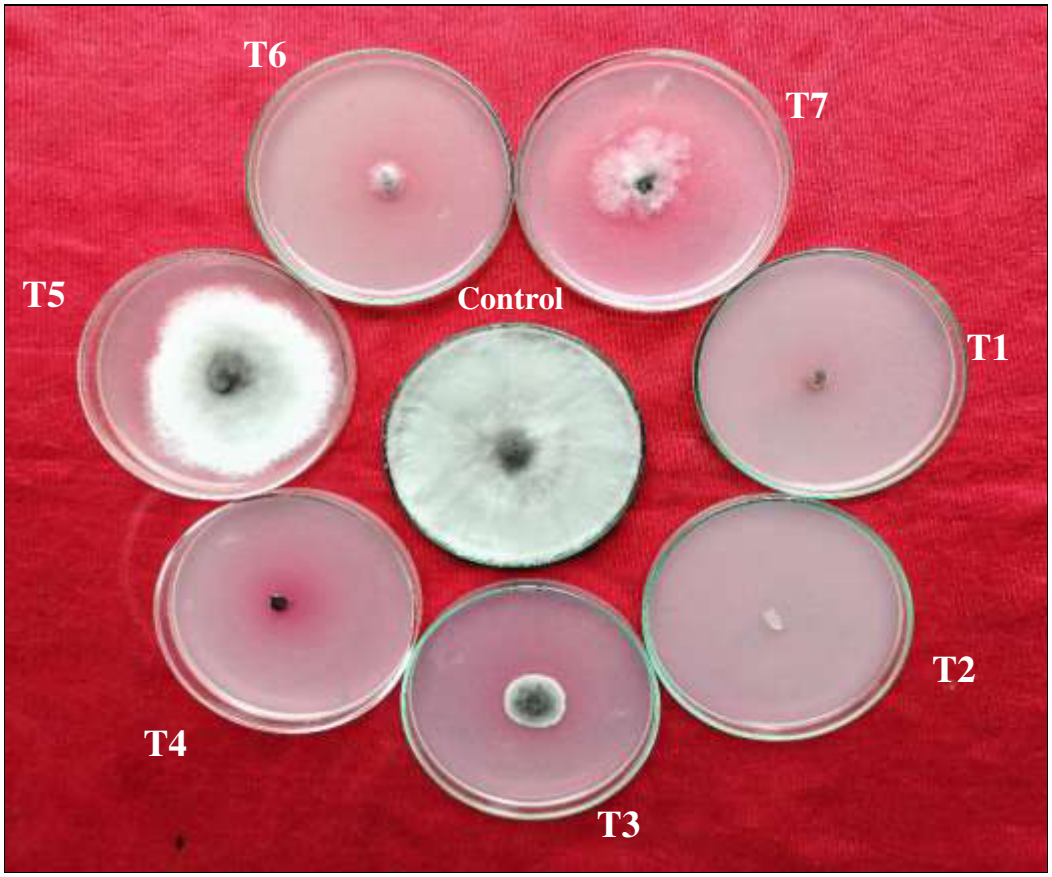
* Figure in parenthesis are angular transformed values.

4.3.3.2: Systemic fungicides at 1000 ppm:

Radial mycelial growth:

The results (Table 4.7) revealed that at 1000 ppm all the systemic fungicide treatments were found statistically significant over untreated control (T_0). The

PLATE – IX



$T_{1=}$ Propiconazole 25% EC

$T_{2=}$ Tebuconazole 29.9% EC

$T_{3=}$ Hexaconazole 5% EC

$T_{4=}$ Carbendazim 50% WP

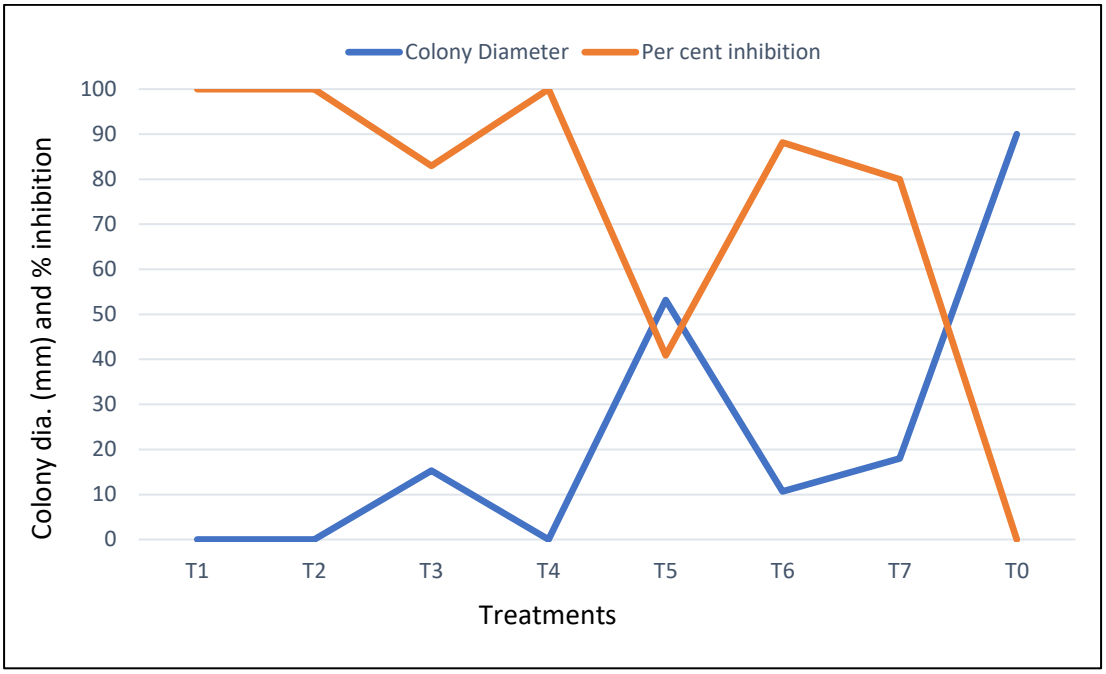
$T_{5=}$ Pyraclostrobin 20% WG

$T_{6=}$ Difenoconazole 25% EC

$T_{7=}$ Azoxystrobin 25% EC

$T_{0=}$ Control

PLATE IX: Effects of 500 ppm systemic fungicides on *Colletotrichum lindemuthianum.*



$T_{1=}$ Propiconazole 25% EC

$T_{2=}$ Tebuconazole 29.9% EC

$T_{3=}$ Hexaconazole 5% EC

$T_{4=}$ Carbendazim 50% WP

$T_{5=}$ Pyraclostrobin 20% WG

$T_{6=}$ Difenoconazole 25% EC

$T_{7=}$ Azoxystrobin 25% EC

$T_{0=}$ Control

4.4: Effects of 500 ppm systemic fungicides on *Colletotrichum lindemuthianum*

fungicides Propiconazole (T_1), Tebuconazole (T_2), Carbendazim (T_4) and Difenoconazole (T_6) were found most effective in controlling the mycelial growth of *C. lindemuthianum*. These treatments did not allow *C. lindemuthianum* mycelium to grow (00.00mm) and were statistically at par with each other. The next effective systemic fungicide found was Hexaconazole (T_3), which recorded 10.00 mm mycelial growth of *C. lindemuthianum* and was statistically significant over rest of the treatments including untreated control (T_0).

The fungicide Pyraclostrobin (T_5) was least effective in controlling the mycelial growth of *C. lindemuthianum* at 1000 ppm (36.83 mm). It was statistically significant over rest of the treatments and followed by Azoxystrobin (T_7) (17.50 mm).

Table 4.7: *In vitro* effects of 1000 ppm systemic fungicides on *Colletotrichum lindemuthianum*

Tr. No.	Fungicides	Radial mycelial growth (mm)	Per cent inhibition of mycelial growth
T_1	Propiconazole 25% EC	00.00	100.00 (90.00)*
T_2	Tebuconazole 29.9% EC	00.00	100.00 (90.00)
T_3	Hexaconazole 5% EC	10.00	88.88 (70.49)
T_4	Carbendazim 50% WP	00.00	100.00 (90.00)
T_5	Pyraclostrobin 20% WG	36.83	59.07 (50.28)
T_6	Difenoconazole 25% EC	00.00	100.00 (90.00)
T_7	Azoxystrobin 25% EC	17.50	88.55 (63.90)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E. (m) ±		**1.95**	**1.34**
C.D. at 1%		**5.92**	**4.07**

* Figure in parenthesis are angular transformed values.

