Leaf Blight of Anthurium (*Anthurium andraeanum*) and its management

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DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
BHUBANESWAR-751003, ODISHA
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A Thesis submitted to the
Odisha University of Agriculture and Technology
in Partial fulfilment of the Requirement for the degree of
Master of Science in Agriculture
(Plant Pathology)

By
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Date: 13.09.2021

CERTIFICATE I

This is to certify that the thesis entitled **"Leaf Blight of Anthurium (*Anthurium andraeanum*) and its management"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Plant Pathology)** to the **Odisha University of Agriculture and Technology, Bhubaneswar** is a faithful record of bonafide and original research work carried out by **Deepali Mohapatra, Adm. No. 191222209** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

13.09.21

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CERTIFICATE II

This is to certify that the thesis entitled **"Leaf Blight of Anthurium (*Anthurium andraeanum*) and its management"** submitted by **Deepali Mohapatra, Adm. No. 191222209** to the **Odisha University of Agriculture and Technology, Bhubaneswar** in partial fulfilment of the requirements for the degree of **Master of Science in Agriculture (Plant Pathology)** has been approved/disapproved by the students' advisory committee and the external examiner.

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LIST OF SYMBOLS AND ABBREVIATION

%	:	per cent
@	:	At the rate
a.i.	:	Active ingredient
CD	:	Critical Difference at 5 per cent level
cm	:	Centimeter
CRD	:	Complete Randomized Design
et al.	:	and other co-workers
etc.	:	and so on
g	:	gram
g^{-1}	:	Per gram
gl^{-1}	:	Grams per litre
hrs	:	hours
i.e	:	That is
ml	:	Millilitre
PDA	:	Potato Dextrose Agar
SEm	:	Standard Error of Mean
T	:	Treatment
Tr. No.	:	Treatment Number
viz.	:	Namely
µg	:	Microgram
µg/ml	:	Micrograms per millilitre

ABSTRACT

Anthurium ranks ninth in the global flower trade and commands a fair price for both cut flowers and whole plants. Anthurium is the latest sensation of Indian floriculture scene and gains its fame and respected status by exhibiting its striking ensemble, which is created unitedly by its spadix and its spathe, within the economically essential ornamentals, allowing its use in interior and exterior decoration and also to its use as a cut flower. As in the most other cultivated plants, Anthurium plant too is subjected to attack by various pathogens during its growth period. Among all the diseases, leaf blight disease caused by *Helminthosporium sp.* and *Colletotrichum gloeosporioides* (Mancini *et aI.,*1973) is newly emerging threat to all the Anthurium growers of Odisha. Therefore, the research work was undertaken on the title "Leaf blight of Anthurium (*Anthurium andraeanum*) and its management". The disease was first reported during 2020 from the gardens of Department of Floriculture and Landscaping, College of Agriculture, OUAT, Bhubaneswar. According to the symptomatology study, the typical brown spots began as a water-soaked lesion on the upper surface of the leaves, which usually developed from the tip or along the margin of the upper surface of the leaf. The size of these spots grew larger with age, until two or more spots merged and formed elongated reddish brown necrotic patches surrounded by a yellow halo. The centres of such spots eventually turn greyish white, with a small brown zone surrounding them. The pathogenicity of the isolated fungi *Helminthosporium sp*. and *Colletotrichum sp.* was tested on potted Anthurium plants and re-isolated fungi were found identical to the original isolated one. The growth of the fungus *Helminthosporium sp.* was best on Potato Dextrose Agar medium (68.20 mm) followed by Potato Carrot Infusion Agar medium (66.80mm) and the best medium for growth for *Colletotrichum sp.*was Potato Dextrose Agar medium (76.88 mm) followed by Carrot Agar medium (74.24mm). *In vitro* evaluation of fungicides against *Helminthosporium sp.* recorded maximum inhibition (100%) by Tebuconazole (0.1%), Tebuconazole 10% + Sulphur 65% (0.1%) and Tebuconazole 50% + Trifloxystrobin 25% (0.1%) and against *Colletotrichum sp.* maximum inhibition was recorded by Tebuconazole (0.1%), Difenoconazole (0.1%), Tebuconazole 10% + Sulphur 65% (0.1%) and Tebuconazole 50% + Trifloxystrobin 25% (0.1%). In vitro efficacy of different bio-control agents revealed that, *Pseudomonas fluorescens* was most effective in inhibiting the growth of *Helminthosporium sp.* (54.44%) and *Trichoderma viride* was most effective against *Colletotrichum sp.* (58.88%). Further, management of the disease under *in vivo* condition is suggested in subsequent years to draw a conclusion.

INTRODUCTION

Floriculture, till recently considered to be a simple garden activity, has become an important agri-business enterprise. Today, floriculture is a lucrative profession with higher potential for returns than most of the field and other horticultural crops. The demand for flowers both in India and International markets is increasing at a faster rate owing to the liberalization of economy and globalization of trade. India contributes 0.6 % to the global floriculture trade. Rose, gladiolus, tuberose, aster, Anthurium, orchid, gerbera and carnation are the principal cut flowers grown all over the country. The popularity of Anthurium is increasing due to higher return per unit area and their beautiful and attractive long lasting flowers.

Floriculture is a fast emerging competitive industry and cultivation of flowers for commercial purposes is common to many countries. It has become one of the high value agricultural industries in many countries of the world (Taj *et al.*, 2013). Global floriculture market is expected to flourish at a CAGR of 8.6% during the forecast period. Further, the market of floriculture is expected to garner 3.65 billion US dollars by the end of 2027.

Flowers occupy an important place in Indian society, often symbol of beauty, love and tranquility (Gajanana and Sudha, 2006). In recent years, commercial cultivation of cut flowers such as rose, Anthurium, lilies, orchids, carnation, gladiolus, etc. has become popular.

India is bestowed with several agro-climatic zones conducive for production of sensitive and delicate floriculture products. This era has seen a dynamic shift from sustenance production to commercial production. As per National Horticulture Database published by National Horticulture Board, during 2015-16 the area under floriculture production in India was 249 thousand hectares with a production of 1659 thousand tonnes loose flowers and 484 thousand tonnes cut flowers.

Government of India has identified floriculture as a sunrise industry and accorded it 100% export oriented status. Owing to steady increase in demand of flower, floriculture has become one of the important Commercial trades in

Agriculture. It has been found that commercial floriculture has higher potential per unit area than most of the field crops and is therefore a lucrative business. India's total export of floriculture products to the world was 16,949.37 MT worth of Rs. 541.61 Crores / 75.89 Million US dollars in 2019-20. More than 50% of the floriculture units are based in Karnataka, Andhra Pradesh and Tamil Nadu. The Indian Floriculture market is expected to reach a value of INR 661 Billion by 2026.With the technical collaborations from foreign companies, the Indian floriculture industry is poised to increase its share in world trade.

Anthurium is a tropical ornamental plant that is highly prized as a cut flower and has a high export potential. Anthurium is ranked ninth in the global flower commerce, and its cut flower and full plant fetch fair prices. Netherland is currently the world's largest producer and exporter, followed by Mauritius. Together with orchids, Anthuriums make up 90% of all tropical flowers imported into the United States (Echeverri *et al.*, 1997). The share of imported Anthuriums sold in the United States has increased steadily in recent times, due to severe attacks of bacterial blight (*Xanthomonas sp.*) affecting production in Hawaii, which has reduced production from this state by over 60%.

Anthurium is the latest sensation of Indian floriculture scene and is the largest genus of the monocot family Araceae, The word Anthurium refers to the tail like spadix in the center of attraction. The Anthurium gains its fame and respected status by exhibiting its striking ensemble, which is created unitedly by its spadix and its spathe, within the economically essential ornamentals, allowing its use in interior and exterior decoration and also to its use as a cut flower. Mr. S. M. Damon imported *Anthurium andreanum* to Hawaii for the first time in 1889 (Higaki and Watson, 1973). Commercially cultivated Anthurium planting materials are primarily imported from the Netherlands.

Anthurium is a Central and South American native with over 900 species in the genus Anthurium. The name Anthurium comes from the Greek words "anthos" which means "flower" and "aura" which refers to the spadix. The popularity of growing Anthurium as cut flowers has sky-rocketed in recent years, and it has now established itself as a significant export crop.

Anthurium (*Anthurium andraeanum*) is a slow-growing perennial flowering plant that requires shady, humid conditions as found in tropical forests. The genus Anthurium is evergreen and belongs to the family Araceae as the plant possesses an underground rhizome with adventitious roots, characteristic of the family. Anthurium characteristically produces numerous inflorescences subtended by brightly colored spathes, which are carried on long, slender peduncles. Spathes are characteristically heart-shaped, flat, puckered and shiny where as flowers have a wide range of spathe colors viz., white, pink, salmon-pink, red, light-red, dark-red, brown, green, lavender, cream or multi-colored. The colorful spathe is long-lasting (Higaki *et al.*, 1983 and Kamemoto *et al.*, 1988).

Anthurium is one of the world's best domestic flowering plants. They are not only attractive, but they also help to purify the air by removing dangerous airborne compounds such as formaldehyde, ammonia, toluene, xylene, and allergies. NASA has included it in the list of air purifier plants because of its relevance in eliminating hazardous compounds from the air. Because of its eye-catching, Anthurium has a higher commercial value. Anthurium is shade loving plant growing best in a well drained soil under highly humid conditions (70 to 80% RH) with a temperature range of 18°C to 28°C. It is generally propagated through seeds and suckers. In recent years, micropropagation is also being employed for commercial production of planting materials.

Similarly to other flower crops, Anthurium is susceptible to a variety of illnesses caused by fungus, bacteria, and viruses in both field and protected culture (Bhatt and Desai, 1989). In Florida, in the Caribbean and the Pacific islands, bacterial pathogens have caused the most catastrophic Anthurium illnesses, limiting the creation of susceptible hybrids. Fungal and fungal-l are two terms for the same thing. Anthracnose (black nose), foliage, stem and root rots caused by *Phytophthora nicotianae*, *P. tropicalis* and *Rhizoctonia solani* are examples of fungal and fungal-like diseases. Viruses have been identified, however they do not inflict significant economic harm. Anthurium decline caused by *Radopholus similis*, a burrowing worm, is currently posing a severe challenge to Anthurium production due to prohibitive costs.

Anthurium leaf blight disease is caused by *Colletotrichum gloeosporioides.* (Mancini *et al.*, 1973) and *Helminthosporium spp.* Resulting in significant loss. If left unchecked, this disease has the potential to wipe out up to 50% of the population of flower unmarketable. The disease usually starts at the tip of the leaf and spread along the margin of the upper surface. The symptoms on leaves were typically observed on the upper surface as water soaked, small, round to oblong, brown spots. The size of these spots grew larger with age, until two or more spots coalesced and formed elongated reddish brown necrotic patches. The centres of such spots finally turn greyish white with small brown zone around. The fruiting body of the pathogen in the form of acervuli is visible as minute black dots under severe infection

The quality and quantity of leaves and flowers are found to be reduced as a result of the leaf blight disease, resulting in economic losses. During January-February, 2021 leaf blight disease was observed on the leaves of Anthurium in the garden of Department of floriculture, College of Agriculture, OUAT, Bhubaneswar. No systematic study has yet been undertaken for documenting the disease, its etiology and management. In view of this the present study on 'Leaf blight of Anthurium and its management' was undertaken with the fallowing objectives:

1. To study the symptomatology of Leaf blight disease of Anthurium
2. To study the etiology, morphological and cultural characteristics of the pathogen
3. Evaluation of novel fungicides and biocontrol agents against the test pathogen

REVIEW OF LITERATURE

2.1 Description of *Anthurium*

Anthurium is the largest and most complicated genus in the Araceae family. It is made up of about 1000 different species (Croat 1992). This genus' range includes northern Mexico and the Greater Antilles, as well as southern Brazil, northern Argentina, and Paraguay (Croat 1983, 1986). The most studied species have 2 n = 30 chromosomes (Sheffer and Kamemoto 1976).

The anthurium (*Anthurium andraeaniim* Lind.) is a tropical or near-tropical ornamental flower. Anthuriums are primarily grown in Hawaii and southern California in the United States, and nearly all are sold as cut flowers (Walker and Smith, 1978). The flower is made up of a modified leaf (spathe) that supports a cylindrical inflorescence known as a spadix (Marutani, 1984). Hundreds of tiny true flowers cover the spadix (Higaki and Watson, 1967). The spathe is approximately 7 cm x 10 cm in size (Walker and Smith, 1978).

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Anthurium andreanum Lind. is a perennial, herbaceous monocotyledon in the family Araceae with cordate leaves and flowers. The commercial "flower" is an inflorescence consisting of conspicuous bract (spathe) and protruding rachis (spadix), on which minute perfect flowers are borne helically. The flowers are protogynous; the stigma is receptive before the pollen is shed. Anatomically, the spathe has a uniseriate upper and lower epidermis, one or two layers of hypodermal cells, and 10 to 12 layers of spongy parenchyma cells. Anthocyanin is localized in the hypodermal cells. The leaf blade is similar to the spathe, except for two layers of palisade parenchyma cells immediately below the upper epidermis. Venation is netted. Chloroplasts are dispersed throughout the mesophyll but are more concentrated in the palisade cells. The peduncle, petiole, and stem are typically monocotyledonous in structure.

Epidermal cells cover the cortex, a layer of sclerified parenchyma cells, and the ground tissue. Vascular bundles are dispersed throughout the ground tissue. Roots are cylindrical, fleshy, epiphytic, and adventitious. The epidermis is developed as a velamen. Raphide and druse crystals are found throughout the plant. Above-ground parts have a thick, waxy cuticle (Higaki *et al.,* 1984).

Anthuriums belong to the family Araceae, which has many genera, including *Anthurium, Dieffenbachiae, Aglaonema, Epipremnum, Xanthosoma, Spathiphyllum, and Philodrendron* (Higaki *et al.,* 1983 and Kamemoto *et al.,* 1988).

Anthurium andraeanum Lind. (Anthurium) is a perennial herbaceous plant grown for its showy, long-lasting flowers. Anthurium, a member of the Araceae family, is native to the tropics of Central and South America, but it is now widely cultivated and traded globally as cut flowers, potted plants, and landscape plants (Gantait *et al.*, 2008).

2.2 Cytology

The most common number of somatic chromosomes discovered was 30, but counts ranged from 2n = 20 to 90. (Sheffer and Kamemoto, 1976). Anthurium chromosome numbers have been reported as 2n = 30 (Gaiser, 1927) and 2n = 32 (Sheffer and Kamemoto, 1976). The chromosome number is 2n = 30 for Anthurium andraeanum Linden ‘Kaumana,' which originated in Kaumana, and 2n = 20 + 2B for ‘Uniwai,' a white-spathe cultivar released by the University of Hawaii in 1962 (Kaneko and Kamemoto, 1978).

2.3 Propagation and growth conditions

Anthuriums thrive in a high organic, well-aerated medium with excellent water retention and drainage. Anthurium plants do not thrive in bright light, and shade must be provided for adequate growth and flowering (Nakasone and Kamemoto, 1962). Anthuriums thrive when the night temperature does not fall below 18°C and the day temperature does not fall below 11°C (Higaki and Watson, 1967).

2.4 Importance of *Anthurium*

Anthuriums are listed in NASA's air purifying plants list. They are one of the best houseplants that purify indoor air. Their large, dark leaves suck up ammonia, formaldehyde, toluene and xylene, so they're a thoughtful present for a workplace (especially around copiers, printers or adhesives) (NASA 1989).

Anthurium andraeanum is toxic to humans and pets because its tissues contain calcium oxalate crystals. If swallowed, a strong burning sensation and an inflammatory reaction involving blisters and edoema may occur (chewing). It is classified as toxic in two ways: category 2 and category 3. If the sap of the plant comes into contact with the skin, it may cause an allergic reaction (Clay *et al.*, 1989).

Anthurium andraeanum Lind. (Anthurium) is a perennial herbaceous plant grown for its showy, long-lasting flowers. Anthurium, a member of the Araceae family, is native to the tropics of Central and South America, but it is now widely cultivated and traded globally as cut flowers, potted plants, and landscape plants (Gantait *et al.*, 2008).

2.5 Diseases of Anthurium

McCulloch and Pirone (1939) first reported *Xanthomonas phaseoli pv. dieffenbachiae* (=*Xanthomonas axonopodis pv. dieffenbachiae*) (Xad) causing bacterial blight on Anthurium (*Anthurium andreanum*) in New Jersey, U. S. A. and described as Bacterium dieffenbachiae. The bacterium occurs in a broad range of ornamental crops, with most reports coming from Anthurium.

Henny *et al.* (1988) reported that Anthurium is very susceptible to bacterial and fungal diseases that can seriously limit commercial production. Bacterial blight caused by *Xanthomonas* is probably the most serious. Root rots caused by *Rhizoctonia, Pythium, Colletotrichum* and *Phytophthora* also occur in Anthurium production.

Plant diseases play major role in the crop production with drastic reductions in quantity and quality of the yield. Several diseases are known to affect the ornamental Anthurium (*Anthurium andraeanum.*) *viz.*, Leaf blight (*Colletotrichum gloeosporioides*), Root rot (*Pythium splendens*), Leaf spot (*Septoria anthurii*), Powdery mildew (*Erysiphe communis*), Bacterial blight (*Xanthomonas compestris Pv. diffenbachiae*) (Bhatt and Desai, 1989).

Pilon (2013) reported a number of fungal pathogens that collectively cause what growers refer to as anthracnose diseases. Some of the most common pathogens are *Colletotrichum*, *Phoma* and *Phyllosticta*; however, there are additional pathogens including *Coniothyrium*, *Cryptocline*, *Diplodia*, Gloeosporium, Glomerella, *Macrophoma* and *Phyllostictina* that cause anthracnose diseases.

Ben H *et al.* (2014) observed a new severe disease on *Anthurium andraeanum* Lind. in the summer of 2011 in Beijing, China. The fungus was isolated from symptomatic leaves, and its pathogenicity was confirmed. Based on the morphological characteristics and molecular analysis, the pathogen was identified as *Myrothecium roridum.*

According to the reports of Alvarez (2018) Anthuriums are highly susceptible to bacterial and fungal infections that can limit commercial production. The major diseases of Anthuriums include bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (Aysan & Sahin, 2003), bacterial wilt caused by *Ralstonia solanacearum* (ZhiQiong *et al.*, 2006), black rot caused by *Phytophthora citrophthora* (Pitta, Siuza and Feichtenberger, 1991) and anthracnose (Kagiwata, 1990) and black nose caused by *Colletotrichum gloeosporioides* (Alvarez, 2018). Black nose, also known as spadix rot, is a major disease that reduces both the quality and quantity of Anthurium pot plants and cut flowers. This disease develops at the tip of the spadix, starting with a small brown spot that later expands to a brown to dark brown lesion, causing dry rot. The causal pathogen of this disease has been identified as *C. gloeosporioides*.

Wilailuck (2020) reported bacterial pathogens that caused the most devastating diseases of *Anthurium* and have limited the production of susceptible hybrids in Florida, the Caribbean and the Pacific islands. Fungal and fungal-like diseases include anthracnose (black nose), foliage, and stem and root rots caused by *Phytophthora nicotianae*, *P. tropicalis* and *Rhizoctonia solani*. Viral diseases have been described but do not cause appreciable economic damage. Anthurium decline caused by the burrowing nematode, *Radopholus similis*, currently poses a major challenge to Anthurium production.