Per cent inhibition of *C. lindemuthianum*:

The results (Figure 4.5 Plate-X) revealed that at 1000 ppm concentration the systemic fungicides Propiconazole (T_1), Tebuconazole (T_2), Carbendazim (T_4) and

Difenoconazole (T_6) completely inhibited the mycelial growth of *C. lindemuthianum* (100.00 %) and were statistically at par with each other and significant over rest of the treatments. The next best systemic fungicide found was Hexaconazole (T_3), which recorded 88.88 % inhibition of radial mycelial growth of *C. lindemuthianum* and was statistically at par with Azoxystrobin (T_7) which recorded 88.55% inhibition over untreated control (T_0).

The fungicide Pyraclostrobin (T_5) (59.07%) was least effective in inhibition of radial mycelial growth of *C. lindemuthianum* at 1000 ppm concentration and was statistically significant over rest of the treatments including untreated control (T_0).

4.3.3.3: Systemic fungicides at 1500 ppm:

Radial mycelial growth:

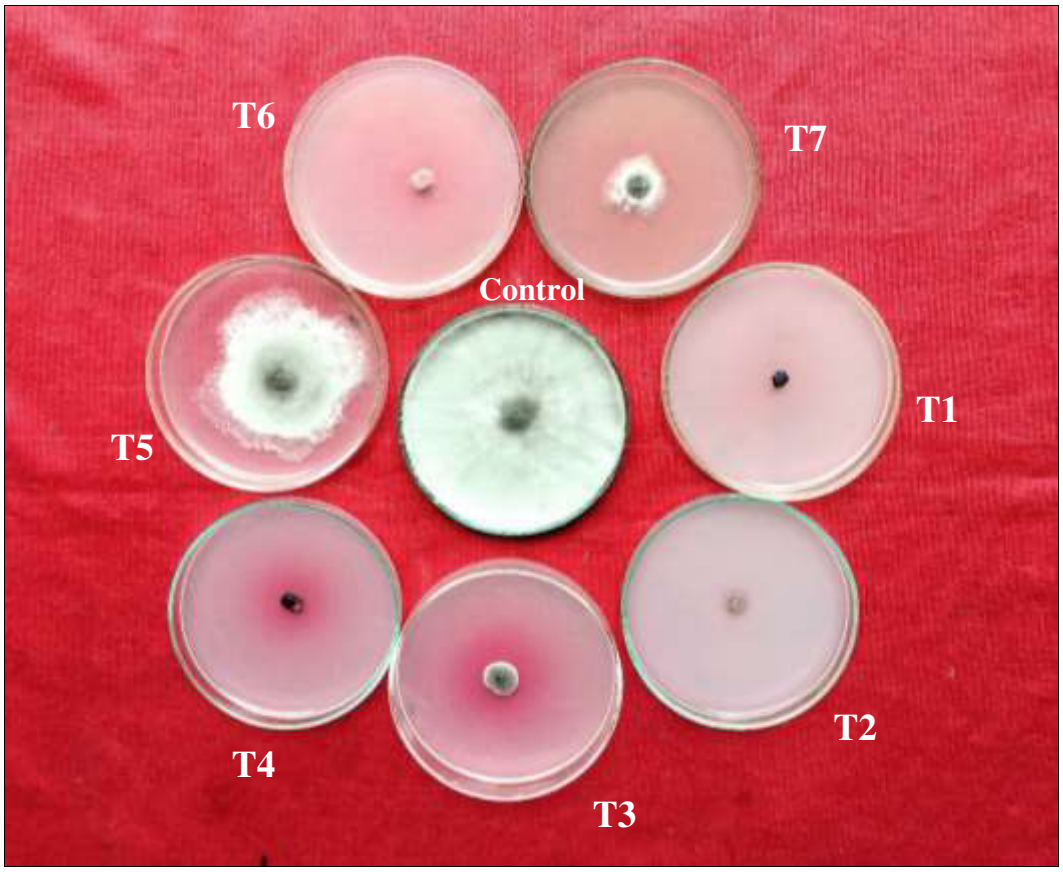
The results (Table 4.8) revealed that at 1500 ppm concentration the systemic fungicide treatments showed the similar trend as for 1000 ppm. The fungicides Propiconazole (T_1), Tebuconazole (T_2), Carbendazim (T_4) and Difenoconazole (T_6) were found most effective in controlling the mycelial growth of *C. lindemuthianum*. These treatments not allowed *C. lindemuthianum* to grow (00.00 mm) and were statistically at par with each other. The next effective systemic fungicide found was Hexaconazole (T_3) (10.00 mm) followed by Azoxystrobin (T_7) (15.67%) which were statistically significant with each other and over rest of the treatments including untreated control (T_0) (90.00 mm).

The fungicide Pyraclostrobin (T_5) was found least effective and allowed 69.50 mm mycelial growth of *C. lindemuthianum*.

Per cent inhibition of *C. lindemuthianum*:

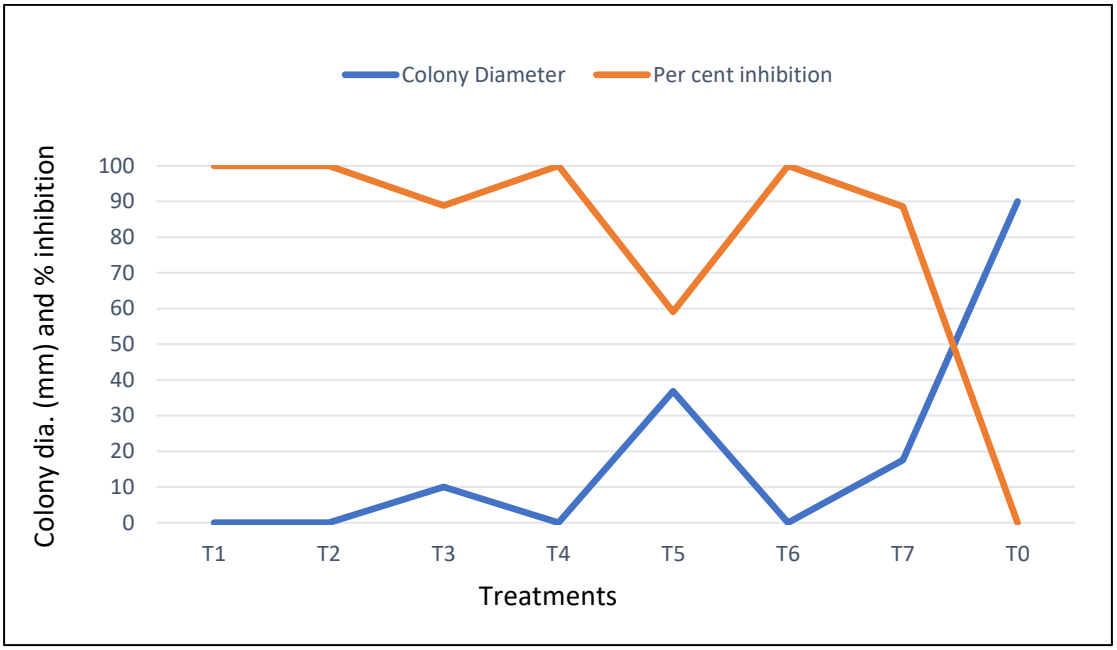
The results (Figure 4.6 Plate-XI) revealed that at 1500 ppm concentration the systemic fungicide treatments showed the similar trend as for 1000 ppm concentration. The fungicides Propiconazole (T_1), Tebuconazole (T_2), Carbendazim (T_4) and Difenoconazole (T_6) were found most effective in inhibiting the mycelial growth of *C. lindemuthianum*. These fungicide treatments completely inhibited the mycelial growth of *C. lindemuthianum* (100.00 %) and were statistically at par with each other. The next effective systemic fungicide found was Hexaconazole (T_3)

PLATE – X



$T_{1=}$ Propiconazole 25% EC
$T_{2=}$ Tebuconazole 29.9% EC
$T_{3=}$ Hexaconazole 5% EC
$T_{4=}$ Carbendazim 50% WP
$T_{5=}$ Pyraclostrobin 20% WG
$T_{6=}$ Difenoconazole 25% EC
$T_{7=}$ Azoxystrobin 25% EC
$T_{0=}$ Control