2.5 Importance and disease severity

During autumn, 1959, *A. andraeanum* growing in the high rainfall areas of Hawaii suffered an epiphytotic of spadix rot caused by *Colletotrichum gloeosporioides* [*Glomerella cingulata*]. The rot developed rapidly with the prevailing high temp, high R.H. and on plants in transit (Aragaki and Ishii, 1960).

Anthurium leaf blight disease caused by *Colletotrichum gloeosporioides was* reported by Mancini *et al.* (1973) who observed that due to this disease, quality and quantity of the leaves and flowers was reduced, leading to economical losses.

Bacterial pathogens have caused the most devastating diseases of Anthurium and have limited the production of susceptible hybrids in Florida, the Caribbean, and the Pacific islands. Fungal and fungal-like diseases include anthracnose (black nose), foliage, stem and root rots caused by *Phytophthora nicotianae*, *P. tropicalis* and *Rhizoctonia solani*. Viral diseases have been described but do not cause appreciable economic damage (Alverez *et al.,* 1989).

Severe anthracnose was observed on *Anthurium andraeanum*, *Ardisia crenata*, *Calathea insignis* [*C. lancifolia*], *Dendrobium phalaenopsis*, *Peperomia clusifolia* and *Phalaenopsis amabilis* in vinyl-housed fields and greenhouses in Gyunggi province, Korea Republic, between Nov. 1992 and Jan. 1993. All isolates collected from diseased plants were identified as *C. gloeosporioides* [*Glomerella cingulata*] and pathogenicity of the isolates was confirmed for each host (Kim *et al.,* 1993).

Anthracnose, known also as spadix rot or black nose, continues to be the most serious disease of Anthurium in Hawaii. It is caused by the fungus *Colletotrichum gloeospotioides* and is particularly severe in high-rainfall areas on the island of Hawaii. Anthracnose (black-nose, spadix rot) is caused by the fungus *Colletotrichum gloeosporioides* and is a common cause for flower rejection in Hawaii. The fungus is common on numerous ornamental, fruit, and vegetable crops grown in tropical and subtropical areas. Many specialized strains that differ in host range are suspected to be included in this large species group (Higaki *et al.*, 1995).

A detailed survey was conducted by B Rex *et al.* (2019) during 2017-2018 in various places of Tamil Nadu. The occurrence of anthracnose disease on Anthurium cultivars was recorded and the result varied between 56.66 percent disease index (PDI) (Dindugal district) and 17.77 percent (Salem district).

2.6 Symptomatology

Mancini *et al.* (1973) observed the anthracnose disease of Anthurium and described the symptoms of the disease under glasshouse conditions. Infection developed on leaf stalks and peduncles of inflorescence.

Sankaran *et al.* (1980) studied *Strychnos nux-vomica* leaf spot disease caused by *C. gloeosporioides*, which produced greyish brown spots surrounded by a yellow halo. Spots grew quickly, coalesced, and frequently covered a large area, and the leaves dried up.

According to Kalra *et al.* (1988), scented geranium (*Pelargonium graveolens* L. Herit) leaf blight caused by *C. gloeosporioides* produced brown necrotic spots that later expanded towards the mid-rib, resulting in complete necrosis and rotting of leaves.

Sutton (1992) documented that black nose can cause havoc in cut-flower and potted-plant production. Flowers and flowering potted plants cannot be sold with this condition. The first symptoms observed are small, brown to black flecks on the floral spadix (nose). These spots rapidly enlarge, become watery, turn brown to black, and may totally encompass the spadix. The spadix may eventually fall off. Growers may also observe black, spore-containing structures (acervuli) on dead leaves and stems.

Natural *et al.* (1994) studied an anthracnose or spadix rot of Anthurium caused by *Colletotrichum gloeosporioides* and reported that the symptoms varied from small dots to blackening of the tip of the spadix.

Anthracnose of crop plants caused by the fungus *Colletotrichum gloeosporioides* (PENZ) is a common disease in the Caribbean. The fungus has caused considerable decline in crop output, notably productivity. Putative toxins were extracted from culture filtrates of the fungus isolated from diseased yam (*Dioscorea* *alata* L. cv. White Lisbon), coffee, and Anthurium plants and tested on a number of plant genotypes. Partially characterized, putative toxins from yam isolates of the fungus induced typical anthracnose symptoms on yam, but not on citrus (*Citrus spp.* L.), mango (*Mangifera indica* L.), avocado (*Persea americana* Mill.), soursop (*Anona muricata* L.) and Anthurium plants. Similarly putative toxins induced foliar symptoms only on the respective host plant from which isolates were obtained. Furthermore, the viability of yam cell suspensions was also affected by the putative toxin (Alleyne, 1995).

Deshmukh (1997) observed symptoms of anthracnose of Anthurium as numerous, small, water soaked, round to oblong, brown spot along the margins on the upper surface of leaves. The size of the spot increased with age and the centers of such spots finally turned greyish white with small brown zone around the spot. The fruiting body of the pathogen in the form of acervulus was visible as minute black dots under severe infection.

The fungus *Colletotrichum gloeosporioides* attacks many temperate and tropical crops and can cause damage to roots, stems, leaves, and flowers. However, in *Anthurium* the pathogen is highly specific, only attacking the spadix portion of the flower (the nose). The first symptoms observed are small, brown to black flecks on the floral spadix (nose). These spots rapidly enlarge, become watery, turn brown to black, and may totally encompass the spadix. The spadix may eventually fall off. Growers may also observe black spore-containing structures (acervuli) on dead leaves and stems (Chen *et al.*, 2003).

Warumby *et al.* (2004) reported that anthracnose is caused by the fungus *Colletotrichum gloeosporioides.* Symptoms start as tiny brownish spots on the flower spadix. During high humidity these spots enlarge, appear water-soaked and turn necrotic. Sometimes the entire spadix will turn black as lesions coalesce. The shape of most lesions is, however, angular due to the shape of the spadix tissue. As the disease becomes more severe, masses of orange coloured spores form on necrotic areas. Leaves and spathes are rarely infected.

Among the *Colletotrichum* strains isolated from *Anthurium andraeanum* in Guangzhou, Nanjing, Yangzhou, there were two groups exhibited different morphology on the culture. Two strains of each group were chose for species identification with the morphological character, pathogenicity on plants, host range and the sequence of ribosomal DNA-ITS. The results indicated that these strains belonged to two species, *Colletotrichum gloeosporioides* and *C. destructivum*, and the latter was first reported as a pathogen in *Anthurium andraeanum*. Both species could infect solely, crossly and latently (HongMei *et al.*, 2009).

Colletotrichum destructivum is the pathogen of anthracnose in *Anthurium andraeanum*. Based on internal transcribed space（ITS）sequences of *Colletotrichum* genus, a pair of specific primers（F1 and ITS4） to detect *C. destructivum* was synthesized. The primer sets amplified a single PCR band of 486 bp with DNA extracted from *C. destructivum* isolated from *A. andraeanum,* while other relative strains within different species had no corresponding band. The detection sensitivity was 10 pg of genomic DNA. When using ITS1/ITS4 as the first round primes and F1/ITS4 as the second round primes, the detection sensitivity increased 10 000-folds to 10 fg. The detection sensitivity for the soil pathogens was 200-conidia per gram soil. The PCR-based method developed here could stably and quickly detect the pathogen from water samples and diseased plants (Hongmei, 2011).

Patel (2012) described typical symptoms of leaf spot of orchid caused by C. *gloeosporioides* as several small pin head circular red colored spots on the leaves. As the development progressesed, the spots enlarged, became angular to circular light brown with dark blackish margin surrounded by brick red area which was further encircled by light yellow halo with black center.

In 2012, rooted mini-cuttings of clones of *Eucalyptus urophylla* x *Eucalyptus grandis* ("urograndis") exhibiting leaf spot and stem girdling symptoms were collected from nurseries in the Brazilian states of Pará and Minas Gerais, and cultures of *Colletotrichum* were obtained from the lesions. The isolates were initially identified to species of the *C. gloeosporioides* species complex, according to searches of the Q-bank Fungi database with the DNA sequences of their internal transcribed spacer (ITS) regions (Rodrigues *et al.,* 2014).

Symptoms of anthracnose in Anthurium begin to appear when the air humidity is 95–99% and temperatures above 20°C. At spore germination sites, solitary dark spots may be seen shortly after infection. Then, in their place, small brown spots with a yellowish or pink border are formed. Arising at the edges of the leaf plates, they increase in size and quantity over time. Patches of dead tissue grow, moving towards the central foliage. Holes are formed in the center of the spots. If untreated, the lesion progresses, the stems may become ulcerated and cracked. The plant becomes weakened. The process of photosynthesis is reduced. The conductivity of the vessels is disrupted - some of them are cut, some are clogged with threadlike formations that feed the mycelium. As a result, the stems and roots dry out. Symptoms can vary depending on the type of specific pathogen that is at the site of the lesion of Kabatiella zeae, small dark spots surrounded by a gray rim will be visible. If *Colletotrichum trichellum* settled on *Anthurium*, it will manifest itself by the appearance of characteristic gray pads, the surface of which is dotted with small black villi (these formations contain spores). In the end, the diseased plant becomes unviable (Rodrigues *et al.,* 2014).

In December 2016, flowers (*Anthurium spp*.) with circular necrotic spot symptoms were collected in Atalaia, state of Alagoas, Brazil. The causal organism was found to be *Colletotrichum gloeosporioides,* Chaves *et al.,* 2020). Anthurium infected with *colletotrichum gleosporoides* developed dark, water soaked lesions on stems, leaves or fruit. The centers of these lesions often became covered with pink, gelatinous masses of spores especially during moist, warm weather.

Anthurium infected with *colletotrichum gleosporoides* showed anthracnose symptoms with circular wavy pattern on leaves and raised fluffy growth in Tamil Nadu. The fungus *C. gloeosporioides* was isolated and the morphological variations were studied among the isolates. The result revealed that all the isolates of *C. gloeosporioides* were circular and wavy pattern and the topography were observed as raised fluffy growth (B Rex, 2019).

In October 2017, typical symptoms of anthracnose were observed on pomegranate flowers (cv. Wonderful) in commercial orchards in Pelotas, Rio Grande do Sul state, Brazil. Initially, symptoms appeared as small, light brown, slightly sunken lesions, circular to irregular in shape. As infection continued, the lesions expanded and became dark brown to black, eventually forming orange conidial masses under high humidity, ultimately causing the flowers to abort (Bellé *et al.,* 2018).

2.7 Pathogenecity

Various workers have proved the pathogenicity of leaf blight pathogen (*Colletotrichum sp*. and *Helminthosporium sp.*) on different host by different method.

Munjal and Gupta (1965) inoculated a seedling of Cock's comb with a pure culture of *C. gloeosporioides* and developed typical lesions on the fifth day following inoculation.

Mhaiskar (1967) discovered *C. gloeosporioides* on *Vinca rosae* in Maharashtra from August to November 1966. When artificially inoculated, the fungus was highly pathogenic and virulent to the twigs and leaves.

Natarajan and Subramanian (1973) successfully proved *C. gleosporioides* pathogenicity on chilli leaves and green and ripe fruits by spray inoculation with conidia after slight injury by pin prick method. Infection was also reported on uninjured leaves.

Sattar *et al.* (1981) isolated the pathogen of soybean crop target spot disease on potato dextrose agar medium and cultured and purified it using single spore isolations. The spores of *Corynespora cassiicola* were isolated from infected leaves and stems.

Sharma *et al.* (1981) isolated *Corynespora cassiicola* cultures from diseased leaves of *Ephelandra sp.*, an ornamental plant, on PDA. They confirmed the pathogenicity of the fungus by spraying conidial suspension on both injured and uninjured plant leaves. Within 7-10 days of being inoculated, the inoculated leaves developed the characteristic symptoms.

Patel (1983) used the pin prick method to demonstrate pathogenicity of *C. gloeosporioides* isolated from jackfruit on detached leaves.

Kalra *et al.* (1988) conducted pathogenicity test of *C. gloeosporioides* by spraying spore suspension (106 conidia/ml) on the leaves of geranium and symptoms appeared two to three days later on detached leaves and potted plants.

Thakare and Patil (1995) demonstrated *C. gloeosporioides* pathogenicity on chrysanthemum by spraying spore suspension on injured leaves. After four days of inoculation, typical watery circular to irregular dark brown spots appeared.

By spraying spore suspension, Deshmukh (1997) from Dapoli demonstrated the pathogenecity of *C. gloeosporioides* isolated from anthurium leaves.

Patel (2000) demonstrated the pathogenicity of *C. gloeosporioides* isolated from turmeric leaves on detached leaves with and without injury by pin-prick and tooth brush and reported that tooth brush injury method produced more superior symptoms than pin-prick and without injury.

Venkataravanappa (2002) reported that pathogenicity was demonstrated by spraying spore suspension (106 spores/ml) of the fungus *C. gloeosporioides* on five-month-old mango seedlings, and infection occurred twelve days later.

Anjos *et al.* (2004) performed a pathogenicity test in a greenhouse and then resolved *H. maydis* from artificially inoculated Pojuca seedlings. They reported that four days after inoculation, symptoms of leaf spot appeared in 100% of the inoculated Pojuca plants.

Jeske (2006) successfully demonstrated pathogenicity by spraying papilionaceous plants with spore suspension of *C. gloeosporioides* (3 x 106 spores mJ^{-l}) and observing characteristic symptoms after one week.

Raut (2011) demonstrated the pathogenicity of *C. gloeosporioides* causing anthracnose of banana (*Musa paradisiaca* L.) by four artificial inoculation methods, namely, cork borer injury, pin prick injury, tooth brush injury, and injury with carborandum powder on banana fruits, and found that the pin prick and cork borer injury inoculation methods were more significant than the carborandum and tooth brush injury methods.

Thangamani *et al.* (2011) used the pin prick method to demonstrate the pathogenicity of (*Colletotrichum musae*) anthracnose disease of banana.

Naz *et al.* (2011) tested the pathogenicity of the fungal strain on susceptible maize varieties in earthen pots (30 cm in diameter) filled with autoclaved sterilised soil. Six pots were inoculated with *Helminthosporium maydis* inoculum (2x104 spores/ml) at 3-4 leaf stages, and six were sprayed with sterilised distilled water (control). These pots were placed in a growth room with the temperature set at 29°C for incubation. After one week of incubation, the leaves were examined for the development of lesions. The fungus was re-isolated from diseased leaves on PDA and subjected to a pathogenicity test.

Patel (2012) demonstrated the pathogenicity of (*Colletotrichum gloeosporioides* penz. and sacc.) from ornamental orchid leaf spot using a pin pricking injury method on the leaves.

Chai *et al.* (2014) investigated the pathogenicity of leaf and fruit spots on hot pepper (*Capsicum annuum* L.) plants caused by the pathogen *Corynespora cassiicola* in Hainan province, China. Dark brown spots on leaves and fruits, as well as black stem lesions, were used to isolate a fungus. Inoculation experiments confirmed the pathogenicity of the isolated fungus.

Pal *et al.* (2015) conducted pathogenicity tests for *Helminthosporium maydis* blight of maize in greenhouse conditions and observed that symptoms appeared 3-4 days after inoculation with small yellow and necrotic spots, later all spots coaleased and produced typical blight symptoms, similar results were obtained by Akram and Singh (2001), Misra (2014).

2.8 Isolation and identification of the causal agent

2.8.1 Causal organism

The genus *Colletotrichum gloeosporioides* taxonomically belongs to the sub division of Deuteromycotina (Fungi Imperfecti), Class Coelomycetes, Order Melanconiales. The genus *Colletotrichum* was established by Corda (1831), for fungi characterized by hyaline, curved, fusiform conidia and setose acervuli. After that, followed a period of uncertainty as to the distinction between *Colletotrichum* and other genera such as *Vermicularia* and *Gloeosporium* (Jeffries *et al.*, 1990). Previously recognized genus *Gloeosporium* Desm. et Mont, was revised by Arx

(1970). In few species, a perfect state was detected and described in the genus *Glomerella* (Stoneman) H. Schrenk et Spauld in the division of Ascomycota (Hindorf, 2000). The most well known generative and vegetative combination was represented with *G. cingulata* and *C. gloeoesporioides.*

Kingdom	: Fungi
Division	: Ascomycota
Class	: Sordariomycetes
Order	: Phyllachorales
Family	: Phyllachoraceae
Genus	: *Colletotrichum*
Species	: *gloeosporioides*
Scientific name	: *C. gloeosporioides* (Penz.) Penz. & Sacc.
Teleomorph	: *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk

Link (1809) established the genus *Helminthosporium* based on H. Velutinum Link ex Gray, which was validated in 1821 by Gray's publication. Persoon proposed the name *Helminthosporium* in 1822 to designate *Helminthosporium*; later that year, in 1824, Link adopted the revised name (Misra, 1973).

Shabana *et al.* (2008) reported that *Helminthosporium oryzae* Breda as the causal pathogen which belongs to family Dematiaceae, order Moniliales and subdivision- Deuteromycotina. This fungus is both inter-and intra-cellular within mesophyll tissue of the leaves.

2.8.2 Morphological characteristics

Kafi and Tarr (1966) found that growth, sporulation and morphology, colour and dimensions (especially length) of conidia produced by *Helminthosporium rostratum* Drechsler, *H. sorghicola* Lefebvre & Sherwin, *H. cynodontis* Marigoni, *H. hawaiiense* Bugnicourt and *H. australiense* Bugnicourt were significantly affected by culture medium, glucose concentration, and carbon source, but only slightly by the accessory growth substances used. *H. rostratum* and *H. hawaiiense* had higher glucose requirements than the others, and production of conidia in all five species occurred over a narrower range of glucose concentration and at a lower range of C/N ratio than vegetative growth. Length and septation of conidia were positively correlated and were significantly affected by the factors studied, mean conidial length from different treatments ranging from 55 to 137µ in *H. rostratum*, 42 to 70µ in *H. sorghicola*, 24 to 39µ in *H. cynodontis*, 19 to 26µ in *H. australiense*, and 17 to 24µ in *H. hawaiiense*. Length was generally greater on natural media, such as malt agar and potato dextrose agar, and at moderately low glucose concentration (up to 1 % depending on species), whereas short conidia developed when galactose was the carbon source. The conidial characteristics of *H. rostratum* appeared to vary more in culture than under natural conditions.

Kapoor and Munjal (1968) reported that *C. gloeosporioides* produced cylindrical conidia with obtuse or rounded ends measuring 14.0-16.0 x 3.5-4.5 µm in size.

Yee and Sakiah (1993) investigated the morphology of papaya isolates of *Colletotrichum gloeosporioides* and discovered circular, dull white to pure white colonies on PDA. Conidia were cylindrical, single celled, straight and round at both ends, and contained one or two oil globules. The spores ranged in size from 14.83× 5.42 µm to 15.83× 5.42 µm.

Conidiogenous cells of c*olletotrichum* cylindrical to narrowly clavate, hyaline to pale brown becoming dark brown or black with age, forming a compact mass on branching conidiophores occasionally percurrently proliferating 12-24 X 2-4 µm. Conidia hyaline, curved fusiform with truncate base 16-34 x 2-4 µm, mostly 21 x 3 µm. Appressoria medium brown, oval to irregularly lobed, 5-18 x 4 µm formed abundantly from germinating conidia and vegetative mycelium (Baxter *et al.,* 1983).

Agostini, 1992 investigated the morphological characteristics of various strains of *Colletotrichum gloeosporioides* isolated from citrus in Florida and discovered that all isolates readily produced appresorium from germinating conidia. Ascospores were hyaline, slightly curved with rounded apices, and 15.5+ 0.5 µm long and 4.5+ 0.9 µm wide.

Colletotrichum gloeosporioides, the pathogen responsible for yam anthracnose in Nigeria, was studied morphologically by Abang (2002). The perithecia, asci, and ascospores of all isolates matched those described. The ascomata of many isolates did not mature and remained as sterile initials (Baxter et al., 1983). Mature perithecia were beaked, erumpent, and dark brown to black in colour, with sparse paraphyses. Hyaline ascospores were 15–18 µm long and 4–6 µm wide, with slightly curved apices. Asci were 35–55 8–10 µm tall.

Photita *et al.* (2005) isolated thirty-four strains of *Colletotrichum spp*. from banana, ginger, soyabean, mango, *Euphatorium thymifolia* and *Draceanans anderiana* and identification was made on the basis of their colony morphology, size and shape of appresoria and conidia. The cultures had sparse, cottony white to pale grey mycelium with abundant mycelia containing bright orange conidial masses produced in concentric rings on the colonies. Conidia were cylindrical and size ranged from length 12-20× 16.1-23.1µm and width 3-6 ×3.88.4µm.

Colletotrichum gloeosporioides was identified as the causal organism of anthracnose of water yam (*D. alata*) by Abang *et al.* (2002) based on acervular fruitification with oval to oblong conidia. In addition, the morphological, cultural, and virulence characteristics were investigated. The growth rate ranged from 5.9 to 10.5 mm/day, with a maximum colony diameter of 87mm and maximum conidial length and width (16.7 and 4.7 µm).

The fungus *Colletotrichum gleosporoides* produced white mycelia, which became dark grey with later formation of numerous salmon pink coloured spore masses. The conidia were hyaline, unicellular, aseptate and oval to cylindrical with rounded ends and were 10–20 µm long and 3–5 µm wide (Vidyalakshmi, 2012).

According to Manamgoda *et al.*, (2014) the hyphae of *H. maydis* are hyaline, pale to dark brown as grey, pale to dark brown conidiophores arose from these hyphae, they are single or branched, sometimes arranged in small groups, straight to flexuous or geniculate.

Sharma *et al.* (2015) reported that there are more than 600 synonyms of *C. gloeosporioides* that showed many morphological and physiological variations reported by Von Arx (1957). Conidia were straight, cylindrical and oval and borne on distinct well developed hyaline conidiophores (Sattar and Malik, 1939). Bose et al., 1973, observed the size of conidia varied from 11-16 x 4-6 µm and 13.8 x 4.8 µm, broad oblong with rounded ends 14.0 x 3.7 µm reported by Simmonds, 1965. Formae speciales of *C. gloeosporioides* was observed by Sutton, 1992 and also recognized the species as a heterogeneous group with a great variation in morphology. Baxter et al., 1985 defined *C. gloeosporioides* aggregate by using morphological methods and reported that conidia were cylindrical with rounded ends and less than 4.5 µm in diameter .

The mycelium of *C. gloeosporioides* is hyaline, septate and branched. The conidiomata are acervular, separate, composed of hyaline to dark brown septate hyphae. In culture the fungus produces sclerotia, which are dark brown, occasionally setose. The setae are long, brown and septate. The conidiogenous cells are enteroblastic, phialidic hyaline, conidia are hyaline, one celled, straight, cylindrical and obtuse at apices. The fungus produces hyaline, one celled, ovoid to oblong, slightly curved or dumbbell shaped conidia, 10-15 µm (average) up to 20 µm in length and 5-7 µm in width (Gautam, 2015).

Deshpande and Deshpande (2015) studied on *Helminthosporium atypicum* and saw that the colony on PDA were fast spreading, reaching a diameter of 90 mm on the 6th day; aerial mycelium abundant, olivaceous to black, wooly. Vegetative hyphae profusely branched, septate, olivaceous 5-7.0 µ, in width, hyphae sometimes give out thread like lateral, rhizoidal proliferations; conidiophores lateral or terminal, usually unbranched, 5—20 septate; when terminal, 2 conidiophores grow side by side from a common point, pale, olivaceous, septate, 291.6—510 X 7—13.8 µ; conidiophore proliferation percurrent, conidia arranged variously, terminal as well as lateral when lateral, uniparous or alternate, cylindrical, 3—13 septate, average distance between two septa 5.7 µ,, olivaceous, 23—126 X 11.5—13.8 µ; conidial proliferation axial; hilum in the form of a short cylindrical protuberance; germination uni or bipolar, percurrent or lateral, producing 1-3 germ tubes from the same cell.

Pal *et al.* (2015) discovered that isolates of *H. maydis* produced dark-colored, fusoid conidia measuring 6.14-23.41 µ X 2.15-5.70 µ and the colour of mycelium varied between media, the colour was black ranging from light black, light grey, light green, and grey.

Sonavane *et al.* (2015) studied on Forty-two sporulating isolates of *Drechslera, Helminthosporium, Bipolaris* and *Exserohilum* obtained from Indian Type Culture Collection, New Delhi. Macroscopic and microscopic studies revealed that, the isolates were assembled into two major groups and three sub-groups; there was a lot of variation in the cultural characters. In group I (28 isolates) aerial mycelium was fluffy, cottony and whitish gray in colour and there was wide variation in radial growth of the isolates [8.45 cm in D19 to 2.2 cm in D7]. In group II (14 isolates), most of the cultures were black in colour and texture was smooth in which radial growth was highest in E7 (8.65 cm) and lowest in D42 (6.25 cm). It was found that germination from two or more cells of conidia separates *Drechslera* from *Bipolaris* and *Exserohilum*. Whereas, presence of strongly protuberant hilum structure differentiates between *Exserohilum* and *Bipolaris* isolates. Thirteen of the isolates having strongly protuberant hilum structure were grouped into the genus *Exserohilum*. Remaining 29 isolates were considered as the genus *Bipolaris* group with no protuberant hilum.

Meehan *et al.* (2015) studied on *Helminthosporium victoriae* and found that the conidiophores of H. *victoriae* were erect, simple, emerging usually singly, rarely in clusters of two to five, from the stomata or between the epidermal cells of diseased culms, pale olivaceous to medium-brown, 60 to 280 by 5.8 to 10 μ, mostly 120 to 160 by 6.5 to 7.8 μ, with 4 to 10, generally 6 to 8 septa; rather closely geniculated, apical spore-producing area is 30 to 80 μ, in length. The fuliginous to dark olivaceous, typically pale olivaceous, slightly curved, rather thin-walled conidia, tapering to a rounded tip, measure 40 to 130 by 11 to 25 (70 by 15) μ and were provided with 4 to 11 (8) septa. Germination was effected by one polar germ-tube from each terminal cell. Conidia produced on water agar are somewhat below normal size and had fewer septa than those formed on the host, while weathered spores on the bases of field plants were often dark brown and of irregular shape, with a thick exospore.

Jaiganesh and Kannan (2019) studied on *Helminthosporium oryzae* causing brown spot of rice and found that the colony morphology of all the isolates showed olivaceous, light brown to black, septate, profuse aerial/submerged and branched mycelium. The colour of the conidia was brown to light brown colour. The conidia were slightly curved with a bulge in the middle and tapering towards the ends and when fully mature they were brownish in colour. The size of the conidia significantly varied among the isolates from 29.3 to 33.2 µm length and 13.5 to 14.8 µm width.

2.8.3 Cultural characteristics

Hau and Rush (1980) discovered that PDA induces abundant conidia production in *H. oryzae* when exposed to 12 hours of black light followed by 12 hours of darkness, whereas continuous illumination or complete darkness does not induce sporulation.

Sinclair (1982) studied the growth and sporulation of the soybean leaf spot pathogen (*Corynespora cassiicola*) on various media and discovered that growth and sporulation were abundant on potato dextrose agar and czapek's dox agar.

All the isolates of *Colletotrichum gleosporoides* showed differential response in requirements of media, temperature and media pH for growth and sporulation. Malt Extract Agar (MEA) medium was best suited for growth in terms of radial mycelial diameter for all the isolates. Among the studied isolates, Cg 72 (from Maharashtra) showed more virulence and maximum sporulation (137.5×103 ml^{-1}) at 28°C and media pH 6. Maximum growth and virulence at 28°C was observed with Cg 62 isolate. Media of pH 6 was found to be most suitable for the growth of respective isolates (s), but Cg 62 which was collected from Bihar was found to be most virulent in this experiment (Banik *et al.,* 1998).

Natural *et al.* (1994) reported the causal fungus of Anthracnose or spadix rot of Anthurium was isolated and studied. Based on morphological and cultural studies and the characteristic symptoms produced on Anthuriums, the fungus was identified as *Colletotrichum gloeosporioides* (Perrz.) Sacc. Symptoms varied from small dots to blackening of the tip of the spadix, thus termed black nose in other countries. The fungus showed varied growth characteristics and conidial production in various media. Widest mycelial colony diameter and maximum conidial production was obtained when *C. gloeosporioides* was grown on PDA adjusted to pH 5.5 and incubated at room temperature. Corn meal agar, carrot decoction agar and tomato fruit decoction agar also supported the growth of *C. gloeosporioides* but the number of conidia produced was much less.

Soltani (2014) investigated the growth of *Colletotrichum gleosporoides* on various culture media, which included potato dextrose agar (PDA), carnation leaf agar (CLA), potato carrot agar (PCA), and water agar (WA). Under continuous light at 28°C, PDA and PCA culture media provided the best conditions for *C. gloeosporioides* growth. Reduced light periods slowed fungal growth. Furthermore, fungal sporulation was found to be highly dependent on light, temperature, and the culture medium used. *C. gloeosporioides* sporulation was at its peak on Fabriano paper placed on PDA medium during a 16/8 h light/dark interval at the same temperature. At a lower temperature, that is 22°C, *C. gloeosporioides* sporulation on the same culture media was highly defected. Furthermore, aging generally increased the fungal sporulation.

Kumar and Singh (2008) discovered that Potato dextrose agar and Richard's agar were the best for radial growth and excellent sporulation of *H. maydis,* with colony diameters of 78.6 mm and 66.0 mm, respectively.

Colletotrichum gloeosporioides was found to cause anthracnose disease of Anthurium. Studies on different nitrogen sources revealed that *C. gloeosporioides* produced maximum vegetative growth on peptone followed by L-cystine. This was followed by urea, ammonium dihydrogen phosphate and methionine. Methionine and sodium nitrate supported profuse sporulation. Poor vegetative growth of the test fungus was recorded when the basal medium was separately supported with sodium nitrate and ammonium sulphate as nitrogen sources. Poor sporulation was recorded with ammonium sulphate, ammonium chloride and L-cystine (Deshmukh and Mehetre, 2010).

Manjunath *et al.* (2011) discovered that *C. gloeosporioides* grew best in a pH range of 6.50–7.00 and a temperature range of 25–30°C. *C. gloeosporioides* mycelial growth was greatest when exposed to alternate cycles of 12 h light and 12 h darkness, as opposed to 24 h exposure to continuous light and 24 h exposure to continuous dark. The host leaf extract medium supported significantly the maximum growth of all ten isolates of *C. gloeosporioides* among the different media tested, followed by potato dextrose agar.

Naz *et al.* (2011) found that Richard's agar was the most effective supporting medium for the growth of *Helminthosporium maydis* after an incubation period of 7 days, followed by PDA (78 mm), Basal medium (70 mm), Corn meal agar (70 mm), and Czapek's medium (70 mm), while Water agar was found to be the least effective (50 mm).

Colletotrichum gloeosporioides was found to cause anthracnose of Anthurium. Studies on various carbon sources revealed that *C. gloeosporioides* produced maximum vegetative growth on dextrose followed by starch and lactose. Vegetative growth on remaining carbon sources was in the order of maltose, fructose, sorbitol, mannitol, citric acid and control. Profuse sporulation was recorded in the media in presence of starch and dextrose. Mannitol, sorbitol, lactose and fructose supported moderate sporulation. Poor sporulation was observed on maltose and citric acid sources. *Colletotrichum gloeosporioides* was found to cause anthracnose of Anthurium. Studies on various carbon sources revealed that *C. gloeosporioides* produced maximum vegetative growth on dextrose followed by starch and lactose. Vegetative growth on remaining carbon sources was in the order of maltose, fructose, sorbitol, mannitol, citric acid and control. Profuse sporulation was recorded in the media in presence of starch and dextrose. Mannitol, sorbitol, lactose and fructose supported moderate sporulation. Poor sporulation was observed on maltose and citric acid sources (Deshmukh, 2011).

Sixteen isolates of *C. musae* were collected from different banana growing areas of Tamil Nadu and their pathogenicity was proved under laboratory conditions. Effect of different pH levels, temperature, light intensity and media were tested against the growth of *C. musae* under *in-vitro* condition. Results indicated that the growth of *C. musae* was maximum at pH range of 6.50-7.00 and temperature range of 25-30°C. Exposure of the fungus to alternate cycles of 12 hr light and 12 hr darkness resulted in the maximum mycelial growth of *C. musae* compared to the 24 hr exposure to either continuous light or dark. Among the different media tested, Potato dextrose agar medium supported significantly the maximum growth of all the sixteen isolates of *C. musae*. Further, the strains were found to vary morphologically between the isolates under the study (Thangamani, 2011).

Tulasi (2012) reported that all of the isolates grew cottony and appeared whitish at first, but turned dark brown with wide variation in radial growth and differed in sporulation initiation and intensity (13.7-40.7 days) with age on different media. The Nlr and Sklm isolates differed slightly when the culture medium was changed. In terms of radial growth, Bpt isolate was the fastest, while Mtu isolate was the slowest. PDA supported better radial growth than CDA and RLE for the majority of isolates, with the exception of Mtu, which grew better on RLE.

Bhavani *et al.* (2016) isolated the fungus *Helminthosporium maydis* from maize and studied its growth on 5 different media and found that the highest mycelial growth was recorded on PDA (35.25 mm) followed by Richard's agar (20.75 mm). The next best media in order of merit were Czapek's agar (15.25 mm) and Oat Meal Agar (11.75 mm) medium. However, the significantly lowest mycelial growth (6.75 mm) was recorded on Peptone sucrose agar medium. The excellent sporulation of *H. maydis* was observed on PDA and Richard's agar medium. Fair sporulation recorded on Oat meal agar, while moderate sporulation was observed on Czapek's agar medium.

Nayak and Hiremath (2019) reported that among the different media tested, potato dextrose agar demonstrated the greatest radial growth in all isolates, followed by host extract dextrose agar.

2.9 Management of anthurium leaf blight

Several Anthurium cultivars are grown commercially for the cut-flower trade. Among the most popular are Kaumana, Kozuhara, and Kansako No.1, but these are highly susceptible to spadix rot in the field. Also popular are Ozaki and Nitta Orange, which are moderately resistant to the disease. Abe Pink and Kansako No. 2 have been recognized by growers to be highly resistant. These have remained virtually free from the disease even under conditions that are very favorable to anthracnose development (Aragaki *et al.,* 1968).

According to Panda (1996), *Trichoderma viride* (T8 isolate) was antagonistic to the test fungus and significantly inhibited *Helminthosporium maydis* spore germination.

Dash (1997) identified *Trichoderma viride, T. Bioderma,* a commercial formulation of *Trichoderma viride* or *T. harzianum* and *Gliocladium virens* to be antagonistic to the pathogen. *T. Harzianum* was equally effective in inhibiting the growth of *Helminthosporium maydis* in vitro.

The *Colletotrichum* fungus produced thousands of small hot-dog-shaped spores that can readily be moved by splashing water, air movement, and workers. A strict sanitation program is crucial to control the spread of this pathogen in a production facility. Fungicides containing mancozeb (Protect T/O™, Dithane®) are effective. Fungicide applications are usually discouraged because chemical residues diminish the marketability of flowers and plants. *Anthurium* plant breeding programs both in Hawaii and Florida have incorporated disease resistance into many of the current cultivars. Newer cultivars are highly resistant to this pathogen and rarely exhibit black nose (Chen *et al.,* 2003).

Poudyal *et al.* (2005) investigated the effects of various chemicals Kresoxim methyl, Azoxystrobin, Propiconiazole, Trifloxystrobin + Tebuconazole, Difeconazole and Tricyclazole on the radial growth of the fungus *Helminthosporium* and discovered that Azoxystrobin and Tricyclazole were the most effective.

Sundravadana *et al.,* (2007) tested efficacy of azoxystrobin on *colletotrichum gloeosporiodes* penz. and the efficacy of azoxystrobin, one of the strobilurin class fungicides, was evaluated both *in-vitro* and *in-vivo* conditions. In *in-vitro* tests, azoxystrobin completely inhibited mycelial growth of *C. gloeosporioides*. In field experiment, azoxystrobin at 1, 2 and 4 ml/l significantly suppressed the development of both panicle and leaf anthracnose.

Sharma and Verma (2007) studied cross pathogenicity on *Colletotrichum gleosporoides* and revealed that complete inhibition of linear growth of *C. gloeosporioides* by systemic fungicides *viz.* Bavistin and Topsin-M proved most effective at the concentration of 100µg/ml. Among the non synthetic fungicides, Kavach and Dithane M-45 provided complete growth inhibition at 1000100µg/ml followed by captaf and Bordeaux mixture.

Barrera-Necha *et al.* (2008) investigated the efficacy of nine essential oils in vitro on conidial germination and mycelial growth inhibition of *Colletotrichum gloeosporioides* isolated from papaya (*Carica papaya* L.) and observed better antifungal effect with *Cinnamomum zeylanicum* and *Syzygium aromaticum* oils which had strong inhibition of conidial germination of *C. gloeosporioides* at 50, 100, 150, 200 and 250 µg ml^{-1} and a dose dependent inhibition of mycelial growth was caused by these oils. Teloxys ambrosioides, Mentha piperita and Ruta chalepensis oils exhibited a moderate action at 150, 200 and 250 μg ml^{-1} on conidial germination and mycelial growth inhibition.

Deshmukh and Mehetre (2010) discovered that systemic fungicides such as propiconazole (Tilt 25 percent EC), carbendazim (bavistin 50 percent WP) at concentrations of 250, 500, and 1000 ppm and non-systemic fungicides such as copper oxychloride (blue copper 50 % WP) at concentrations of 1000, 2000, and 3000 ppm which were found to be highly fungitoxic at all concentrations to *C. gloeosporioides*, which caused Anthurium leaf blight.

The anti fungal effects of some plants extracts namely tobacco leaf, keora seed, keora, mahogoni, gaint indian milky weed, garlic and ginger at different concentrations (30%, 40%, 50%, 60% and 70%) on the growth and development of *C. gloeosporioides*, causal agent of anthracnose of mango were evaluated. Radial growth of *C. gloeosporioides* was recorded. The growth inhibition increased with the increase of concentration of all the plant extracts. Highest mycelial growth inhibition (74.35%) was observed in case of garlic extracts at 70% concentration. Garlic extract at 50% and 60% concentration were also effective than other treatments (Mukherjee *et al.,* 2011).

Hossain *et al.* (2011) investigated the effectiveness of Bion (benzothiodiazole), Amistar (azoxystrobin), and Tilt (propiconazole) in controlling Brown spot and Narrow brown spot in rice cv. BR11 (Mukta). Amistar @ 50 mg/L and Tilt @ 1 ml/L were sprayed at the tillering and panicle initiation stages, respectively, and resulted in a reduction of brown spot and a significantly increased number of grains/ear number of healthy grains/ear and higher grain yield compared to the untreated control.

Gomathinayagam *et al.* (2012) collected cultures of *T. harzianum* and *T. viride* for the control of brown spot diseases in rice and obtained satisfactory results in both biocontrol agents against the rice pathogen *Helminthosporium oryzae*.