PLATE X: Effects of 1000 ppm systemic fungicides on *Colletotrichum lindemuthianum*



$T_{1=}$ Propiconazole 25% EC

$T_{2=}$ Tebuconazole 29.9% EC

$T_{3=}$ Hexaconazole 5% EC

$T_{4=}$ Carbendazim 50% WP

$T_{5=}$ Pyraclostrobin 20% WG

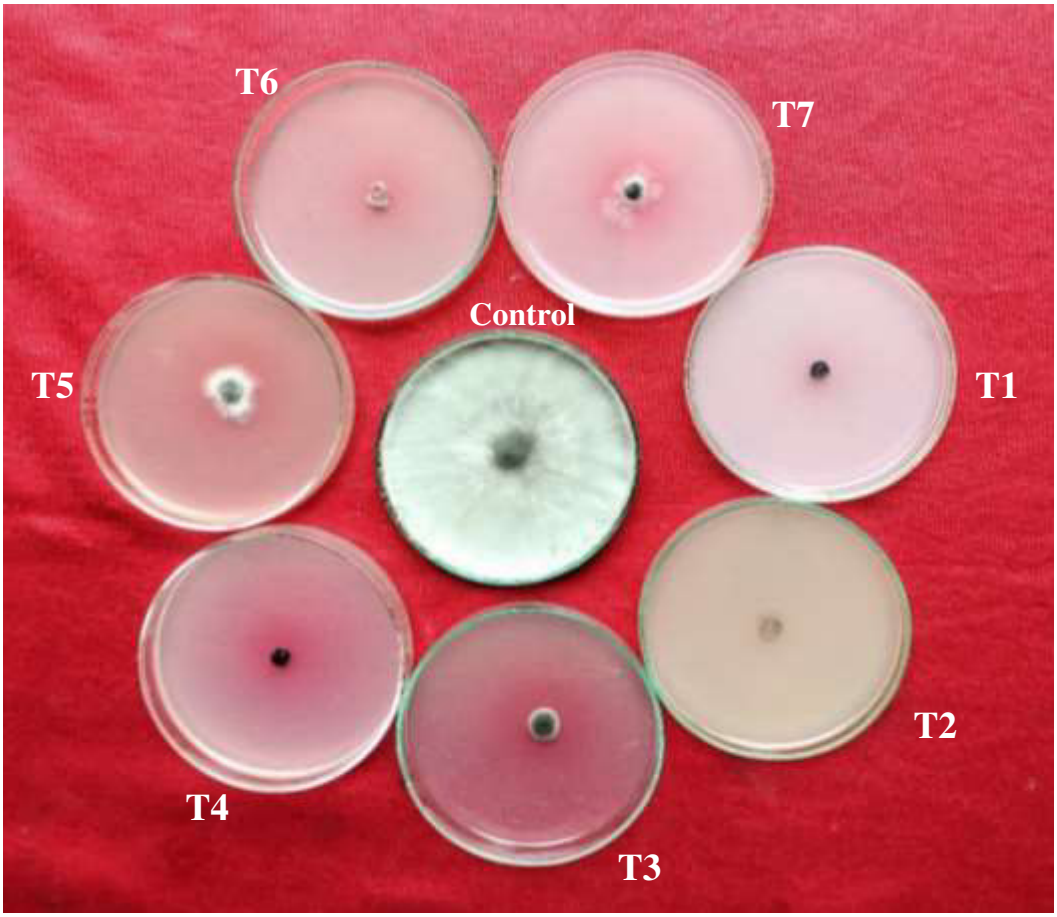
$T_{6=}$ Difenoconazole 25% EC

$T_{7=}$ Azoxystrobin 25% EC

$T_{0=}$ Control

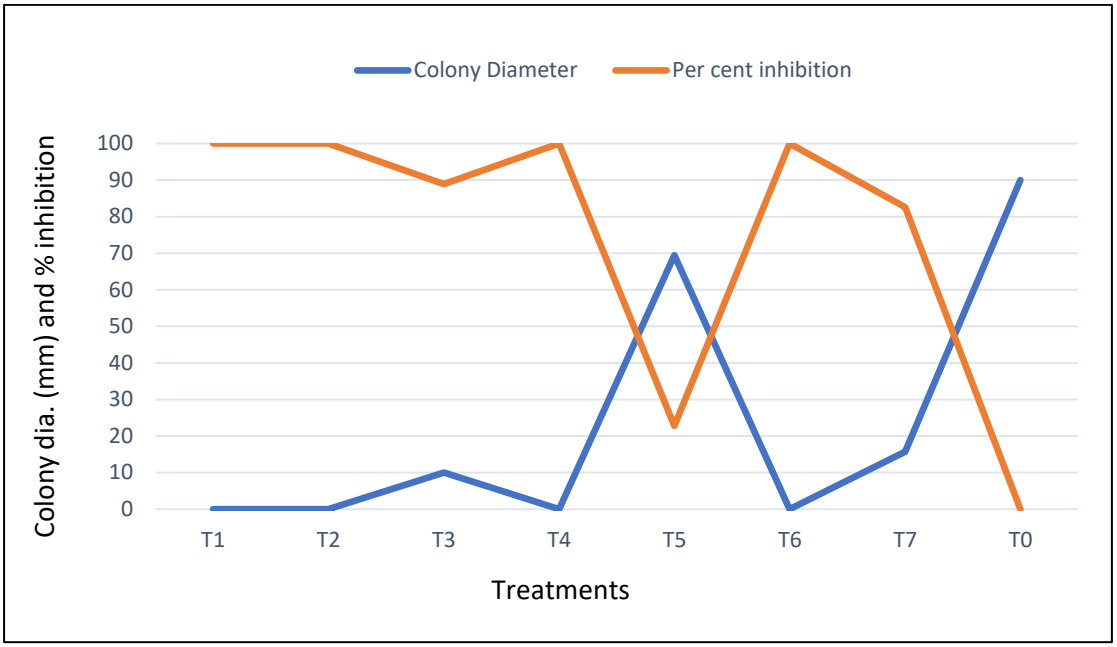
4.5:Effects of 1000 ppm systemic fungicides on *Colletotrichum lindemuthianum*

PLATE – XI



$T_{1=}$ Propiconazole 25% EC
$T_{2=}$ Tebuconazole 29.9% EC
$T_{3=}$ Hexaconazole 5% EC
$T_{4=}$ Carbendazim 50% WP
$T_{5=}$ Pyraclostrobin 20% WG
$T_{6=}$ Difenoconazole 25% EC
$T_{7=}$ Azoxystrobin 25% EC
$T_{0=}$ Control

PLATE XI: Effects of 1500 ppm systemic fungicides on *Colletotrichum lindemuthianum*



$T_{1=}$ Propiconazole 25% EC

$T_{2=}$ Tebuconazole 29.9% EC

$T_{3=}$ Hexaconazole 5% EC

$T_{4=}$ Carbendazim 50% WP

$T_{5=}$ Pyraclostrobin 20% WG

$T_{6=}$ Difenoconazole 25% EC

$T_{7=}$ Azoxystrobin 25% EC

$T_{0=}$ Control

4.6:Effects of 1500 ppm systemic fungicides on *Colletotrichum lindemuthianum*

(88.88%) followed by Azoxystrobin (T_7) (82.58%) which were statistically significant with each other and over rest of the treatments including untreated control (T_0) (00.00%).

The fungicide Pyraclostrobin (T_5) was found least effective and inhibited only 22.77 % mycelial growth of *C. lindemuthianum* over untreated control (T_0).

Table 4.8: *In vitro* effects of 1500 ppm systemic fungicides on *Colletotrichum lindemuthianum*

Tr. No.	Fungicides	Radial mycelial growth (mm)	Per cent inhibition of mycelial growth
T_1	Propiconazole 25% EC	00.00	100.00 (90.00)*
T_2	Tebuconazole 29.9% EC	00.00	100.00 (90.00)
T_3	Hexaconazole 5% EC	10.00	88.88 (70.52)
T_4	Carbendazim 50% WP	00.00	100.00 (90.00)
T_5	Pyraclostrobin 20% WG	69.50	22.77 (28.50)
T_6	Difenoconazole 25% EC	00.00	100.00 (90.00)
T_7	Azoxystrobin 25% EC	15.67	82.58 (65.33)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E. (m) ±		**0.90**	**1.71**
C.D. at 1%		**2.73**	**5.18**

* Figure in parenthesis are angular transformed values.