Abd-Alla and Haggag (2013) studied the efficacy of some essential oils like Basil oil (*Ocimum basilicum*), Orange oil (*Citrus sinensis*), Lemon oil (*Citrus Medica*) and Mustard oil (*Brassica juncea*) in reducing the postharvest losses induced by *Colletotrichum gloeosporioides* (Penz.) in mango fruits. At low concentration (50μg/ml) orange oil caused 10.0% reduction in fungal growth, while at 100μg/ml it caused 72.2% and at high tested concentration (150μg/ml) it caused a complete reduction in mycelium linear growth of pathogenic fungus. Meanwhile, at low tested concentration (50 μg/ml), mustard oil caused a highly significant reduction of the percentage of fungal spore germination by 70.8% followed by basil oil by 64.7%. At low concentration of 250 ppm, mustard oil caused a high reduction of anthracnose incidence of mango fruits by 79.9% followed by basil oil with 66.7%. On the other hand, orange and lemon oil at low concentration (250ppm) showed a high effect in reducing the percentage of rotting of fruit tissue by 84.5 and 75.0%, respectively if compared with other treatments and un-treated fruits.

Bautista-Rosales *et al.,* (2014) studied the Mechanisms of action and effectiveness of the antifungal yeast *Cryptococcus laurentii* [(Kuff.) C.E. Skinner] strain L5D, which were examined against the causal agent of anthracnose *Colletotrichum gloeosporioides* (Penz. & Sacc.) in mango (*Mangifera indica* L.). *C. laurentii* showed a high antagonistic potential *in vivo*, with significant inhibition of anthracnose (75.88%). Different mechanisms of action were examined in *C. laurentii* and among them competition for nutrients, specifically for sucrose ($p < 0.05$) was observed.

Ashwini and Srividya (2014) found that a soil bacterium, *Bacillus subtilis*, isolated from the rhizosphere of Chilli, showed high antagonistic activity against *Colletotrichum gloeosporioides* OGC1. A clear inhibition zone of 0.5–1 cm was observed in dual plate assay. Microscopic observations showed a clear hyphal lysis and degradation of fungal cell wall. In dual liquid cultures, the *B. subtilis* strain inhibited the *C. gloeosporioides* up to 100 % in terms of dry weight. This strain also produced a clear halo region on chitin agar medium plates containing 0.5 % colloidal chitin, indicating that it excretes chitinase. The strain also produced other mycolytic enzymes—glucanase and cellulase, demonstrated by a clear zone of hydrolysis of yeast cell wall glucan (YCW 0.1 % v/v) and carboxymethylcellulose (CMC 0.1 % v/v). In liquid cultures, the strain showed appreciable levels of chitinase, glucanase and cellulase activities and hydrolytic activity with *C. gloeosporioides* OGC1 mycelia in the substrate.

Antifungal activities of 20 plant species were tested against *Colletotrichum gloeosporioides*, the causal agent of mango anthracnose. Culture sensitivity tests of the plant extracts were done using paper disc diffusion inhibition method. Among the twenty plant species extracted with ethyl acetate *Ruta chalepensis* and *Eucalyptus globulus* showed the highest activity. Methanol extracts of three plants, namely *Datura stramonium*, *Adhatoda schimperiana* and *Eucalyptus globulus* also showed remarkable activity. The extracts also significantly inhibited conidial germination of the test fungi over the control (Alemu *et al.*, 2014)

Begum *et al.* (2015) studied on *in-vitro* efficacy of fungicides against four isolates of *Colletotrichum capsici* was evaluated by poisoned food technique. Eight commercial fungicides, viz., Bavistin 50 WP (Carbendazim), Nativo 75 WP (Trifloxystrobin + Tebuconazole), Folicur 25.9 EC (Tebuconazole), Flint 50 WG (Trifloxystrobin), Amistar 23 SC (Azoxystrobin), Kocide 77 WP (Copper hydroxide), Indofil M-45 75 WP (Mancozeb) and Saaf 75 WP (Carbendazim + Mancozeb) were tested at four different concentrations i.e., 50 µg/ml, 100 µg/ml, 150 µg/ml and 200 µg/ml. All the fungicides except Carbendazim efficiently inhibited the linear growth of the four isolates of the fungus. Tebuconazole, Mancozeb and Trifloxystrobin + Tebuconazole each @ 150 µg/ml and Carbendazim + Mancozeb @ 200 µg/ml were significantly superior to all other treatments in inhibiting mycelial growth of isolates SCC1 , SCC2 and SCC4 by 100%. Whereas complete inhibition against SCC3 was obtained by Tebuconazole and Trifloxystrobin + Tebuconazole each @ 150 µg/ml and Carbendazim + Mancozeb at 200 µg/ml. Out of the eight fungicides tested carbendazim showed the least Correspondence inhibition of mycelial growth i.e., only upto 25% even at the concentration of 200 µg/ml.

Lal *et al.* (2015) tested two fungicides (Carbendazim 50 percent WP and Tricyclazole 75 percent WP) against *Helminthosporium sp.* in-vitro using the poisoned food technique, as well as the bioagent *Pseudomonas fluorescens* (2108 cfu/ml). Helminthosporium *oryzae* was cultured in the laboratory using a dual culture technique. Carbendazim 50 percent WP showed the greatest inhibition (100%) at 0.1 percent, 0.2 percent, and 0.4 percent concentrations at 4, 8, and 12 days, while Tricyclazole 75 percent WP showed the greatest inhibition (100%) at 0.06 percent and 0.12 percent, followed by 98.93 percent inhibition at 0.03 percent concentration at 4, 8, and 12 days, and the bioagent *P. fluorescens* was also found to be inhibit the mycelial growth of *Helminthosporium* significantly (59.72 %).

Eleven yeasts from avocado (three from fruits, four from leaves and four from rhizospheric soil) were isolated; of which three showed *in vitro* antagonistic activity against *C. gloeosporioides* and *C. acutatum*. ITS sequence analysis of the isolated yeasts revealed that the strains obtained from fruits belonged to *Candida intermedia* while those isolated from leaves belonged to *Wickerhamomyces anomalus*. *C. intermedia* reduced disease incidence caused only by *C. gloeosporioides*, whereas, *W. anomalus* caused a significant reduction in the incidence and severity of disease caused by both C. *gloeosporioides* and *C. acutatum* (Campos-Martínez *et al.*, 2016)

Sawant *et al.* (2016) isolated two hundred and ninety-three bacteria from the grape ecosystem of 43 spatially distant vineyards in peninsular India. Of these, 25 bacteria substantially inhibited the radial growth of *C. gloeosporioides* in '*in vitro'* studies and 18 bacteria significantly reduced infections *in vivo*. Of these 18 bacteria, 5 and 3 bacteria also significantly reduced percent disease index (PDI) of downy and powdery mildew diseases, respectively. These bacteria were labeled as strains, DR-38, DR-39, TL-171, DRo-198, TS-204, TS-205, and DR-219, and were identified as *Bacillus* species based on morphological and molecular characterisation. Aqueous suspensions of all these strains applied as foliar sprays at 1×10^8 cfu/ml on field grown vines significantly lowered the PDI and the AUDPC (area under disease progress curve) of anthracnose. Strains TS-204 and TL-171 were identified for biocontrol of anthracnose in grapes.

Agaraki and Ishii (2016) studied that the new fungicide benzovindiflupyr was highly active against all nineteen isolates of *Colletotrichum* species, including *C. gloeosporioides*, *C. acutatum*, *C. orbiculare*, *C. cereale* and *C. truncatum.* Benzovindiflupyr exhibited very high inhibitory activity against the germination of *Colletotrichum* species.

In-vitro biological control of *Colletotrichum gloeosporioides* by *Aspergillus flavus* gave good results and appeared to be the most effective against the test pathogen followed by *Aspergillus niger* and *Trichoderma harzianum*, while *Aspergillus fumigatus* gave poor results. Out of six fungicides tested against *Colletotrichum* *gloeosporioides*, systemic fungicides gave better results than non systemic fungicides. Least colony growth was observed in case of Derosal which gave effective control against *C. gloeosporioides* followed by Bayleton, Daconil, Ridomil Gold, Mancozeb and Alliete. By the application of these strategies the anthracnose problem could be managed properly with better economic benefits and small risk of health hazard effects (Haider *et al.*, 2016).

Chaudhuri and Gohel (2016) tested nine fungicides at three concentrations under *in vitro* condition against C. gloeosporioides by poisoned food technique, propiconazole, carbendazim(12%) + mancozeb (63%) and propineb completely inhibited the mycellial growth and proved to be most effective over rest of the treatments. The next best were azoxystrobin + difeconazole and trifloxystrobin + tebuconazole.

Liu (2018) evaluated the a total of 216 killer yeasts *Saccharomyces cerevisiae*, isolated from wine, for control of *Colletotrichum gloeosporioides*, a pre-harvest anthracnose agent of grape. Three of these yeast isolates were tested positive for antagonizing *C. gloeosporioides* and were further evaluated for their mechanisms as biological control agents (BCAs): production of antifungal compounds, production of hydrolytic enzymes, inhibition of *C. gloeosporioides* conidia germination, colonization on grape berry, and efficiency in controlling anthracnose of grape. The results showed that all three *S. cerevisiae* isolates produced antifungal compounds, inhibited *C. gloeosporioides* conidia germination and produced β-1, 3-glucanase and chitinase.

Oliveira *et al.* (2020) evaluated the efficacy of succinate-dehydrogenase-inhibitor (SDHI) fungicides like penthiopyrad and benzovindiflupyr in managing Colletotrichum crown rot (CCR) caused by *Colletotrichum gloeosporioides.* The EC50 for benzovindiflupyr and penthiopyrad varied from 0.08 to 1.11 and 0.45 to 3.17 µg/ml, respectively.

Seven strains were isolated and identified as *Trichoderma harzianum*, *T. guizhouense*, *T. atroviride* and *T. koningiopsis* through morphological and molecular analyses. Five of them showed effective antagonistic performance *in vitro* against the pathogens. The strain *T. harzianum* IC-30 was the best biological control agent *in vivo*, obtaining a reduction of rot percentage around 80% after 3 weeks of infection of oranges with *P. digitatum A21* (resistant to pyrimethanil). This strain also showed the highest chitinase and glucanase activities (Ferreira *et al.*, 2020)

Yangyang *et al.* (2021) studied the efficacy of novel fungicide mefentrifluconazole against *Colletotrichum Scovillei* and found that the sensitivity of *C. scovillei* to mefentrifluconazole was determined by mycelial growth and germ tube elongation assays using 157 single-spore isolates with mean 50 percent effective concentration values of 0.462 ± 0.138 and 0.359 ± 0.263 mg/liter, respectively. The *in vivo* data also showed that mefentrifluconazole had favourable protective and curative effects against pepper anthracnose. Mefentrifluconazole significantly affected *C. scovillei* infection on pepper by reducing appresorium formation and sporulation, shrivelling spores and germ tubes, and causing the abnormal development of appresoria and conidiophores.

Gama *et al.* (2021) evaluated the sensitivity of blueberry *Colletotrichum gloeosporioides* to azoxystrobin, benzovindiflupyr, penthiopyrad, pydiflumentofen, boscalid, thiophanate-methyl, fluazinam and fludioxonil and found that all isolates were sensitive to benzovindiflupyr, penthiopyrad, fluzinam and fludioxonil.

MATERIALS AND METHODS

The present investigation was carried out in the Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar. Laboratory works were conducted in the Laboratory of the Department.

The details of the materials used and the methods adopted in the present investigation are described here as under:

3.1 Materials

3.1.1 Host Plant

Anthurium plants were collected from the garden of the Department of Floriculture and Landscaping, College of Agriculture, OUAT, Bhubaneswar, for testing and pathogen isolation.

3.1.2 Diseased sample

Leaves of the diseased plants were collected from the garden in sterilized poly bags and were taken to the laboratory.

3.1.3 Glasswares

The glasswares that were used in the investigation were of Borosil make. Petri plates, test tubes, 250 ml, 500 ml, and 1000 ml conical flasks, funnel, beakers, pipettes, measuring cylinder, slides, cover slips, and glass rods were among the glasswares utilised.

3.1.4 Equipments used

Standard laboratory equipments *viz.* autoclave, laminar air flow, research microscope, stereoscopic binocular microscope, refrigerator, hot air oven, digital weighing balance, Bunsen burner, pH meter, water distillation unit etc. were used.

3.1.5 Culture Medium

The microbes were isolated, cultured, and sub-cultured on potato dextrose agar (PDA) medium. Different nutritional media, such as Potato dextrose agar (PDA), Carrot agar, Oat meal Agar, Water Agar, Czapek dox Agar, and Potato carrot infusion

Agar, were used to study the pathogen's growth behaviour. The ingredients of different media are as follows

a) Potato Dextrose Agar medium

Peeled and sliced potatoes	200.0 g
Dextrose	20.0 g
Agar	20.0 g
Distilled water	1000.0 ml

b) Carrot agar medium

Carrot agar	200.0 g
Dextrose	20.0 g
Agar	20.0g
Distilled water	1000.0 ml

c) Oat meal agar medium

Oat meal	72.0 g
Agar	20.0 g
Distilled water	1000.0 ml

d) Water agar medium

Agar	20.0 g
Distilled water	1000.0 ml

e) Czapex dox agar medium

Sodium Nitrate	2.0 g
Potassium dihydrogen phosphate	1.0 g
Magnesium sulphate	0.5 g
Potassium chloride	0.5 g
Distilled water	1000.0 ml

f) Potato carrot infusion agar medium

Potato	200.0 g
Carrot	200.0 g
Agar	15.0 g
Water	1000 ml

3.1.6 Organics, Bioagents and Chemicals

A commercial formulation of combination of Tebuconazole 50% and Trifloxystrobin 25% WG available in the Trade name of 'Nativo', combination of Captan 70% and Hexaconazole 5% WP available in the Trade name of 'Taqat', alone Hexaconazole 5% EC available in the Trade name of 'Contest', and` Copper Oxychloride 50% WP available in the Trade name of 'Blitox 50 WP', a contact fungicide, were the chemicals used in case of chemical treatment.

3.1.7 Other Materials used in Laboratory and Field

Blotter paper, non-absorbent cotton, muslin cloth, cork borer, inoculation needle, forcep, sterilized soil, pots etc. were used during research programme.

3.1.8. Maintenance of aseptic condition

All the operations in the laboratory were carried out inside an inoculation chamber under laminar air flow and prior to it, it was disinfected by spraying formalin and with U.V light to maintain aseptic condition.

3.2 Methods

3.2.1 Observation of diseases

Anthurium plants present in the garden of Department of Floriculture and Landscaping, College of Agriculture, OUAT, Bhubaneswar were randomly observed at regular intervals to see the presence of diseases.

3.2.2 Collection of Diseased Samples

In sterile plastic bags, diseased leaves of Anthurium plants were collected. The polythene bags were tagged and kept at 4°C until the pathogen was isolated.

3.2.3 Cleaning and sterilization of glasswares

Petridishes, culture tubes, conical flasks, beakers, and other glasswares used in the study were cleaned by dipping them in chromic acid solution for four hours to remove any oil on the surface. The chromic acid was made by dissolving 60g of potassium dichromate in one litre of distilled water, then gently adding 60ml of concentrated sulphuric acid. The glassware was then properly cleaned with mild detergents, rinsed three to four times in distilled water, and then dried. All cleaned glassware was air dried and wrapped before being sterilised in a hot air oven for 2 hours at 160°-180°C. The sanitised glasswares were stored in an aseptic manner for future use.

3.2.4. Sterilization of plant materials

To eliminate surface-borne microbes, the collected plant materials were washed 2-3 times in tap water before being dipped in 0.1 percent mercuric chloride solution for 2 minutes and rinsed with sterile water three times to remove traces of $HgCl_2$ solution.

3.2.5 Sterilization over flame

The prickling needle, inoculation needle, spear-head needle, glass rod, forceps, and nichrome wire loop were sterilised by soaking them in 70% ethanol and then passing them over a spirit lamp. Just before use, the cover slip and slide were placed over a flame.

3.2.6. Sterilization of blotting paper

It was sterilized in hot air oven for 2 hours at 160^0C by placing it inside Petri dish.

3.2.7. Sterilized moist chamber

Sterilized blotting paper were placed in Petri dishes aseptically and sterilized cotton swab was placed in it. It was moistened with sterile water.

3.2.8. Sterilization of water

Distilled water was put in a conical flask and plugged with non-absorbent cotton and sterilized in an autoclave at 15 psi for 20 minutes

3.2.9 Sterilization of other materials

The prepared media during the study were sterilized by autoclaving at 15 psi for 20 minutes at 121°C.

3.3 Preparation of Media

3.3.1 Potato dextrose agar medium

Potatoes were peeled and cut into small pieces and boiled in 500 ml of distilled water in a 1000 ml beaker until softened. A double-layered muslin cloth was used to filter the extract. Another 500 ml of distilled water was taken in another 1000 ml beaker, 20g of agar was added and melted until dissolved. Both solutions were combined in another 1000 ml beaker containing 20 g of dextrose. The medium's final volume was increased to 1000 ml by adding distilled water. The pH of the medium was adjusted to 6.8 using the pH metre and 1 N NaOH or 1 N HCl, depending on the case. The medium was poured into culture tubes (8.0ml) and conical flasks (100 ml), respectively. The media was autoclaved at 15 psi for 15 minutes.

3.3.2 Carrot Agar

Peeled carrot 250 g was cut into pieces and boiled in a container with 500 ml of distilled water until softened. The extract was collected by filtering it through a clean double layer muslin cloth. In a separate container 500 ml of distilled water was taken in which 20 g of agar was melted. The carrot decoction and melted agar were thoroughly combined, and the final volume was increased to 1000 ml. Slants were made by placing 7-8 ml of medium in a culture tube and plugged with non-absorbent cotton. The conical flask and culture tubes containing the medium were autoclave sterilised at 15 psi for 15 minutes.

3.3.3 Oat meal agar medium

All of the ingredients for the Oat meal agar medium were mixed in 500ml of distilled water. Agar agar was melted in 500 ml of distilled water before being mixed with the above solution. The medium was autoclaved for 15 minutes at 15 psi.

3.3.4 Water agar medium

Water agar medium was prepared by melting 20g of agar agar in 1000 ml of distilled water. The medium was autoclaved for 15 minutes at 15 psi.

3.3.5 Czapek’s Dox medium

Czapek's Dox medium was made by combining all of the ingredients in 500ml of distilled water. Agar agar was melted in 500 ml of distilled water before being mixed with the above solution. The medium was autoclaved for 15 minutes at 15 psi.

3.3.6 Potato carrot infusion agar medium

Potato 200g and carrot 200g were thoroughly washed and peeled with sterile distilled water. In a 1000 ml beaker, potato and carrot were sliced into small pieces. The beaker is then filled with 500 ml of water and placed in a microwave oven for 20 minutes to boil. The potato and carrot pieces were removed, and the only supernatant was collected in a 1000ml volumetric flask and filled to a volume of 1000 ml. The volumetric flask was then filled with 15 g of agar and thoroughly mixed. The medium was then sterilised in an autoclave at 15 psi (121°C) for 15 minutes. It was poured into sterile Petri dishes after cooling.

3.4 General procedure followed

In all the *in vitro* studies, different replications were used for each set of treatments. In general, 15-20 ml of potato dextrose agar medium supplemented with streptomycin was poured into each Petri dish to prevent unwanted bacterial contamination. Wherever growth studies were conducted, a seven-mm disc of pure culture of *C. gloeosporoides* and *Helminthosporium spp* was used to inoculate medium in Petri dishes using a cork borer. For three days, the inoculated plates were incubated at 27°C ± 1°C. After inoculation, observations on growth were taken daily and recorded.

3.5 collection of diseased sample

The diseased leaf samples of Anthurium were collected from the garden of the Department of Floriculture and Land scaping, College of Agriculture, OUAT, Bhubaneswar (situated at 20.27^0N 85.84^0E with an altitude of 58 m above mean sea level)

3.6 Collection and isolation of the fungus

The leaves of Anthurium showing typical symptoms of blight i.e. circular to angular, light to dark brown spots with a dark red or blackish margin were collected from the Department of Floriculture and Landscaping, OUAT, Bhubaneswar. The fresh infected leaves were subjected to microscopic examination and tissue isolation for the causal agent. The symptoms on the leaves observed in nature were critically observed and recorded. The standard tissue isolation procedure was followed to isolate the pathogen. The infected leaf pieces along with some healthy portions were surface sterilized with 1 : 1000 mercuric chloride ($HgCl_2$) solution for 30 second and followed by subsequent three washing with sterilized distilled water and then transferred aseptically under laminar air flow system on sterilized petriplates containing 20 ml potato Dextrose Agar (PDA) medium. Such Petri plates were incubated at room temperature ($25^0 \pm 1$°C) and observed periodically for the growth of the organism which developed from the pieces. The organism was transferred to PDA slants and incubated at $25^0 \pm 1^0$C for 7 days.

3.7 Identification of the pathogen

A small outgrowth of the fungus was taken from infected host and mounted on a clean glass slide. One hundred conidia were observed under low power (lOx) microscope. The measurements *viz*, length and breadth of spores were recorded using stage and ocular micrometer. Similarly the morphological characters of the fungus from culture were also observed and compared.

3.8 Proving the pathogenicity

To prove the Koch's postulates, Anthurium plants raised in earthen pots were sprayed with distilled water. They were then covered with polythene bags for 24 hrs. The spore suspension from 10 days old culture was prepared in sterile distilled water. The spore suspension was spray inoculated on plants by using hand sprayer. Similarly control plants were sprayed with sterile distilled water for comparison. The seedlings covered with polythene bags were incubated for 3 days; the polythene bags were removed and the seedlings were kept under green house condition. Regular observations were made for the appearance and development of symptoms. The symptoms appeared within 15 days. Re-isolations were made from the affected tissues. The isolates obtained were compared with the original cultures for confirmation. The inoculation was carried out on three leaves of the Anthurium plants after pin pricking.

3.8.1 Spraying of spore suspension

The foliar Inoculation was done on three healthy leaves of Anthurium by spraying with spore suspension prepared from the sporulating 8 days old culture of *Colletotrichum* sp., by homogenization of culture in the distilled sterile water. The leaves to be inoculated were washed thoroughly with distilled water before inoculation.

3.8.2 Pin pricking

Sterilized pins were used to prick the leaves and inoculum was applied with sterilized cotton swab. Suitable controls with only pin pricking were maintained.

3.9 Maintenance of the culture

The fungus was subcultured on PDA slants and allowed to grow at 27 ± 2° C for 5 days and such slants were preserved in a refrigerator and renewed once again in about 45 days.

3.10 Innoculation

Five days old culture grown on PDA plates was used for the physiological studies and inoculations were made by placing 5mm discs cut from the periphery of the culture.

3.10.1 Agar agar based medium (Solid medium)

These sterilised agar agar media were poured aseptically into 90mm diameter pre-sterilized (oven temperature I80^{0}C for 2 hour) petriplates @ 20ml plate^{-l}. After solidification, a 5mm diameter culture block from an 8-day-old pure pathogen culture was placed in the centre of the petriplates using a sterilised cork borer. Three replications were maintained for recording observations on colony diameter and fungus characteristics. The petriplates were incubated at a temperature of 27 $\pm$ 2^0 C. The radial growth of the fungal mycelium was measured on a daily basis until the plates were completely covered with it. The resulting data was statistically analysed.

3.11 Cultural Studies

3.11.1 Effect of media

To know the effect of different media viz. Potato dextrose agar, Carrot agar , Oat meal agar, water agar, Czapex dox agar and potato carrot infusion agar media were used to find out the best medium for the growth and sporulation of *Colletotrichum gleosporoides* and *Helminthosporium spp.* 20 ml of media was poured in petriplate, 5mm discs of seven days old culture of test fungi were placed in the center of medium. Four replications were maintained for each treatment and observation for radial growth was recorded on 5th day after inoculation.

3.12 Morphological characterization and identification of the pathogen associated with *Anthurium* leaf blight

The fungi causing Anthurium leaf blight were isolated on potato dextrose agar medium and was examined periodically and identified basing on morphological characteristics *viz.* colour, growth pattern and morphology of spores and sporophores structure.

3.13 Management studies

3.13.1 *In-vitro* evaluation of fungicide by poisoned food technique

The relative efficacy of different chemical fungicides on inhibition of the mycellial growth of the fungi was studied at specified concentrations by following the poisoned food technique. (Nene and Thapliyal, 1993). The required quantity of fungicides were weighed and mixed in the molten potato dextrose agar medium by thorough shaking for uniform mixing of the fungicide before pouring in to petridishes so as to get the desired concentration of active ingredients of each fungicide. Six different concentrations (0.05%, 0.1%, 0.15%, 0.2%, 0.25% and 0.3%) of each chemical were used. Four replications were maintained for each fungicide for each of its concentrations in CRD. To avoid bacterial contaminations a little amount of streptomycin was added in each flask before plating; five mm disc was cut with the help of sterilized cork borer from 7 day old culture of the test fungi and was placed in the center of the medium in the reversed position to maintain continuous contact of the pathogen with poisoned medium. PDA plates without fungicides served as control.

Table 1. Details of Sole compound fungicides employed in the present investigation

Sl. No.	Trade name	Common name	Chemical name	Formulation	Manufacturing Company
1	Blitox50	Copper Oxychloride	Dicopper dichloride trihydroxide	50% WP	Tata Rallis
2	Mirador	Azoxystrobin	Methyl(*E*)-2-[2-[6-(2-cyanophenoxy)pyrimidin-4-yl]oxyphenyl]-3-methoxyprop-2-enoate	23% WP	Adama
3	Tebu	Tebuconazole	1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol	25.9 % EC	Heranba industries
4	Score	Difenoconazole	1-[[2-[2-chloro-4-(4 chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-methyl-1,2,4-triazole	25% EC	Syngenta
5	Kocide	Copper hydroxide	Copper dihydrate	53.8% DF	Dupont
6	Contaf	Hexaconazole	2-(2,4-dichlorophenyl)-1-(1,2,4-triazol-1-yl)hexan-2-ol	5% EC	Tata Rallis

Table 2. Details of combination compounds of fungicides employed in the present investigation

Sl. No.	Trade name	Common name with formulations	Chemical name	Source of supply
1	Cultivo	Tebuconazole 10% + Sulphur 65% WG	1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol + Sulphur	hpm
2	Nativo	Tebuconazole 50% +Trifloxystrobin 25% WG	1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol + Methyl 2-methoxyimino-2-[2-[[1-[3 (trifluoromethyl)phenyl]ethylideneamino]oxymethyl]phenyl]acetate	Bayer
3	Vitavax power	Carboxin 37.5% + Thiram 37.5% WP	6-methyl-*N*-phenyl-2,3-dihydro-1,4-oxathiine-5-carboxamide + dimethylcarbamothioylsulfanyl *N*,*N*-dimethylcarbamodithioate	Dhanuka
4	Clutch	Metiram 55% + Pyraclostrobin 5% WG	Tris[ammine[ethylenebis(dithiocarbamato)]zinc(2+)][tetrahydro-1,2,4,7-dithiadiazocine-3,8-dithione] + methyl *N*-[2-[[1-(4-chlorophenyl)pyrazol-3-yl]oxymethyl]phenyl]-*N*-methoxycarbamate	Pi industries

3.13.2 In vitro evaluation of Bioagents

Dual culture method (Dennis and Webster, 1971)

The pathogen and the test organism were grown on PDA medium, and from an 8-day-old culture, a 5mm disc of the test organism (antagonist) was cut aseptically from the colony's periphery and placed at one end of a petriplate containing 20 ml solidified PDA. A similar disc of the pathogen was aseptically placed in the opposite side, approximately 70mm away from the antagonist disc. Three repetitions of each treatment were maintained, and the plates with only pathogen served as control. The plates were incubated at room temperature (27 ± 2°C) for 10 days, and the radial growth of the test organism and pathogen was measured, and the percentage growth inhibition (PGI) was calculated using Vincent's formula (1927).

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

Where,

I = Per cent inhibition of mycelial growth

C = Colony diameter in control (mm)

T = Colony diameter in treatment (mm)

3.15 Statistical analysis

Statistical analysis was carried out by following the standard procedure through OPSTAT Software. Data in percentage were transformed to angular values before analysis.

EXPERIMENTAL RESULTS

The study entitled **"Leaf blight of Anthurium (*Anthurium andraeanum*) and its management"** was undertaken during the period 2019-21 in the Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar (situated at 20.270N 85.840E with an altitude of 58 m above mean sea level) and the results of the various studies conducted on **"Leaf blight of Anthurium (*Anthurium andraeanum*) and its management"** have been discussed below.

4.1 Symtomatology

The infected plants of Anthurium were observed in the garden of the Department of Floriculture and Landscaping, College of Agriculture, OUAT , Bhubaneswar for the symptoms of leaf blight. The symptoms comprised of black or brown, sunken spots observed on Anthurium leaves [Fig. 1-4]. The disease usually developed from the tip or along the margin of the upper surface of the leaf. On leaves the symptoms were typically observed on upper surface as water soaked, small, round to oblong, brown coloured spot. The size of these spots increased with age and finally two or more spots coalesced and developed into elongated reddish brown necrotic patches and surrounded by yellow colour zone. The centres of such spots finally turned greyish white with narrow brown zone around the spots surrounded by yellow halo. These spots rapidly enlarged, became watery, turned brown to black, and totally encompassed the spadix. The spadix eventually fell off. The fruiting bodies of the pathogen in the form of acervuli were visible as minute black dots under severe infection.

4.2 Collection, isolation, identification and purification of fungal pathogen associated with leaf blight of Anthurium and proving pathogenicity as per Koch's postulates

4.2.1 Collection of the sample, isolation and purification of the pathogen

The diseased leaf sample of the Anthurium plants were collected from the garden of Department of Floriculture and Landscaping, College of Agriculture, OUAT, Bhubaneswar. The infected leaves were brought in to the laboratory, and were subjected to microscopic examination and tissue isolation for the causal agent.

Tissue isolation from infected leaves resulted in getting pure cultures of *Colletotrichum sp*. and *Helminthosporium sp* .The cultures were further purified using the single spore isolation technique and was kept on Potato dextrose agar (PDA) for further investigation.

4.2.2 Identification

The pathogens were identified on the basis of morphological characters and cultural characters like colour and shape of mycelial growth, size and shape of conidiophores and conidia.The fungus *Helminthosporium sp.* in pure culture produced colonies with profuse cottony mycelium, initially white, later turning in to an ashy black colour mycelial mat, fluffy in margin, regular distinct rings which were produced after 10-12 days of inoculation (Figure 5).The mycelium was septate and hyaline. Whereas the fungus *Colletotrichum sp*. produced moderate to rapid growth on potato dextrose agar with grayish white to dark pinkish color mycelia after 10-12 days of inoculation (Figure 6).

4.2.3 Pathogenecity

To prove the Koch's Postulate, the pathogenic fungi (*Colletotrichum sp. and Helminthosporium sp*.) isolated from diseased Anthurium leaves were inoculated by spraying spore suspension with and without pin prick injury as described earlier. The inoculated fungus and water-inoculated (control) leaves were placed in sterilised desiccators containing sterile distilled water. Twelve Anthurium leaves were taken for the study, and four leaves of Anthurim of nearly equal size and age were surface sterilised with 1% Sodium hypochlorite and inoculated separately with the pathogen *Colletotrichum sp.* using the spore suspension with and without pin prick injury and four leaves were inoculated with *Helminthosporium sp.* following the same method. Four leaveswere sprayed with sterile distilled water. The onset of symptoms began 15 days after the inoculation (Figure 7-8)

The results pertaining to pathogenicity tests are presented in Table-3 and Table-4 and Figure 9-12. From the table, maximum symptoms on leaves were observed in the method of injury made by pin prick method (100%) followed by spraying spore suspension (50.00%) in case of *Colletotrichum sp.* where as in case of *Helminthosporium sp.* maximum symptoms were recorded in case of pin prick method (100%) following spraying spore suspension method (66.66%). Spraying sterile distilled water on an injured (uninoculated) surface produced no disease symptoms on Anthurium leaves (control). Re-isolations from artificially inoculated leaves consistently produced the same organism. Uninoculated leaves did not produce any symptoms, and tissues remained sterile after re-isolation. All methods of inoculation demonstrated pathogenicity. However both the pathogen showed similar trend in showing maximum symptoms in pin prick method followed by spore suspension method. Thus isolated *Colletotrichum sp.* and *Helminthosporium sp*. were proved pathogenic to Anthurium beyond doubt, satisfying the Koch's postulates.

Table 3. Pathogenicity test of *Colletotrichum sp.* on Anthurium leaves under laboratory condition

Sl. No.	**Innoculation Technique**	**No of leaves**		**% of Diseased leaves**
		Innoculated	**Infected**	
1	Spraying of spore suspension without injury	4	2	50.00
2	Spraying of spore suspension after injury by pin prick	4	4	100.00
3	Control (Spraying sterile distilled water on uninjured surface)	4	0	0.00

Table 4. Pathogenicity test of *Helminthosporium sp.* on Anthurium leaves under laboratory condition

Sl. No.	**Innoculation Technique**	**No of leaves**		**% Diseased leaves**
		Innoculated	**Infected**	
1	Spraying of spore suspension without injury	4	3	66.66
2	Spraying of spore suspension after injury by pin prick	4	4	100.00
3	Control (Spraying sterile distilled water on uninjured surface)	4	0	0.00

4.4 Studies on cultural characteristics of the pathogens

4.4.1 Effect of different media on growth and sporulation of the fungus

The majority of microorganisms require nutrients to survive. Their development, sporulation, reproduction and other physiological activities are determined by the amount of nutrient that is available in the media. The dietary requirements of different fungi are different and there is no selective medium available on which they can thrive. As a result, the current study was carried out in order to determine the best medium for *Colletotrichum sp.* and *Helminthosporium sp*. After 7 days of inoculation, radial mycelial growth was measured along two diameters at right angles to each other in each replication in the six different solid media. After 10 days of inoculation, sporulation was also observed and the results are presented in Table 5 and 6 and Figure 18-19.

Table 5. Growth of *Helminthosporium sp.* on different culture media

Tr. No.	Solid media	Radial growth of colony (mm)*	Sporulation
T1	Potato Dextrose Agar	68.20	+ + +
T2	Carrot Agar	48.33	+ +
T3	Oat meal Agar	50.82	-
T4	Potato carrot infusion agar	66.80	+ + +
T5	Czapex dox Agar	60.23	-
T6	Water Agar	52.16	-
C.D.		4.852	
SE(m)		1.558	

*Average of four replications + Sporulation present - Sporulation absent

Table 6. Growth of *Colletotrichum sp.* on different culture media

Tr. No.	Solid media	Radial growth of colony (mm)*	Sporulation
T1	Potato Dextrose Agar	76.88	+ + +
T2	Carrot Agar	74.24	+ +
T3	Oat meal Agar	44.72	+
T4	Potato carrot infusion agar	54.36	+ + +
T5	Czapex dox Agar	49.73	-
T6	Water Agar	56.53	-
C.D.		4.378	
SE(m)		1.405	

*Average of four replications + Sporulation present - Sporulation absent

It is evident from the result that among all the six media tested, significantly highest growth of the fungus *Helminthosporium sp*. was observed on Potato Dextrose Agar medium (68.20 mm) followed by Potato Carrot Infusion Agar medium (66.80mm). On the other hand Czapex dox Agar medium, water Agar medium and Oat meal Agar medium showed radial mycellial growth of 60.23mm, 52.16mm and 50.82mm respectively. The minimum radial growth was observed on Carrot Agar medium (48.33mm). Maximum sporulation was seen on Potato Dextrose Agar medium followed by Potato Carrot Infusion Agar medium and Carrot Agar medium. No sporulation was observed on Oat meal Agar medium, water Agar medium and Czapex Dox Agar medium.

In case of *Colletotrichum gloeosporioides* maximum radial growth of the fungus was observed on Potato Dextrose Agar medium (76.88mm) followed by Carrot Agar medium (74.24mm). The next best in the order were Water Agar medium (56.53mm), Potato Carrot Infusion Agar medium (54.36mm) and Czapex dox Agar medium (49.73mm) and Oat meal Agar medium (44.72mm). Maximun sporulation was observed on Potato Carrot Infusion Agar medium and Potato Dextrose Agar medium followed by Carrot Agar medium and Oat meal Agar medium. No sporulation was observed on water Agar medium and Czapex Dox Agar medium.

Table 7. Cultural characters of *Helminthosporium spp.* on different media

Sl. No.	Medium	Colony colour	Colour of hyphae	Sporulation	Growth pattern
1.	Potato Dextrose Agar	Greyish at center, white at periphery	Hyaline	Present	Creamish white growth producing aerial mycelium having sporulation at periphery at maturity of the growth which later turned olivaceous in colour.
2.	Carrot Agar	Greyish at center, white at periphery	Hyaline	Present	At the point of inoculation puffy growth of mycelium and sporulation was seen at colony edges at maturity of growth.
3.	Oat Meal Agar	White	Hyaline	Absent	Initially whitish dense mycelial growth at the point of inoculation and no sporulation was observed.
4.	Water Agar	Whitish olivine	Hyaline	Absent	Initially whitish scanty mycelial growth which later turned greyish. No sporulation was observed.
5.	Czapek dox agar	White	Hyaline	Absent	Puffy mycelial growth was observed and no sporulation is seen.
6.	Potato Carrot infusion Agar	Greyish at center, white at periphery	Hyaline	Present	Whitish to dirty white thinner and irregular growth of mycelium was observed. Sporulation was observed.

Table 8. Cultural characters of *Colletotrichum sp.* on different media

Sl. No.	Medium	Colony colour	Colour of hyphae	Sporulation	Growth pattern
1.	Potato Dextrose Agar	White	Hyaline	Present	Creamish white growth producing aerial mycelium having sporulation at periphery at maturity of the growth.
2.	Carrot Agar	White	Hyaline	Present	At the point of inoculation puffy growth of mycelium and sporulation was seen at colony edges at maturity of growth.
3.	Oat Meal Agar	White	Hyaline	Present	Initially whitish dense mycelial growth at the point of inoculation and thinner growth towards periphery. Later turned dirty white and sporulation was observed.
4.	Water Agar	White	Hyaline	Absent	Initially whitish dense mycelial growth which later turned yellowish. No Sporulation was observed.
5.	Czapek dox agar	White	Hyaline	Absent	Puffy mycelial growth was observed which became dull white in later stage and no sporulation is seen.
6.	Potato Carrot infusion Agar	Dirty white	Hyaline	Present	Whitish to dirty white thinner and irregular growth of mycelium was observed. Sporulation was observed.

4.5 Morphological characterization of the pathogens

The morphological characteristics of the pathogens isolated from leaves of infected plant showing typical symptom were investigated by growing the pathogen on PDA medium in 9 cm Petri plates at 27 ±2^{O} C, as described in Materials and Methods. The Petri plates were checked on a regular basis, and the final data was recorded after seven days.The results on the basis of observation are summarised in Table 9 and also shown in Figures 15-16.