The similar results were earlier reported by Aggarwal *et al.* (2015), who reported that the cent per cent inhibition mycelial growth of *C. lindemuthianum* was recorded with Tebuconazole at 250, 500, 1000 and 2500 ppm concentration. Wagh *et al*. (2015) reported that the fungicide Propiconazole at 500, 1000 and 1500 ppm was completely inhibited the mycelial growth of *C. capsici*. Chacko and Gokulapalan (2015) reported that Propiconazole @ 0.05% and Tebuconazole 0.1% completely inhibited mycelial growth of *C. capsici.* Some of other researchers also reported similar finding such as Badgujar *et al*. (2017), Katediya *et al*. (2019) and Rajashree *et al.* (2020).

4.3.3.4: Combi products and non-systemic fungicides at 1000 ppm:

Radial mycelial growth:

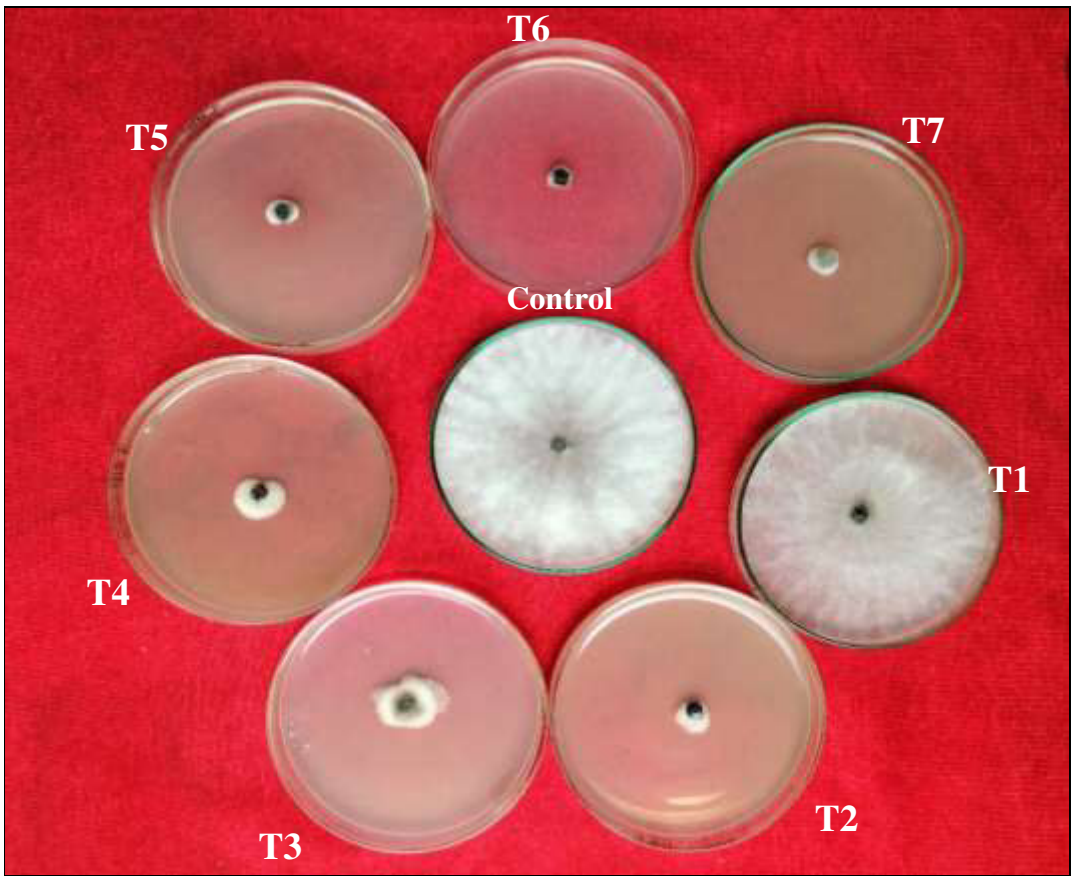
In present studies total seven (4 non-systemic and 3 combi product) fungicides were evaluated for their efficacy against *C. lindemuthianum* by applying Poisoned Food Technique (Nene and Thapliyal, 1993) using PDA as basal media as detailed in material and methods. Effects of these fungicides on radial mycelial growth and inhibition was recorded and the results obtained are presented here.

The results (Table 4.9) revealed that at 1000 ppm concentration the combi product fungicide Captan + Hexaconazole (T_5) was found most effective and not allowed *C. lindemuthianum* mycelium to grow (00.00 mm). This treatment was statistically significant over rest of the treatments including untreated control (T_0) (90.00 mm). This was followed by the combi product fungicide Carbendazim + Mancozeb (T_6), which recorded only 07.83 mm mycelial growth of *C. lindemuthuanum.* The next effective non-systemic fungicide found was Chlorothalonil (T_2) (11.00 mm), which was statistically at par with the combi product fungicide Metalaxyl + Mancozeb (T_7) (11.17 mm). The next best effective non-systemic fungicide found was Propineb (T_4) (15.67 mm) followed by Copper oxychloride (T_3) (18.83 mm). The non-systemic fungicide Mancozeb (T_1) was not effective and allowed full mycelial growth of *C. lidemuthianum* (90.00 mm), which was statistically at par with untreated control (T_0) (90.00 mm).

Per cent inhibition of *C. lindemuthianum*:

The result (Figure 4.7 Plate-XII) revealed that at 1000 ppm concentration the combi product fungicide Captan + Hexaconazole (T_5) has completely inhibited the mycelial growth of *C. lindemuthianum* (100%). This treatment was statistically significant over rest of the treatments including untreated control (T_0). This was followed by combi product fungicide Carbendazim + Mancozeb (T_6), which recorded 91.30% inhibition of radial mycelial growth of *C. lindemuthianum*. The next effective non-systemic fungicide found was Chlorothalonil (T_2) which recorded 87.77% inhibition over untreated control (T_0) and was statistically at par with combi product fungicide Metalaxyl + Mancozeb (T_7) (87.58%). It was followed by non-systemic fungicides Propineb (T_4) (85.00%) and Copper oxychloride (T_3) (79.07%). The

PLATE – XII



$T_{1=}$ Mancozeb 75% WP

$T_{2=}$ Chlorothalonil 75% WP

$T_{3=}$ Copper oxychloride 50% WP

$T_{4=}$ Propineb 70% WP

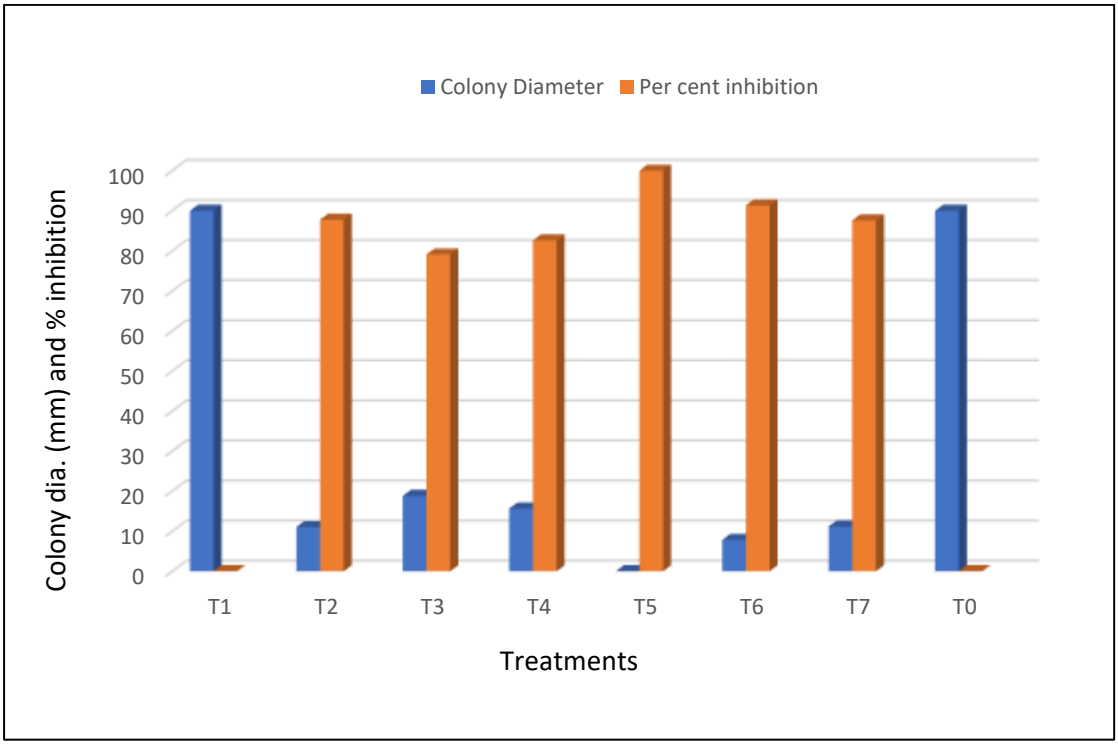
$T_{5=}$ Captan 70% + Hexaconazole 5% (75% WP)