Table 9. Morphological characters of the pathogen *Helminthosporium spp.* and *Colletotrichum gleosporoides*

Pathogen	Morphological characters
Helminthosporium sp.	The colony grew rapidly on PDA, white aerial mycelia at first**,** but later turned grayish to olivaceous colour The mycelium was hyaline, branched and septate**.** Conidiophores were brown to dark brown in colour, erect, parallel walled and ceasing to elongate when the terminal conidium was formed. Conidia were multicellular (six celled), large, solitary and pale to dark brown in colour and were slightly curved with a bulge in the middle and tapering towards the ends and located along the sides of the conidiophores and their wider end was towards the conidiophore
Colletotrichum sp.	The colony grew rapidly on PDA, white aerial mycelia at first, but later turned orange to deep pinkish in colour. Later blackish pigmentation was seen on the petriplate due to formation of acervuli which was prominently visible at the periphery of the petriplates. The mycelium was hyaline, branched and septate**.** Conidiophores were brown to dark brown in colour, erect, parallel walled and ceasing to elongate when the terminal conidium was formed. Conidia were aseptate, hyaline and mostly ellipsoidal in shape

4.6 *In vitro* bioassay with fungicides and bio-control agents

4.6.1 *In vitro* evaluation of fungicides against *Helminthosporium sp.* and *Colletotrichum sp.*

Evaluation of fungicides *in vitro* is a handy tool to screen large number of fungicides at different concentrations. In the present study, the laboratory evaluation of fungicides revealed significant results for various fungicides evaluated with different concentrations. A set of ten chemicals (Six sole compound chemicals and four combination compound chemicals) at different concentrations (0.1%, 0.15%, 0.2%, 0.25%,0.3% and 0.5%) comprising of systemic, contact and the combination product along with control were evaluated against *Helminthosporium sp.* and *Colletotrichum sp.* by adopting poisoned food technique as described in "materials and methods". The results on the basis of observation in respect of sole compound chemicals are presented in Table 10, 11 and Figure 17, 18 While Table 12, 13 and Figure 19, 20 reflect the results of different combination compound chemicals.

As regards to the efficacy of different sole compound chemicals in respect of percent inhibition of mycelia growth, data in the Table 10 revealed that all the tested fungicides significantly inhibited the mycelia growth of *Helminthosporium sp.* and *Colletotrichum sp.* over control. Among the six tested sole compound chemicals against *Helminthosporium sp.*, Tebuconazole recorded 100.0% mycelial growth inhibition in all the tested concentrations while Difenoconazole showed maximum percent inhibition (100%) at a concentration of 0.2% onwards. The fungicide Hexaconazole showed maximum percent inhibition (100.0%) at a concentration of 0.25 % and onwards, Azoxystrobin recorded maximum percent inhibition (100.0%) at a concentration of 0.3% to onwards, Copper hydroxide and Copper oxychloride fungicides showed an increased inhibition with increasing concentration and maximum inhibition of 97.26% and 94.04%, respectively at a concentration of 0.5%. Similarly data in the Table 11 reveal that in case of *Colletotrichum sp.* out of the six tested sole compound chemicals, Tebuconazole showed a similar trend in mycelial growth inhibition i.e. 100% inhibition at all tested concentration where as Difenoconazole showed maximum percent inhibition (100%) at a concentration of 0.2% onwards. Hexaconazole and Azoxystrobin showed maximum percent inhibition (100.0%) at a concentration of 0.25 % and 0.3%, respectively. Copper hydroxide fungicide showed an increased inhibition with increasing concentration and maximum inhibition of 94.11% achieved at a concentration of 0.5%.

Table 10. *In vitro* evaluation of different sole compound fungicide at different concentrations in inhibiting the mycelia growth of *Helminthosporium spp.*

Tr. No.	Fungicide	0.1% conc.		0.15% conc.		0.2% conc.		0.25% conc.		0.3% conc.		0.5% conc.	
		Radial growth (mm)	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**
T1	Copper Oxychloride	38.74	13.91 (21.81)	32.26	28.37 (32.14)	26.35	41.42 (40.03)	16.23	63.93 (53.08)	12.48	72.26 (52.28)	2.68	94.04 (75.89)
T2	Azoxystrobin	23.56	47.64 (43.62)	13.85	69.22 (56.33)	6.39	85.69 (67.81)	2.01	95.53 (77.94)	0	100 (90)	0	100 (90)
T3	Tebuconazole	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T4	Difenoconazole	12.20	72.88 (58.60)	5.32	88.17 (70.05)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T5	Copper hydroxide	28.22	37.28 (37.60)	24.51	45.53 (42.41)	18.42	59.06 (50.21)	7.54	83.24 (65.84)	4.25	90.55 (80.82)	1.23	97.26 (80.82)
T6	Hexaconazole	11.24	75.02 (60.01)	9.65	78.55 (62.44)	2.34	94.79 (76.93)	0	100 (90)	0	100 (90)	0	100 (90)
T7	Control	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)
	SEm (±)		**3.161**		**1.445**		**1.071**		**0.861**		**0.626**		**0.810**
	CD(0.05)		**1.032**		**4.425**		**3.280**		**2.637**		**1.918**		**2.482**

*Average of three replications. **Figure given in parenthesis are angular transformed values

Table 11. ***In vitro*** **evaluation of different sole compound fungicide at different concentrations in inhibiting the mycelia growth of** ***Colletotrichum sp.***

Tr. No.	Fungicide	0.1% conc.		0.15% conc.		0.2% conc.		0.25% conc.		0.3% conc.		0.5% conc.	
		Radial growth (mm)	*Inhibition (%)	Radial growth (mm)	*Inhibition (%)	Radial growth (mm)	*Inhibition (%)	Radial growth (mm)	*Inhibition (%)	Radial growth (mm)	*Inhibition (%)	Radial growth (mm)	*Inhibition (%)
T1	Copper Oxychloride	42.10	6.34 (12.14)	29.68	34.64 (35.97)	12.56	71.08 (57.45)	2.52	94.40 (76.29)	0	100 (90)	0	100 (90)
T2	Azoxystrobin	27.39	39.13 (38.69)	10.86	75.86 (60.61)	4.18	90.71 (72.30)	0	100 (90)	0	100 (90)	0	100 (90)
T3	Tebuconazole	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T4	Difenoconazole	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T5	Copper hydroxide	34.62	23.06 (28.60)	23.67	47.40 (43.51)	18.25	59.04 (50.43)	13.25	70.55 (57.13)	7.43	83.48 (66.09)	2.65	94.11 (76.10)
T6	Hexaconazole	5.82	87.06 (68.92)	2.04	95.46 (77.68)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T7	Control	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)
	SEm (±)		**2.394**		**1.260**		**0.811**		**0.486**		**0.482**		**0.626**
	CD(0.05)		**7.333**		**3.860**		**2.484**		**1.489**		**1.476**		**1.918**

*Average of three replications. **Figure given in parenthesis are angular transformed values

Table 12. ***In vitro*** **evaluation of different combination compound fungicides at different concentrations in inhibiting the mycelia growth of *Helminthosporiumm spp.***

Tr. No.	Fungicide	0.1		0.15		0.2		0.25		0.3		0.5	
		Radial growth (mm)	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**
T1	Tebuconazole 10% + Sulphur 65%	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T2	Tebuconazole 50%+ Trifloxystrobin25%	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T3	Carboxin 37.5% + Thiram 37.5%	25	44.44 (41.78)	16.40	63.44 (52.78)	10.29	77.13 (61.43)	8.15	81.88 (64.81)	2.85	93.66 (75.65)	0	100 (90)
T4	Metiram 55% + Pyraclostrobin 5%	11.25	75.00 (60.00)	6.75	85.00 (67.22)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T5	Control	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)
	SEm (±)		**0.573**		**0.584**		**0.427**		**0.381**		**0.746**		
	CD(0.05)		**1.742**		**1.775**		**1.298**		**1.159**		**2.27**		

*Average of four replications. **Figure given in parenthesis are angular transformed values

Table 13. ***In vitro*** **evaluation of different combination compound fungicides at different concentrations in inhibiting the mycelia growth of *Colletotrichum sp.***

Tr. No.	Fungicide	0.1% conc.		0.15% conc.		0.2% conc.		0.25% conc.		0.3% conc.		0.5% conc.	
		Radial growth (mm)	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**	**Radial growth (mm)**	***Inhibition (%)**
T1	Tebuconazole 10% + Sulphur 65%	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T2	Tebuconazole 50%+ Trifloxystrobin25%	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)	0	100 (90)
T3	Carboxin 37.5% + Thiram 37.5%	21.75	51.66 (45.93)	15.96	64.53 (53.44)	10.38	76.93 (61.32)	7.25	83.88 (66.37)	2.98	93.37 (75.16)	0	100 (90)
T4	Metiram 55% + Pyraclostrobin 5%	10.36	76.97 (61.31)	5.81	87.08 (69.00)	1.08	97.60 (81.50)	0	100 (90)	0	100 (90)	0	100 (90)
T5	Control	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)	45	0 (0)
	SEm (±)		**0.589**		**0.731**		**0.919**		**0.535**		**0.499**		
	CD(0.05)		**1.792**		**2.223**		**2.796**		**1.627**		**1.519**		

*Average of four replications. **Figure given in parenthesis are angular transformed values

Data from the Table 12, 13 in respect of efficacy of different combination compound chemicals against the test pathogen showed that all the combination compound chemicals were statistically very much effective with regard to inhibition of the mycelial growth of *Helminthosporium sp.* and *Colletotrichum sp* over control. Among the 4 tested combination compound chemicals, 2 fungicides namely, Tebuconazole 10% + Sulphur 65% and Tebuconazole 50% + Trifloxystrobin 25% recorded 100.0% mycelial growth inhibition at all the concentrations against both the pathogens. While, fungicide like Metiram 55% + Pyraclostrobin 5% recorded maximum growth inhibition of 100.0% at a concentration of 0.2% and onwards against *Helminthosporium sp* and 0.25% and onwards against *Colletotrichum sp*. Carboxin 37.5% + Thiram 37.5% recorded 100.0 % mycelial growth inhibition at a concentration of 0.5% for both the pathogens *Helminthosporium sp.* and *Colletotrichum sp*.

4.6.2 *In vitro* evaluation of bioagents against *Helminthosporium sp.* and *Colletotrichum sp.*

Management of pathogen through potential bioagents is an important method of non- chemical means of management of diseases. In this study four bioagents were assessed against *Helminthosporium sp. and Colletotrichum sp.* through dual culture technique which is described in “material and methods” and the results are presented in Table 14, 15.

It is evident from the Table 14 that *Pseudomonas fluorescens* showed maximum inhibition of radial growth (54.44%) as compared to *Trichoderma viride* (46.66%) against the pathogen *Helminthosporium sp*. On the other hand Table 15 reveal that maximum inhibition of *Colletotrichum sp.* was achieved by *Trichoderma viride* (58.88%) as compared to *Pseudomonas fluorescens* (45.55%).

Table 14. *In vitro* evaluation of bioagents against *Helminthosporium spp.*

Tr. No.	Bioagents	*Mean colony diameter of test fungus in control (mm)	*Mean colony diameter in dual culture (mm)	*Percentage growth inhibition
T1	*Pseudomonas fluorescens*	90	41.00	54.44
T2	*Trichoderma viride*	90	48.20	46.66

*Average of four replications

Table 15. ***In vitro*** **evaluation of bioagents against** ***Colletotrichum sp.***

Tr. No.	**Bioagents**	***Mean colony diameter of test fungus in control (mm)**	***Mean colony diameter in dual culture (mm)**	***Percentage growth inhibition**
T1	*Pseudomonas fluorescens*	90	49.26	45.55
T2	*Trichoderma viride*	90	37.02	58.88

*Average of four replications

Figure 1: Initial symptoms of Leaf Blight of Anthurium

Figure 2: Later Stage of Leaf Blight symptoms on Leaf

Figure 3: Severely affected plant with leaf blight disease

Figure 4: Severely affected leaves with leaf blight disease

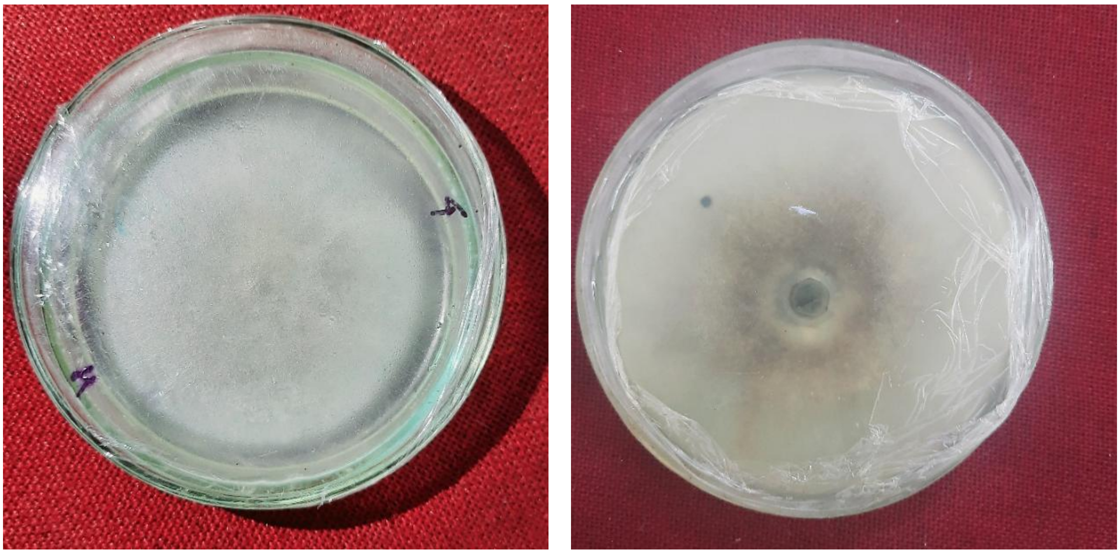


Figure 5: Pure culture of *Helminthosporium sp.*

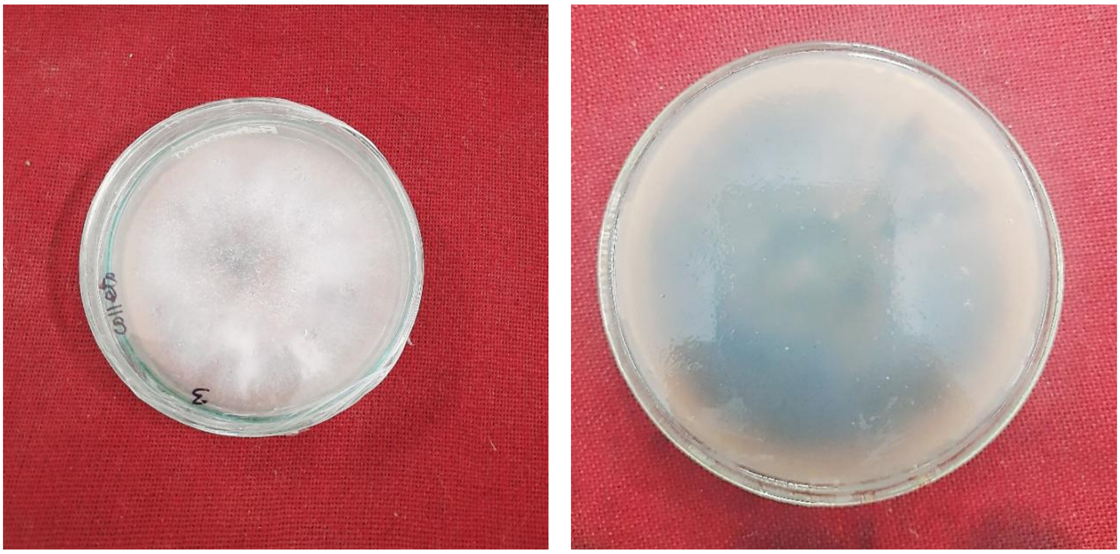


Figure 6: Pure culture of *Colletotrichum sp.*

Figure 7: Pathogenecity test of *Helminthosporium sp.* on potted Anthurium plants

Figure 8: Pathogenecity test of *Colletotrichum sp.* on potted Anthurium plants

Figure 9: Healthy control plant after 10 days of inoculation with *Helminthosporium sp.*

Figure 10: Symptoms observed on artificially inoculated plant wih *Helminthosporium sp.* after 10 days

Figure 11: Healthy control plant after 10 days of inoculation with *Colletotrichum sp.*

Figure 12; Symptoms observed on artificially inoculated plant with *Colletotrichum sp.* after 10 days

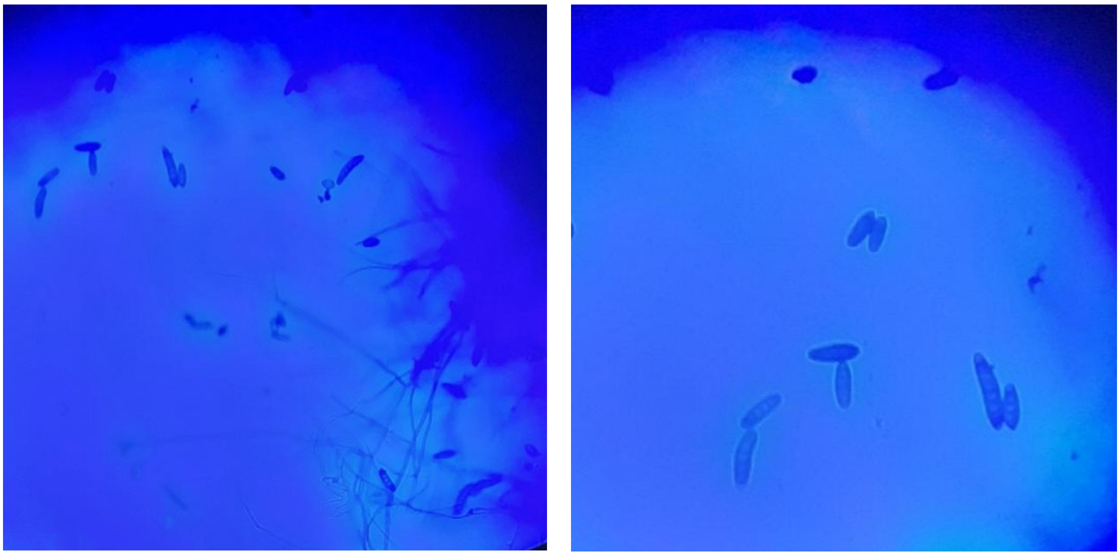


Figure 13: Microscopic photograph of *Helminthosporium sp.* showing mycelium and conidia