$T_{6=}$ Carbendazim 12% + Mancozeb 63% (75%WP)

T7=Metalaxyl 8% + Mancozeb 64% (72% WP)

$T_{0=}$ Control

PLATE XII: Effects of 1000 ppm combi products and non-systemic fungicides on *Colletotrichum lindemuthianum.*



$T_{1=}$ Mancozeb 75% WP

$T_{2=}$ Chlorothalonil 75% WP

$T_{3=}$ Copper oxychloride 50% WP

$T_{4=}$ Propineb 70% WP

$T_{5=}$ Captan 70% + Hexaconazole 5% (75% WP)

$T_{6=}$ Carbendazim 12% + Mancozeb 63% (75%WP)

T7=Metalaxyl 8% + Mancozeb 64% (72% WP)

$T_{0=}$ Control

4.7: Effects of 1000 ppm combi products and non-systemic fungicides on *Colletotrichum lindemuthianum*

fungicide Mancozeb (T_1) was failed to inhibit the mycelial growth of *C. lindemuthianum* (00.00%). This treatment was statistically at par with the untreated control (T_0).

Table 4.9*: In vitro* effects of 1000 ppm combi products and non-systemic fungicides on *Colletotrichum lindemuthianum*.

Tr. No.	Fungicides	Radial mycelial growth (mm)	Per cent inhibition of mycelial growth
T_1	Mancozeb 75% WP	90.00	00.00 (00.00)*
T_2	Chlorothalonil 75% WP	11.00	87.77 (69.50)
T_3	Copper oxychloride 50% WP	18.83	79.07 (62.76)
T_4	Propineb 70% WP	15.67	82.58 (65.31)
T_5	Captan 70% + Hexaconazole 5% (75% WP)	00.00	100.00 (90.00)
T_6	Carbendazim 12% + Mancozeb 63% (75%WP)	07.83	91.30 (72.83)
T_7	Metalaxyl 8% + Mancozeb 64% (72% WP)	11.17	87.58 (69.35)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E.(m) ±		**0.43**	**0.39**
C.D. at 1%		**1.32**	**1.19**

* Figure in parenthesis are angular transformed values.

4.3.3.4: Combi products and non-systemic fungicides at 2000 ppm:

Radial mycelial growth:

The results (Table 4.10) revealed that at 2000 ppm concentration the combi product fungicide Captan + Hexaconazole (T_5) and Metalaxyl + Mancozeb (T_7) were found most effective and not allowed *C. lindemuthianum* mycelium to grow (00.00 mm). These treatments were statistically at par with each other and significant over rest of the treatments including untreated control (T_0) (90.00 mm). This was followed by combi product fungicide Carbendazim + Mancozeb (T_6) (08.17 mm) and non-

systemic fungicide Chlorothalonil (T_2) (12.33 mm), which were statistically at par with each other.

The next best non-systemic fungicide found was Propineb (T_4) (13.50 mm), which was statistically at par with Chlorothalonil (T_2) and followed by Copper oxy Chloride (T_3) which recorded 21.00 mm mycelial growth of *C. lindemuthianum*. The non-systemic fungicide Mancozeb (T_1) was not effective and allowed full mycelial growth (90.00 mm) of *C. lindemuthianum*, which was statistically at par with the untreated control (T_0) (90.00 mm).

Table 4.10: *In vitro* effects of 2000 ppm combi products and non-systemic fungicides on *Colletotrichum lindemuthianum*

Tr. No.	Fungicides	Radial mycelial growth (mm)	Per cent inhibition of mycelial growth
T_1	Mancozeb 75% WP	90.00	00.00 (00.00)*
T_2	Chlorothalonil 75% WP	12.33	86.30 (68.75)
T_3	Copper oxychloride 50% WP	21.00	76.66 (61.09)
T_4	Propineb 70% WP	13.50	85.00 (67.22)
T_5	Captan 70% + Hexaconazole 5% (75% WP)	00.00	100.00 (90.00)
T_6	Carbendazim 12% + Mancozeb 63% (75%WP)	08.17	90.92 (72.48)
T_7	Metalaxyl 8% + Mancozeb 64% (72% WP)	00.00	100.00 (90.00)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E.(m) ±		**1.56**	**1.42**
C.D. at 1%		**4.73**	**4.29**

* Figure in parenthesis are angular transformed values.

Per cent inhibition of *C. lindemuthianum*:

The result (Figure 4.8 Plate-XIII) revealed that at 2000 ppm concentration the combi product fungicide Captan + Hexaconazole (T_5) and Metalaxyl + Mancozeb (T_7) has completely inhibited the mycelial growth of *C. lindemuthianum* (100%) and were

PLATE – XIII

$T_{1=}$ Mancozeb 75% WP

$T_{2=}$ Chlorothalonil 75% WP

$T_{3=}$ Copper oxychloride 50% WP

$T_{4=}$ Propineb 70% WP

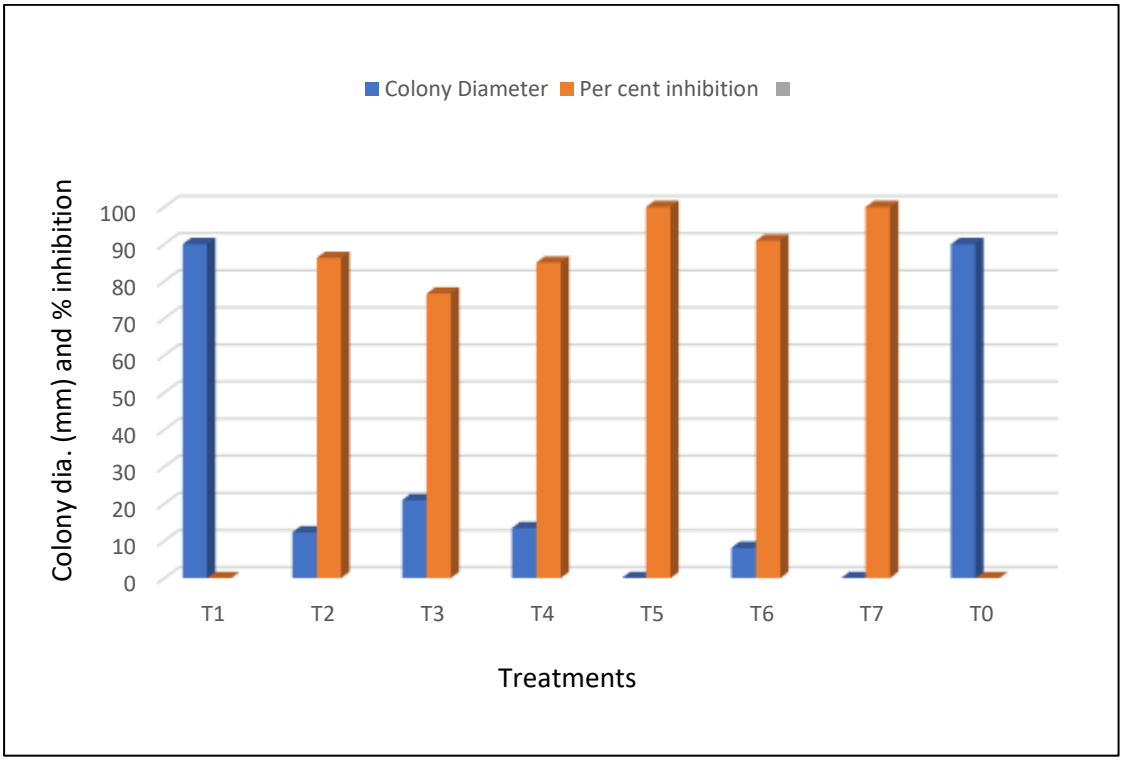
$T_{5=}$ Captan 70% + Hexaconazole 5% (75% WP)