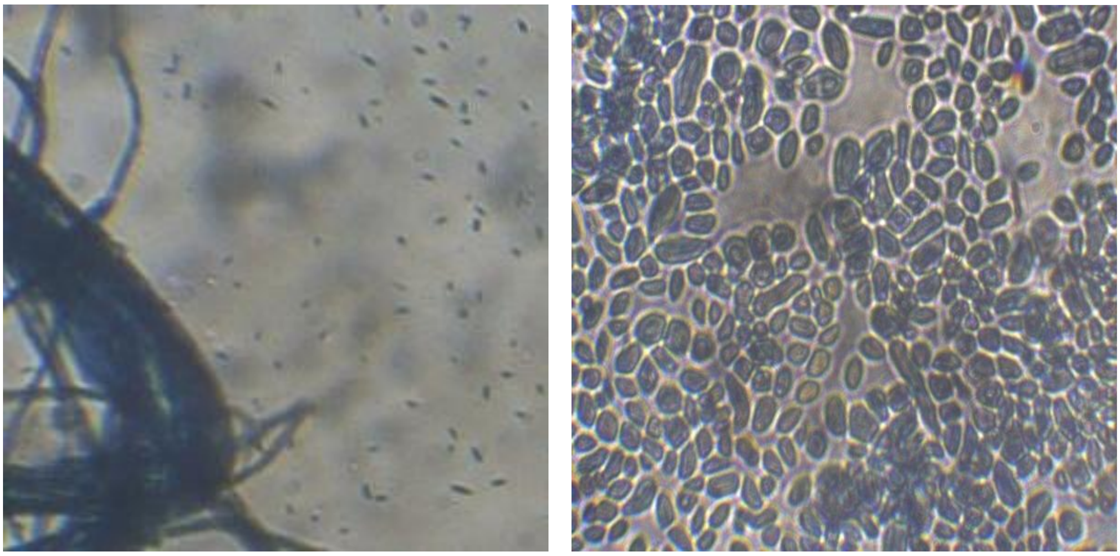
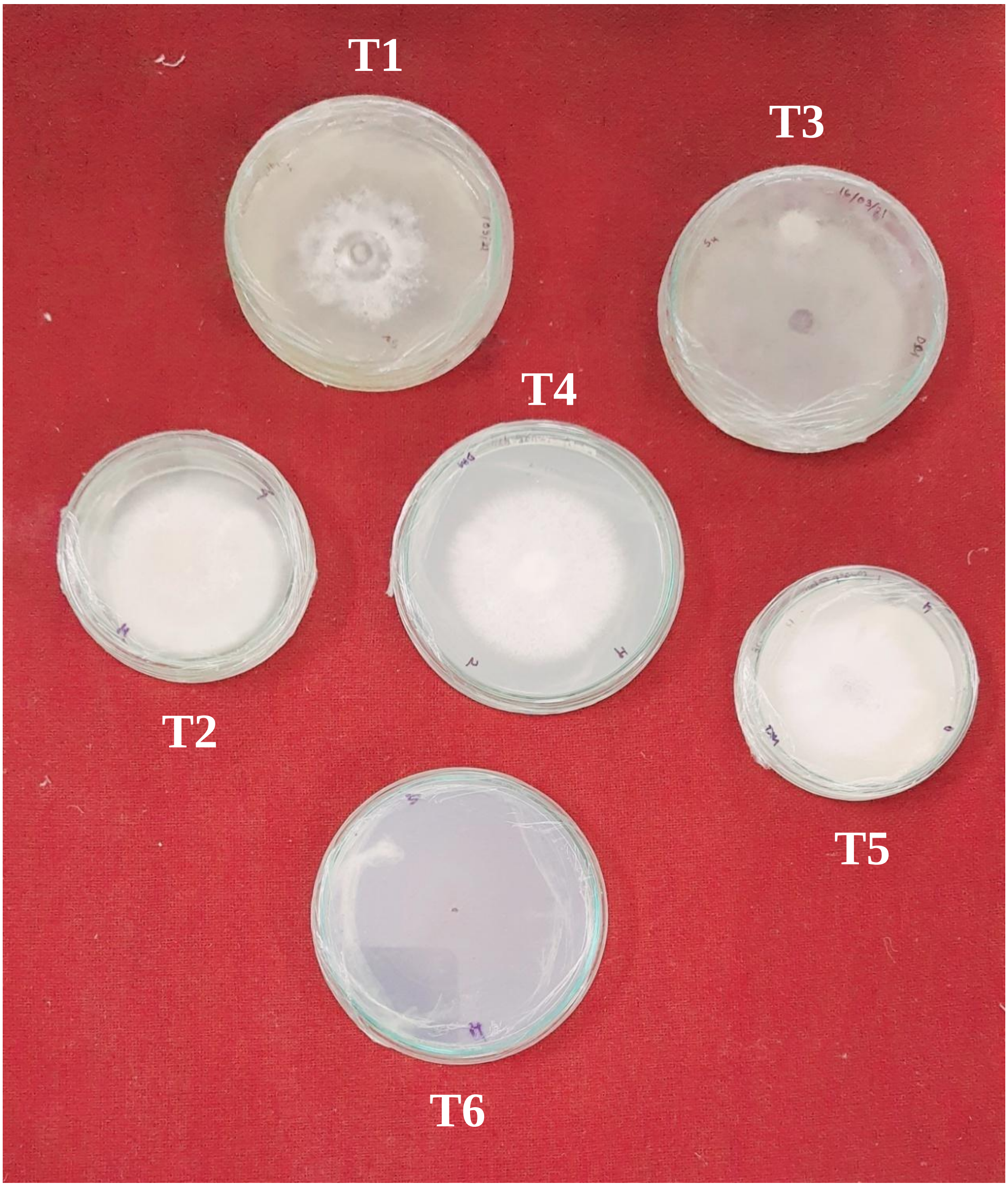


Figure 14: Microscopic photograph of *Colletotrichum sp.* showing mycelium and conidia



T1 : Potato Dextrose Agar

T2 : Carrot Agar

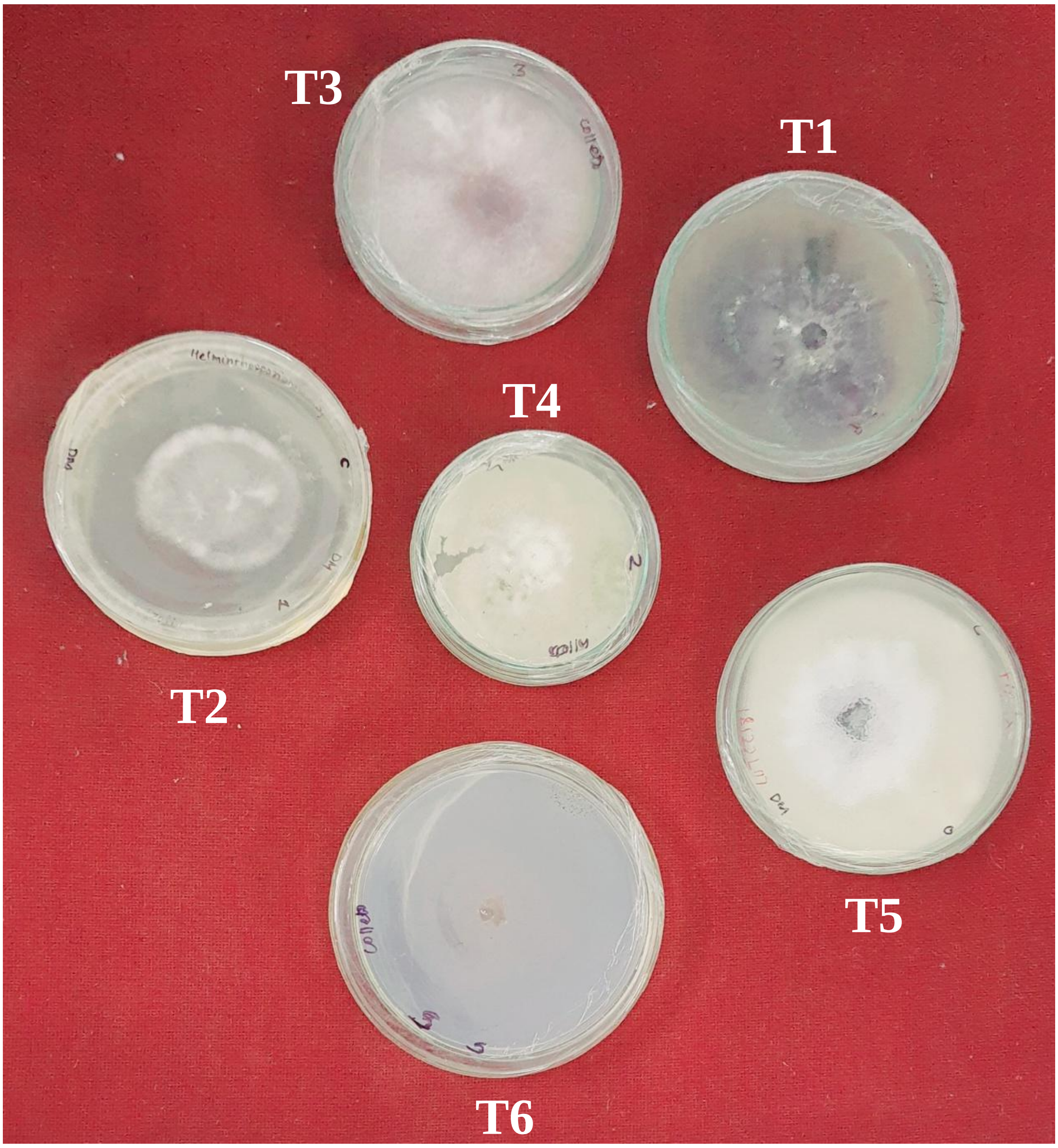
T3 : Oat meal Agar

T4 : Potato carrot infusion agar

T5 : Czapex dox Agar

T6 : Water Agar

Figure 15: Effect of different media on mycelial growth of *Helminthosporium sp.*



T1 : Potato Dextrose Agar

T2 : Carrot Agar

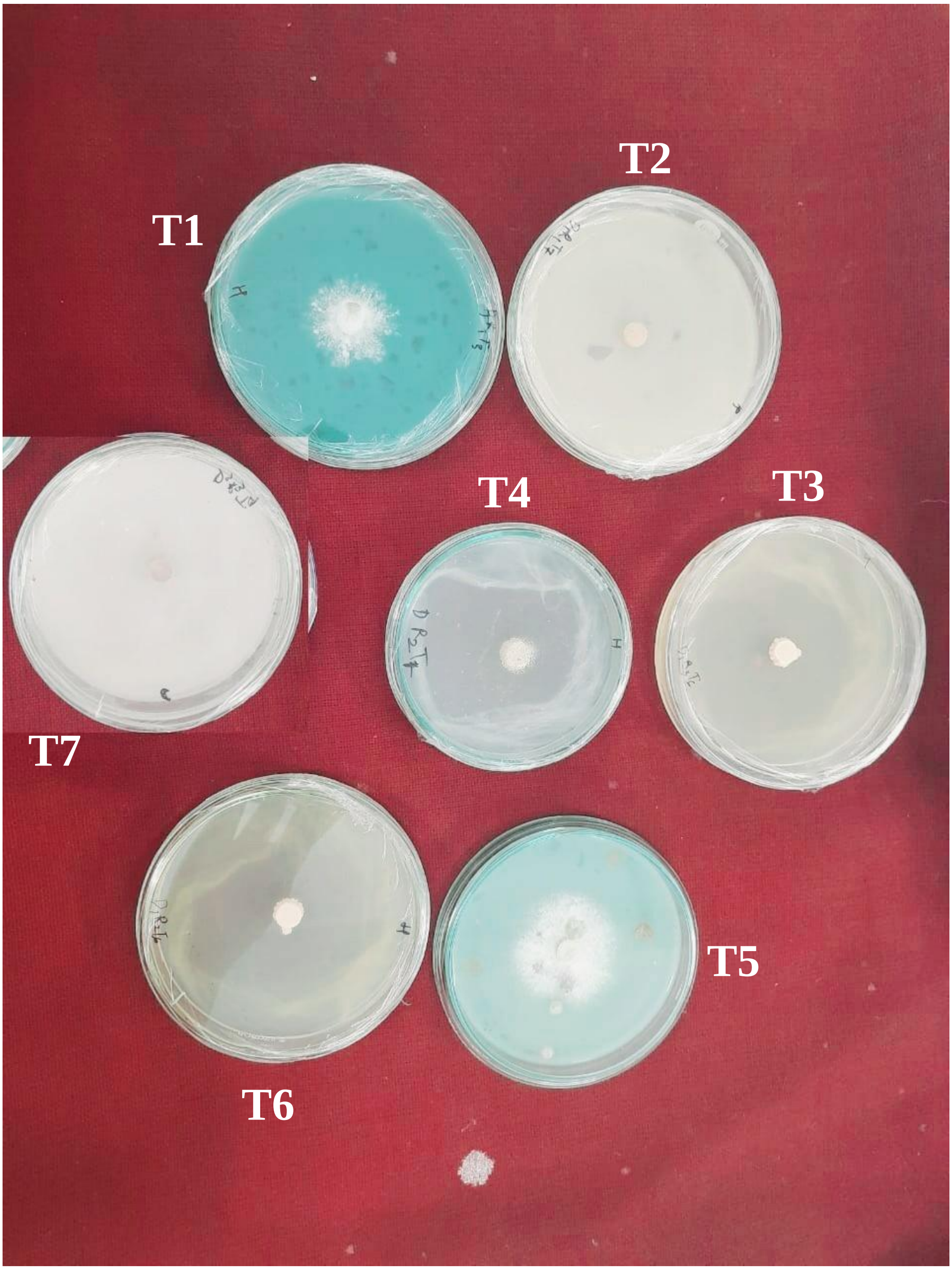
T3 : Oat meal Agar

T4 : Potato carrot infusion agar

T5 : Czapex dox Agar

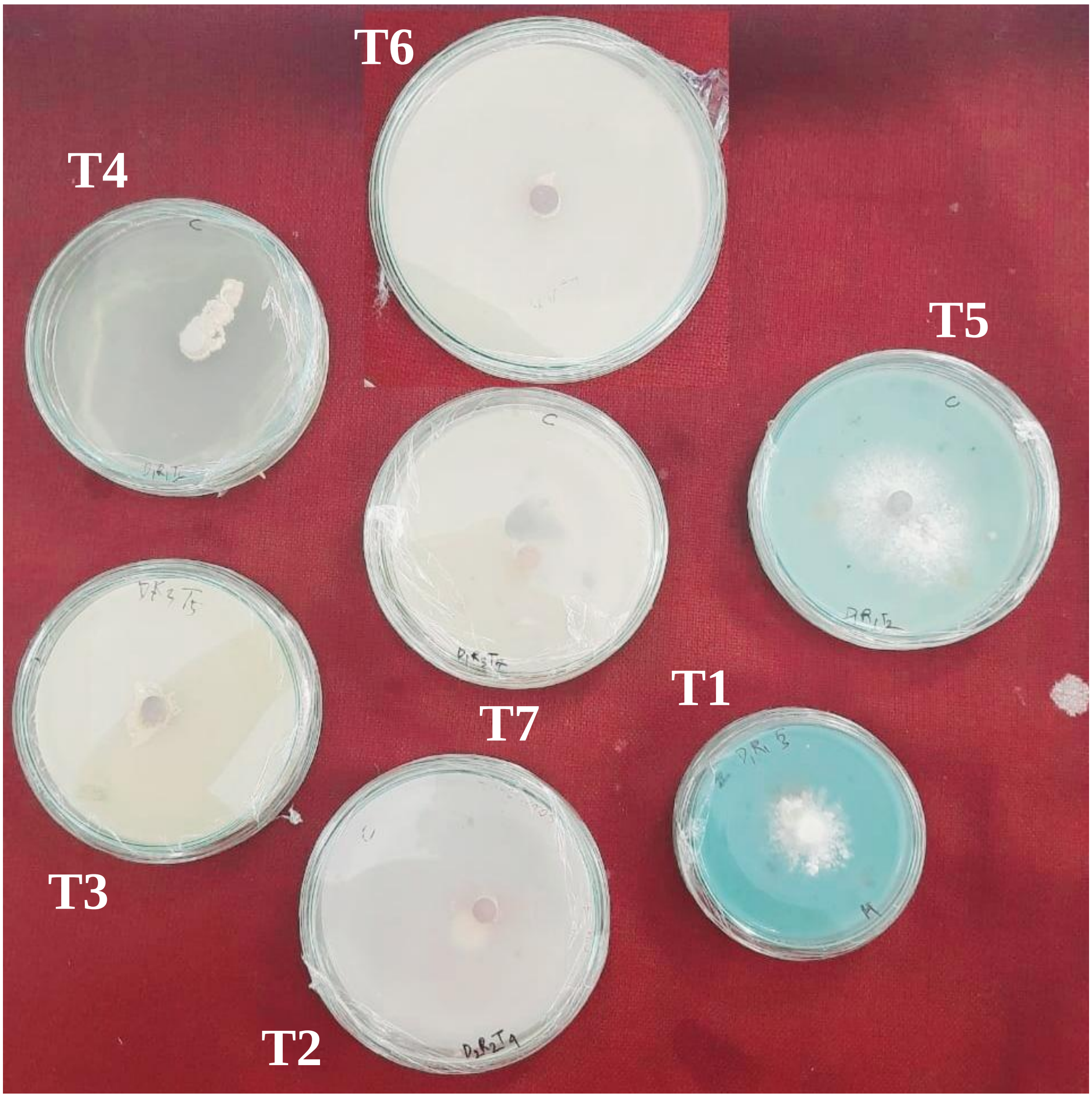
T6 : Water Agar

Figure 16: Effect of different media on mycelial growth of *Colletotrichum sp.*



T1 : Copper Oxychloride
T2 : Azoxystrobin
T3 : Tebuconazole
T4 : Difenoconazole
T5 : Copper hydroxide
T6 : Hexaconazole
T7 : Control

Figure 17: Effect of different fungicide (sole compound chemical) on mycelia growth of *Helminthosporium sp.*



T1 : Copper Oxychloride

T2 : Azoxystrobin

T3 : Tebuconazole

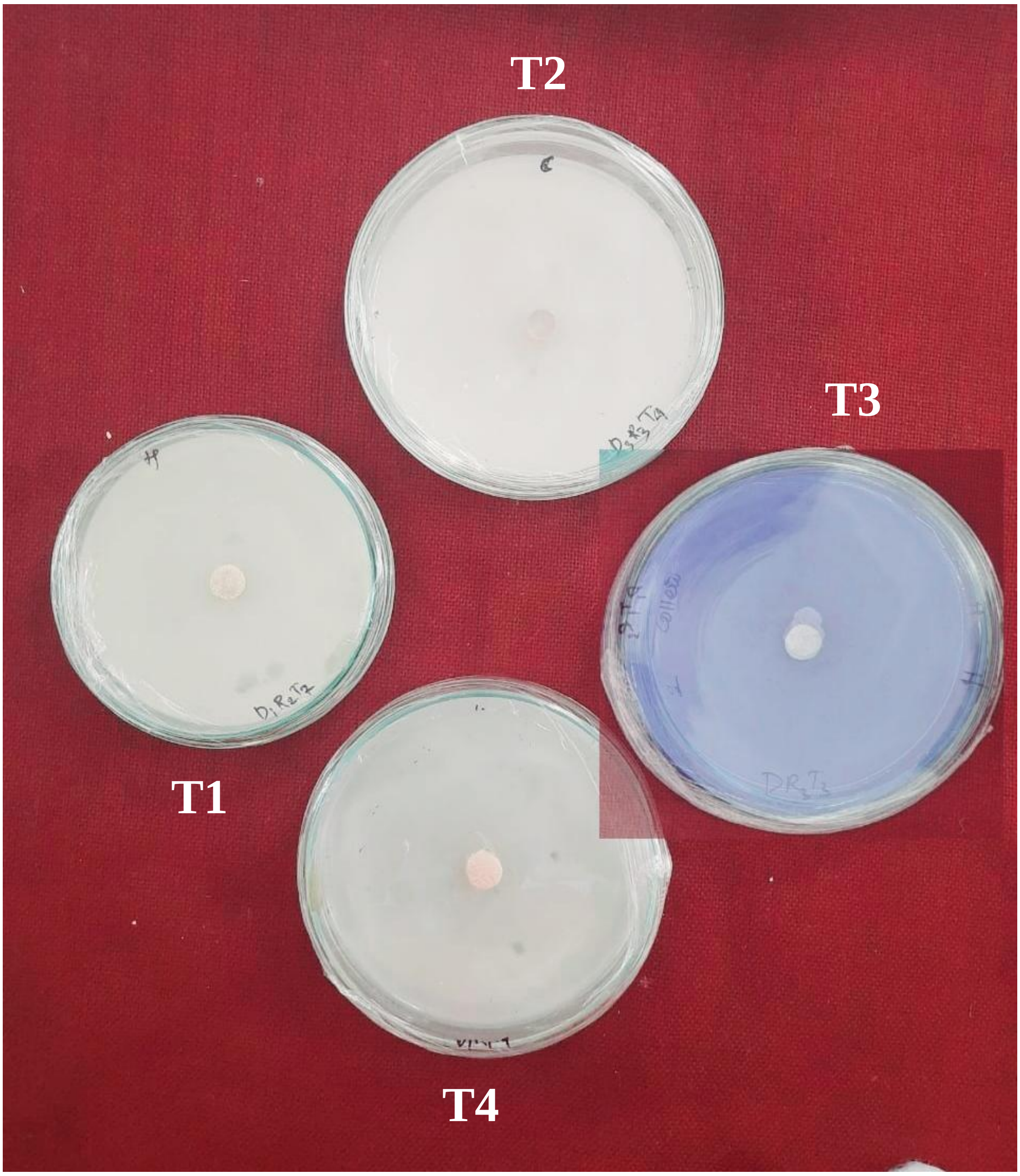
T4 : Difenoconazole

T5 : Copper hydroxide

T6 : Hexaconazole

T7 : Control

Figure 18: Effect of different fungicide (sole compound chemical) on mycelia growth of *Colletotrichum sp.*