$T_{6=}$ Carbendazim 12% + Mancozeb 63% (75%WP)

T7=Metalaxyl 8% + Mancozeb 64% (72% WP)

$T_{0=}$ Control

PLATE XIII: Effects of 2000 ppm combi products and non-systemic fungicides on *Colletotrichum lindemuthianum.*



T_1= Mancozeb 75% WP

T_2= Chlorothalonil 75% WP

T_3= Copper oxychloride 50% WP

T_4= Propineb 70% WP

T_5= Captan 70% + Hexaconazole 5% (75% WP)

T_6= Carbendazim 12% + Mancozeb 63% (75%WP)

T7=Metalaxyl 8% + Mancozeb 64% (72% WP)

T_0= Control

4.8: Effects of 2000 ppm combi products and non-systemic fungicides on *Colletotrichum lindemuthianum*

statistically at par with each other and significant over rest of the treatments. This was followed by Carbendazim + Mancozeb (T_6), which recorded 90.92% inhibition of radial mycelial growth of *C. lindemuthianum* and was statistically at par with non-systemic fungicide Chlorothalonil (T_2) which recorded 86.30% inhibition over untreated control. The next best non-systemic fungicide found was Propineb (T_4) (85.00%) which was statistically at par with Chlorothalonil (T_2) (86.30%) and followed by Copper oxychloride (T_3) which recorded 76.66% mycelial growth of *C. lindemuthianum*. The fungicide Mancozeb (T_1) was failed to inhibit the mycelial growth of *C. lindemuthianum* (00.00%). This treatment was statistically at par with the untreated control (T_0)

4.3.3.5: Combi products and non-systemic fungicides at 2500 ppm:

Radial mycelial growth:

The results (Table 4.11) revealed that at 2500 ppm concentration the combi product fungicide Captan + Hexaconazole (T_5), Carbendazim + Mancozeb (T_6) and Metalaxyl + Mancozeb (T_7) were found most effective and not allowed *C. lindemuthianum* mycelial to grow (00.00mm). These treatments were statistically at par with each other and with Propineb (T_4) which allowed only 02.00 mm mycelial growth and significant over rest of the treatments including untreated control (T_0) (90.00 mm). The next best non-systemic fungicide found was Chlorothalonil (T_2) which recorded 16.50mm mycelial growth of *C. lindemuthianum*. This was followed by Copper oxychloride (T_3) (21.00mm). The fungicide Mancozeb (T_1) was not effective and allowed full mycelial growth (90.00 mm) of *C. lindemuthianum*, which was statistically at par with the untreated control (T_0) (90.00 mm).

Per cent inhibition of *C. lindemuthianum*:

The result (Figure 4.9 Plate-XIV) revealed that at 2500 ppm concentration the combi product fungicide Captan + Hexaconazole (T_5), Carbendazim + Mancozeb (T_6) and Metalaxyl + Mancozeb (T_7) completely inhibited the mycelial growth of *C. lindemuthianum* (100%) and were statistically at par with each other and with Propineb (T_4) which inhibited the mycelial growth 97.77% over untreated control (T_0) and significant over rest of the treatments. The next best non-systemic fungicide found was Chlorothalonil (T_2) which recorded 81.66% inhibition of *C.*

lindemuthianum. This was followed by Copper oxychloride (T_3) (76.66%). The fungicide Mancozeb (T_1) was failed to inhibit the mycelial growth of *C. lindemuthianum* (00.00%) and was statistically at par with the untreated control (T_0).

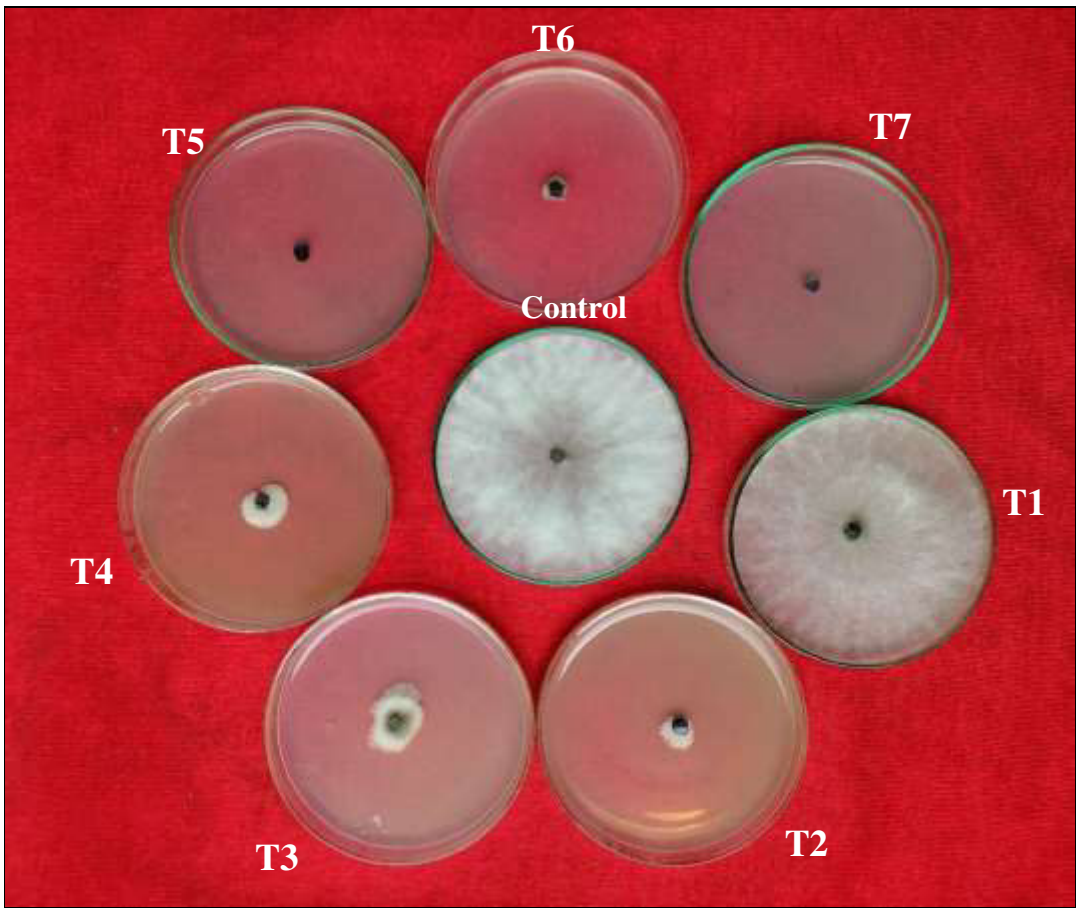
Table 4.11*: In vitro* effects of 2500 ppm combi products and non-systemic fungicides on *Colletotrichum lindemuthianum*

Tr. No.	Fungicides	Radial mycelial growth (mm)	Per cent inhibition of mycelial growth
T_1	Mancozeb 75% WP	90.00	00.00 (00.00)*
T_2	Chlorothalonil 75% WP	16.50	81.66 (64.76)
T_3	Copper oxychloride 50% WP	21.00	76.66 (61.13)
T_4	Propineb 70% WP	02.00	97.77 (85.00)
T_5	Captan 70% + Hexaconazole 5% (75% WP)	00.00	100.00 (90.00)
T_6	Carbendazim 12% + Mancozeb 63% (75%WP)	00.00	100.00 (90.00)
T_7	Metalaxyl 8% + Mancozeb 64% (72% WP)	00.00	100.00 (90.00)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E.(m) ±		**1.35**	**2.00**
C.D. at 1%		**4.08**	**6.05**

* Figure in parenthesis are angular transformed values.

The similar results were reported by earlier workers *viz*., Asalkar *et al.* (2019) reported that the cent per cent (100%) mycelial growth inhibition of *C. gloeosporioides* was recorded with Carbendazim + Mancozeb followed by Azoxystrobin (99.50%). Katediya *et al.* (2019) reported that the Captan 70% + Hexaconazole 5% and Carbendazim 12% + Mancozeb 64% completely inhibited mycelial growth of *C. capsici.* Some of other researchers also reported similar finding such as Bagade *et al*. (2020) and Rajashree *et al.* (2020).

PLATE – XIV



T1= Mancozeb 75% WP

T2= Chlorothalonil 75% WP

T3= Copper oxychloride 50% WP

T4= Propineb 70% WP

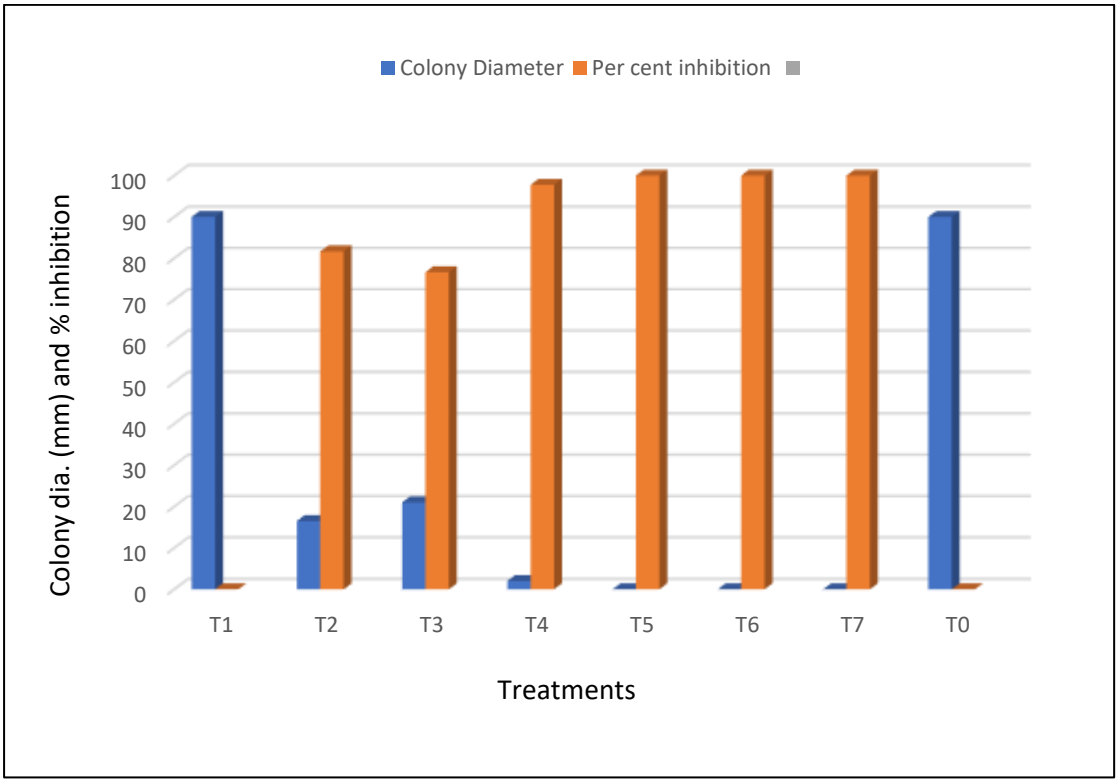
T5= Captan 70% + Hexaconazole 5% (75% WP)

T6= Carbendazim 12% + Mancozeb 63% (75%WP)

T7=Metalaxyl 8% + Mancozeb 64% (72% WP)

T0= Control

PLATE XIV: Effects of 2500 ppm combi products and non-systemic fungicides on *Colletotrichum lindemuthianum.*



$T_{1=}$ Mancozeb 75% WP

$T_{2=}$ Chlorothalonil 75% WP

$T_{3=}$ Copper oxychloride 50% WP

$T_{4=}$ Propineb 70% WP

$T_{5=}$ Captan 70% + Hexaconazole 5% (75% WP)

$T_{6=}$ Carbendazim 12% + Mancozeb 63% (75%WP)

T7=Metalaxyl 8% + Mancozeb 64% (72% WP)

$T_{0=}$ Control

4.9: Effects of 2500 ppm combi products and non-systemic fungicides on *Colletotrichum lindemuthianum*

4.3.3.6: Screening of cowpea varieties against *C. lindemuthianum*

Twelve cowpea varieties *viz,* Cowpea Safal-209, Cowpea Safal-311, Maharani, Ardhaveli, Faguni uphar-31, Ankur VU-5, KSP-150, Savita, KSP-145, KSP-5335, KSP-178, and KSP-170 were screened against *C. lindemuthianum* in *in vitro* conditions. Results (Table 4.12) revealed that, out of these twelve varieties, except Ardhaveli (40 %) and KSP-150 (50 %) all were germinated satisfactorily *i.e.* above 70%.

KSP-178 and Ankur VU-5 flowered early and took 84 and 86 days to 50% flowering. KSP-150, Savita and Maharani varieties of cowpea exhibited 50% flowering at 102 and 100 days after sowing. Pods were not formed on Ankur VU-5 and KSP-150 varieties. Ardhaveli and KSP-5335 produced purple coloured pods and other varieties produced green coloured pods.

Table 4.12: *In vitro* screening of cowpea varieties against *C. lindemuthianum*.

Sr. no	Varieties	Total no of seeds planted	No of seed germi nated	% Germination	Days to 50 % flowering	Pod colour
1	Cowpea Safal-209	10	9	90	90	Green
2	Cowpea Safal-311	10	10	100	94	Green
3	Maharani	10	9	90	100	Green
4	Ardhaveli	10	4	40	98	Purple
5	Faguni uphar-31	10	8	80	90	Green
6	Ankur VU-5	10	10	100	86	No pod
7	KSP-150	10	5	50	102	No pod
8	Savita	10	8	80	100	Green
9	KSP-145	10	7	70	96	Green
10	KSP-5335	10	10	100	94	Purple
11	KSP-178	10	10	100	84	Green
12	KSP-170	10	9	90	100	Green

After artificial inoculation the disease development on these varieties was recorded as detailed under material and methods and the data obtained is presented in Table 4.13.

The results (Table 4.13 and Plate-XV) revealed that out of twelve varieties screened against *C. lindemuthianum*, eight varieties *viz*, Cowpea Safal-209, Faguni uphar-31, KSP-150, Savita, KSP-145, KSP-5335, KSP-178, and KSP-170 showed absolute resistant reaction and four varieties *viz,* Maharani (29.62 %), Ankur VU-5 (22.22 %), Ardhaveli (18.51 %) and Cowpea Safal 311 (14.18 %) showed moderately resistant reaction against *C. lindemuthianum*.

Table 4.13: Reaction of cowpea varieties to *C. lindemuthianum* (Pot culture).

Sr.no	Varieties	% Disease Incidence	% Disease Severity	PDI	Varietal reaction
1	Cowpea Safal-209	45	0	0.00 (0.00)*	AR
2	Cowpea Safal-311	47	2	14.81 (22.62)	MR
3	Maharani	50	3	29.62 (32.95)	MR
4	Ardhaveli	49	2	18.51 (25.47)	MR
5	Faguni uphar-31	45	0	0.00 (0.00)	AR
6	Ankur VU-5	43	2	22.22 (28.11)	MR
7	KSP-150	51	0	0.00 (0.00)	AR
8	Savita	50	0	0.00 (0.00)	AR
9	KSP-145	48	0	0.00 (0.00)	AR
10	KSP-5335	47	0	0.00 (0.00)	AR
11	KSP-178	42	0	0.00 (0.00)	AR
12	KSP-170	50	0	0.00 (0.00)	AR
	S.E.(m) ±			**0.144**	
	C.D. at 1%			**0.423**	

* Figure in parenthesis are angular transformed values.

Similar results were earlier reported by Sharma *et al*. (2012), Awori *et al*. (2018) and Gupta *et al*. (2022).