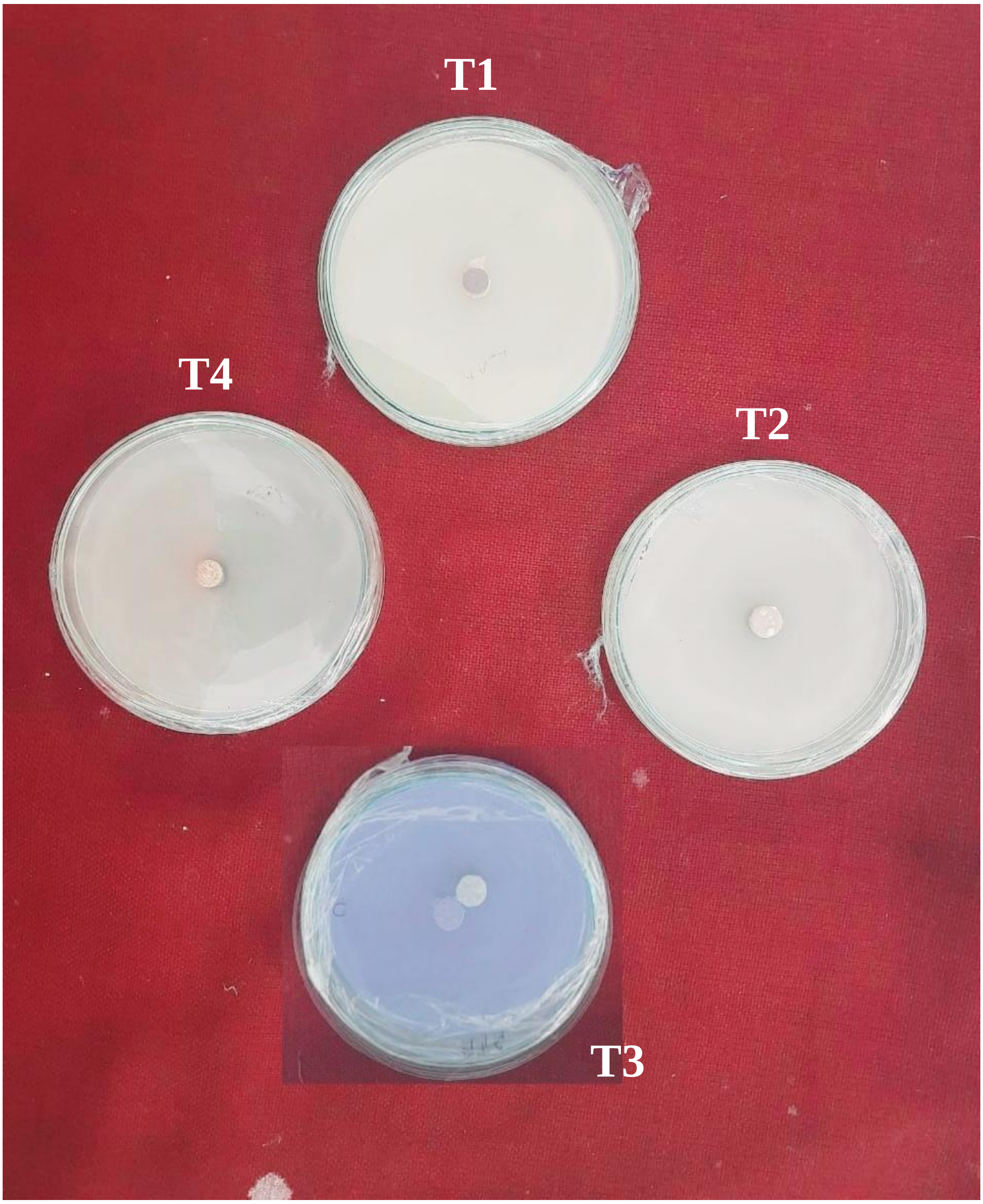
T1 : Tebuconazole 10% + Sulphur 65%

T2 : Tebuconazole 50%+ Trifloxystrobin25%

T3 : Carboxin 37.5% + Thiram 37.5%

T4 : Metiram 55% + Pyraclostrobin 5%

Figure 19: Effect of different fungicide (combination compound chemical) on mycelia growth of *Helminthosporium sp.*



T1 : Tebuconazole 10% + Sulphur 65%

T2 : Tebuconazole 50%+ Trifloxystrobin25%

T3 : Carboxin 37.5% + Thiram 37.5%

T4 : Metiram 55% + Pyraclostrobin 5%

Figure 20: Effect of different fungicide (combination compound chemical) on mycelia growth of *Helminthosporium sp.*

DISCUSSION

Anthurium (*Anthurium andraeanum*) is a tropical plant of great beauty and grown either for the showy cut flowers or for their unusually attractive foliage. They are very popular with flower arrangers because of bold effect and lasting qualities of cut flowers. These contribute to the elegance and attractiveness which are the prerequisites for a quality design. *Anthurium andraeanum* Lind. (Anthurium) is a perennial herbaceous plant grown for its showy, long-lasting flowers. Anthurium, a member of the Araceae family, is native to the tropics of Central and South America, but it is now widely cultivated and traded globally as cut flowers, potted plants, and landscape plants (Kuehnle *et al.,* 1992; Gantait *et al.,* 2008). The global area under floriculture is 3.15 lakh hectares with economic return of US $ 50 billion (Misra & Kumar, 2008). As per the record of National Horticulture Board (NHB, 2013), the country's production of anthurium cut flower during 2012-13 was 320270 MT and more than 97 percent of this quantity was contributed by the North Eastern states.

Anthurium is affected by a number of diseases incited by fungi, bacteria and viruses, under field as well as protected cultivation. Among various diseases infecting Anthurium, leaf blight caused by *Colletotrichum sp.* and *Helminthosporium sp.* has become a severe problem in field as well as protected cultivation. Considering the severity of the problem and lack of complete information on this disease, the present investigations were carried out on various aspects such as isolation, identification, cultural and morphological characterization, epidemiology and management practices that included bioagents and chemicals *in-vitro* to generate scientific information on this important pathological problem. The result obtained in different studies are presented and discussed as below.

The disease appeared initially on the lower leaves of the plants. The characteristic spots of the disease was exhibited as water-soaked circular lesions that were yellow to brown in color usually encircled by a yellow halo. These spots gradually enlarged giving blighting appearance and these spots started from the margin and gradually moved towards the center of the leaves. Later on the spots coalesced with each other to form irregular and large patches covering considerably huge areas on leaf surface. These findings are in compliance with the findings of Deshmukh (1997) whose experiment was based on the *Colletotrichum gleosporoides* on leaves of Anthurium.

The pathogenicity test was performed to prove the Koch's postulate with the isolated fungus in two different method *viz.* spraying of spore suspension with and without pin prick injury method and the later one showed more effectiveness in producing symptoms than the first one. The causal fungi could induce typical symptom on test plant after 10-15 days after artificial inoculation whereas the control did not exhibit any kind of symptom. The current findings corroborate with the earlier findings of Natarajan and Subramanian (1973), who demonstrated the pathogenicity of *C. gloeosporioides* with pin prick method on chilli leaves and green and ripe fruits. Deshmukh (1997) demonstrated the pathogenicity of *C. gloeosporioides* isolated from Anthurium leaves via spore suspension and Patel (2012) demonstrated pathogenicity of (*Colletotrichum gloeosporioides* Penz. and Sacc.) from ornamental orchid leaf spot via pin pricking injury to the leaves and the experimental findings of Pal *et al*. (2015) who demonstrated the pathogenicity of *Helminthosporium maydis* on maize plants under protected cultivation. These earlier findings are confirmed in the present investigation.

The fungi associated in the diseased leaf were examined for its morphological features. The microscopic investigations revealed that in the case of *Colletotrichum sp.* the conidia were hyaline, thin walled, straight, oblong, bacilliform, slightly depressed in the middle with rounded ends. Mycelial growth was cottony white turned into light pink finally brown coloured. The hyphae were thin, branched, hyaline and septate. Numbers of tiny black dots were noticed on infected portion of the leaves which were confirmed as acervuli upon microscopic observations. Acervuli were cushion shaped, brownish black in colour with dark pointed setae tapering towards the apex that looked like bristeles and intermingled with conidiophores. The above morphological description of *Colletotrichum sp*. corresponded closely with the description given by Deshmukh (1997), indicating that the morphological characters of *C. gloeosporioides* isolated from Anthurium leaves. Alam et al. (1978) described the morphology of *C. gloeosporioides*, which caused anthracnose in *Dioscorea composite* followed by other workers like Patel (1978), Korade (1995) and Patel (2000). In the case of other fungi i.e. *Helminthosporium sp.* the conidia were fusiform, pale to mild dark brown, widened at the middle, gradually tapering to round ends possessing 2-3 pseudosepta. The conidiophores were solitary or in groups, emerging from dark brown to black stromata, straight or flexous, sometimes geniculate, mid to dark brown and pale near apex and smooth. Such findings are in agreement with Manamgoda *et al.* (2014) and Pal *et al.* (2015) who worked on *Helminthosporium maydis*.

The investigation for the most suitable medium for mycelial growth and sporulation of the pathogens revealed that *Helminthosporium sp.* exhibited most superior growth in Potato dextrose agar (68.02 mm) followed by Potato carrot infusion agar (66.80mm) and Czapex dox agar (60.23mm) after seven days of inoculation. More or less it is in agreement to the results of Kumar and Singh (2008) on *Helminthosporium maydis*. The *fungus Colletotrichum sp.* showed maximum growth on Potato dextrose agar (76.88 mm) followed by Carrot agar medium (74.24mm) and Water agar medium (56.53mm). These findings are exactly similar with those observed by Deshmukh (1997) from Dapoli who studied the mycelial growth of *C. gloeosporioides* isolated from Anthurium leaves and Natural et al (1994) who studied the mycelial growth of *Colletotrichum gloeosporioides* causing anthracnose of Anthurium on different solid media, The fungus *Colletotrichum sp.* showed widest mycelial colony diameter and maximum conidial production when grown on PDA. Similar results were earlier reported by Aishra and Mahmood (1960), Gullino *et al.* (1985) and Korade (1995) which is confirmed in the present finding.

Use of fungicide is most effective management practice for any pathogen. In this present investigation 6 sole compound chemicals and 4 combination compound chemicals at six different concentrations (0.05%, 0.1%, 0.15%, 0.2%, 0.25% and 0.3%) were tested against *Helminthosporium sp.* and *Colletotrichum sp.* by following poisoned food technique. The present investigation revealed that the combination compound chemicals were far more effective in inhibiting the radial growth of both the pathogens than that of the sole compounds. Out of the 6 sole compound chemicals effective chemicals in reducing the radial growth (100%) of the fungus are in the order of Tebuconazole (0.1%), Difenoconazole (0.2%), Hexaconazole (0.25%), Azoxystrobin (0.3%) for *Helminthosporium sp.* and for *Colletotrichum sp.* the order is Tebuconazole(0.1%), Difenoconazole (0.1%), Hexaconazole(0.2%), Azoxystrobin

(0.25%) and Copper Oxychloride (0.3%). Whereas among the compound chemicals, Tebuconazole 10% + Sulphur 65% and Tebuconazole 50% + Trifloxystrobin 25% showed 100% inhibition at concentrations as low as 0.1% against both the pathogens and the next in the order are Metiram 55% + Pyraclostrobin 5%(0.2%) and Carboxin 37.5% + Thiram 37.5%(0.5%) for *Helminthosporium sp.* and Metiram 55% + Pyraclostrobin 5%(0.25%) and Carboxin 37.5% + Thiram 37.5% (0.5%) for *Colletotrichum sp.* . The current findings corroborate the earlier findings of Begum *et al.* (2015) who worked on *Colletotrichum gloeosporioides* and Lal *et al.* (2015) who worked on *Helminthosporium sp.* and had reported similar findings.

An eco-friendly, effective and an alternate approach to chemical management in any disease management strategy is biological control. These bio agents defense the pathogen attack by performing different metabolic activity viz., lysis, antibiosis, competition and hyper parasitism (Vidyasekaran, 1999). Use of bioagents is best and long-term effective practice that also overcome the residual problems associated with chemical management practices. Hence two bioagents namely *Trichoderma viride* and *Pseudomonas fluorescens* were tested against both the test pathogens i.e. *Helminthosporium sp.* and *Colletotrichum sp.* Between these bioagents, *Pseudomonas fluorescens* inhibited the mycelial growth more in case of *Helminthosporium s.p* (54.44%) than that in case of *Colletotrichum sp.*(46.66%) Whereas *Trichoderma viride* was more effective in reducing the growth of *Colletotrichum sp.*(58.88%) as compared to that of *Helminthosporium sp*.(45.55%). The current findings are supported by the earlier reports of Patel (2000) who reported an antagonistic effect of *T. viride, T. harzianum,* and *A. niger* against *Colletotrichum gloeosporioides*. *T. viride* and *T. harzianum* were identified by Raut (2011) as strong and potent antagonists against *C. gloeosporioides*, the causative agent of banana anthracnose. *T. viride, T. harzianum,* and *T. fasciculatum* were identified as potent antagonists against *C. gloeosporioides*, the causal agent of orchid leaf spot, according to Patel (2012). The current findings are completely in agreement with the above mentioned earlier findings of the preceding workers.

SUMMARY AND CONCLUSION

Anthurium is ranked ninth in the global flower trade and commands a fair price for both cut flowers and whole plants. The anthurium cut flower industry in India is still in its infancy. Anthurium is the latest sensation of Indian floriculture scene and Anthurium gains its fame and respected status by exhibiting its striking ensemble, which is created unitedly by its spadix and its spathe, within the economically essential ornamentals, allowing its use in interior and exterior decoration and also to its use as a cut flower. Among various pathogens attacking Anthurium plants, *Helminthosporium sp.* and *Colletotrichum sp.* causing leaf blight disease of Anthurium are in recent trends in coastal areas of Odisha. Hence, an attempt has been made to isolate and characterize the pathogens, to study the correlation of leaf blight disease incidence and to manage the disease through chemicals and bioagents.Keeping in view the outline of objectives, studies on leaf blight disease of Anthurium caused by *Helminthosporium sp.* and *Colletotrichum sp.* were carried out during 2019-21.

The findings of the investigation on **"Leaf blight of Anthurium (*Anthurium andreanum*) and its management"** are summarized below.

The symptomatological studies revealed that the disease is characterized by spots which were water-soaked circular lesions that ranged in colour from yellow to brown and were usually surrounded by a yellow halo. These spots gradually grew larger and joined together to form irregular and large patches that covered greater areas of the leaf surface.

The detailed investigation showed the constant association of *Helminthosporium sp.* and *Colletotrichum sp*. with the infected leaves. On potted Anthurium plants, the pathogenicity of the test fungus was demonstrated. The test fungi were re-isolated and confirmed through microscopic examination, and the results were the same.

In one case (*Helminthosporium sp.*) the conidia were observed as fusiform, pale to mid-dark brown, widened in the middle, gradually tapering towards rounded ends with 2-3 pseudosepta. Conidiophores were solitary or in groups, straight or flexuous, sometimes geniculated, mid to dark brown and pale near the apex, and smooth, emerging from dark brown to black stromata. In case of the second pathogen (*Colletotrichum sp*.) the conidia were found to be hyaline, one-celled, ovoid to oblong and dumbbell shaped where as the conidiophores were small and erect.

Out of all the six media tested Potato Dextrose Agar medium produced significantly the highest radial growth (68.20 mm) in case of the fungus *Helminthosporium sp.*followed by Potato Carrot Infusion Agar medium (66.80mm), Czapex dox Agar medium(60.23mm), water Agar medium(52.16mm) and Oat meal Agar medium(50.82mm) and Carrot Agar medium (48.33mm).But the fungus *Colletotrichum sp*. recorded maximum radial growth on Potato Dextrose Agar medium (76.88mm) followed by Carrot Agar medium (74.24mm). The next best in the order were Water Agar medium (56.53mm) , Potato Carrot Infusion Agar medium(54.36mm) and Czapex Dox Agar medium(49.73mm) and Oat meal Agar medium (44.72mm).

Ten fungicides were tested *in-vitro* against *Helminthosporium sp.* and *Colletotrichum sp.* using the poisoned food technique. The majority of the fungicides tested were found to be effective in inhibiting the growth of both the pathogens as compared to the control. Among all the fungicide tested Tebuconazole(0.1%), Tebuconazole 10% + Sulphur 65% (0.1%) and Tebuconazole 50% + Trifloxystrobin 25% (0.1%) were found to be most effective chemicals in inhibiting the growth of the fungus *Helminthosporium sp.*The next best in the order were Difenoconazole(0.2%) and Metiram 55% + Pyraclostrobin 5%(0.2%). Similarly in case of *Colletotrichum sp.* the chemicals namely Tebuconazole(0.1%), Difenoconazole(0.1%), Tebuconazole 10% + Sulphur 65% and Tebuconazole 50% + Trifloxystrobin 25% were found to be most effective followed by Metiram 55% + Pyraclostrobin 5%.

Investigations on screening of two biocontrol agents i.e. *Pseudomonas fluorescens* and *Trichoderma viride* against *Helminthosporium sp.* and *Colletotrichum sp.* by dual culture method revealed that *Pseudomonas fluorescens* wasfound to be most effective against *Helminthosporium sp.* whereas *Trichoderma viride* identified as strong and potent antagonists against *Colletotrichum sp.*

Thus the present findings on the causal organisms of leaf blight disease of Anthurium and their management through chemicals as well as biocontrol agents as reported above will help the farmers of the state to save their crop from the ravages of this deadly disease and there by mitigating their financial losses owing to this disease. As much work could not be conducted due to COVID 19 pandemic situation, further investigation on the efficacy of the above-mentioned chemicals and biocontrol agents under *in vivo* condition should be carried out in the subsequent years to get a concrete conclusion.

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