PLATE – XV

PLATE XV: Screening of cowpea varieties against *Colletotrichum lindemuthianum*

PLATE – XVI

A) Plants of cowpea

B) Spraying of conidial suspension

C) Symptoms on leaves

D) Symptoms on stem

PLATE XVI: Symptoms of cowpea anthracnose developed on cowpea varieties

CHAPTER - V

SUMMARY AND CONCLUSIONS

CHAPTER – V

SUMMARY AND CONCLUSIONS

Cowpea [*Vigna unguiculata* (L.) Walp.], is an important multi utility crop locally known as lobiya, chowla (chowli), Southern pea or black eye pea, that is adopted to warm conditions and cultivated in the tropics and sub-tropics for dry grains, green edible pods for vegetable as well as fodder (Gupta *et al*., 2017a). In India, during 2019-2020 area under beans was 221 thousand ha. with annual production of 2226 thousand MT (Anon, 2021).

Among the various biotic factors responsible for low production and productivity of cowpea, anthracnose caused by *Colletotrichum lindemuthianum* is a major constraint. The anthracnose infection may occur on both sides of the leaf and on the petiole. Early signs of infection usually appear on the lower leaf surface along the veins, which showed brick red to purplish red discoloration. Later, such discoloration also appeared on the upper leaf surface. At the same time, brown lesions of various sizes, with black, brown, or purplish red margins developed around small veins, eventually conidiophores ruptured through the host cuticle and formed acervuli on the plant surface.

The *C. lindemuthianum* was isolated from anthracnose diseased cowpea samples on Potato Dextrose Agar (PDA) medium. The pathogen was purified and maintained for further studies.

The pathogenicity test of *C. lindemuthianum* was proved in screen house by inoculating spore culture on the healthy plants of local cowpea grown in earthen pots.

Result obtained on *in vitro* evaluation of various water-based plant extracts at 10 and 20% concentration revealed that highest inhibition of mycelial growth of *C. lindemuthianum* was recorded with *Curcuma longa*. This was followed by *Allium sativum* and *Zingiber officinale*. Whereas *Azadirachta indica, Ocimum sanctum, Annona squamosa* and *Allium cepa* were not effective at 10% concentration.

Plant extracts at 20 % concentration revealed that highest inhibition of mycelial growth of *C. lindemuthianum* was recorded with *Allium sativum* and

Zingiber officinale, followed by *Curcuma longa*, *Allium cepa* and *Ocimum sanctum*. *Azadirachta indica* and *Annona squamosa* were not effective at 20% concentration.

Result obtained on *in vitro* evaluation of various ethanol-based plant extracts at 10 and 20% concentration revealed that the highest inhibition of mycelial growth of *C. lindemuthianum* was recorded with *Allium sativum, Curcuma longa and Zingiber officinale*. This was followed by *Allium cepa, Ocimum sanctum* and *Azadirachta indica.* Whereas *Annona squamosa* was not effective.

Result obtained on *in vitro* evaluation of various ethanol-based plant extracts at 20% concentration revealed that the highest mycelial growth inhibition of *C. lindemuthianum* was recorded with *Curcuma longa*. This was followed by *Allium sativum* and *Zingiber officinale*. Whereas *Allium cepa*, *Azadirachta indica*, *Ocimum sanctum* and *Annona squamosa* were found comparatively less effective.

Result obtained on *in vitro* evaluation of various bioagents revealed that highest mycelial growth inhibition of *C. lindemuthianum* was obtained with *Trichoderma harzianum*. This was followed by *T. asperellum* and *T. virens*. Whereas *Aspergillus niger*, *Metarhizium anisopliae*, *Paecilomyces lilacinus* and *Verticillium lecanii* were found comparatively less effective.

Result obtained on *in vitro* evaluation of various systemic fungicides revealed that highest mycelial growth inhibition of *C. lindemuthianum* at 500 and 1000 ppm concentration was recorded with Propiconazole, Tebuconazole and Carbendazim. This was followed by Difenoconazole, Hexaconazole and Azoxystrobin. Pyraclostrobin was found comparatively less effective at both the concentrations.

At 1500 ppm Propiconazole, Tebuconazole, Carbendazim and Difenoconazole recorded cent percent mycelial inhibition over untreated control. This was followed by Hexaconazole and Azoxystrobin. Whereas Pyraclostrobin was found not effective at 1500 ppm also.

Result obtained on *in vitro* evaluation of various combi products non-systemic and fungicides revealed that at 1000 ppm concentration, highest inhibition of mycelial growth of *C. lindemuthianum* was recorded with Captan 70% + Hexaconazole 5% WP followed by Carbendazim 12% + Mancozeb 63% WP, Chlorothalonil 75% WP

alone, Metalaxyl 8% + Mancozeb 64% WP, Propineb 70% WP alone and Copper oxychloride 50% WP alone.

At 2000 ppm concentration, Captan 70 % + Hexaconazole 5% WP and Metalaxyl 8 % + Mancozeb 64% WP recorded cent percent mycelial inhibition of *C. lindemuthianum* over untreated control. This was followed by Carbendazim 12% + Mancozeb 63% WP, Chlorothalonil 75% WP alone, Propineb 70% WP alone and Copper oxychloride 50% WP alone.

At 2500 ppm concentration Captan 70% + Hexaconazole 5% WP, Carbendazim 12% + Mancozeb 63% WP and Metalaxyl 8% + Mancozeb 64% WP were recorded cent percent mycelial inhibition over untreated control. This was followed by Propineb 70% WP alone, Chlorothalonil 75% WP alone and Copper oxychloride 50% WP alone. Whereas Mancozeb 75% WP was not effective at all the three concentrations tested.

Out of twelve varieties screened against *C. lindemuthianum* in pot culture by artificial inoculation in screen house, eight varieties *viz*, Cowpea Safal-209, Faguni uphar-31, KSP-150, Savita, KSP-145, KSP-5335, KSP-178, and KSP-170 showed absolute resistant reaction and four varieties *viz,* Maharani, Ankur VU-5, Ardhaveli and Cowpea Safal 311 showed moderately resistant reaction against *C. lindemuthianum.*

CONCLUSIONS:

From the results obtained on various aspects during present investigations on anthracnose disease of cowpea. It can be concluded that:

Colletotrichum lindemuthianum (Sacc. & Magnus) Brivosi & Cavara is one of the most destructive pathogens causing anthracnose disease of cowpea causing both quantitative and qualitative losses.

C. lindemuthianum was successfully isolated on Potato Dextrose Agar (PDA) medium from naturally infected cowpea leaves and the pathogen was purified and maintained for further studies.

Pathogenicity of *C. lindemuthianum* was successfully proved by artificial inoculation on cowpea susceptible local variety in screen house under controlled environment.

All the phytoextracts evaluated *in vitro* were found antifungal against *C. lindemuthianum.* The extracts of *Allium sativum, Curcuma longa* and *Zingiber officinale* were found fungistatic against the pathogen with significant inhibition of mycelial growth of test pathogen.

All the bioagents evaluated *in vitro* were found antifungal against *C. lindemuthianum.* Out of these bioagents, *Trichoderma harzianum, T. asperellum* and *T. virens* were found fungistatic against the pathogen with significant inhibition of mycelial growth of test pathogen.

Among the systemic fungicides evaluated in *in vitro,* Propiconazole 25% EC, Tebuconazole 29.9% EC and Carbendazim 50% WP were found most effective in inhibiting the mycelial growth of *C. lindemuthianum* followed by Difenoconazole 25% EC and Hexaconazole 5% EC.

Among the non-systemic and combi product fungicides evaluated in *in vitro,* Captan 70% + Hexaconazole 5% WP and Metalaxyl 8% + Mancozeb 64% WP were found most effective in inhibiting the mycelial growth of *C. lindemuthianum* followed by Carbendazim 12 % + Mancozeb 63 % WP and Propineb 70 % WP alone.

Out of twelve varieties screened against *C. lindemuthianum* in pot culture by artificial inoculation in screen house, four varieties showed moderately resistant reaction and eight varieties showed absolute resistant reaction. But to draw the valid conclusion about the exact reaction of these varieties, screening under natural epiphytotic conditions is necessary.

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APPENDIX

APPENDIX-I

Composition of media used

I) Potato Dextrose Agar medium (PDA)

Peeled potatoes – 200 gm

Agar – 20 gm

Dextrose – 20 gm

Water – 1000 ml

II) Potato Dextrose Broth

Peeled Potatoes : 200 gm

Dextrose : 20 gm

Distilled Water :1000 ml

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