# STUDY ON ANTHRACNOSE OF STRAWBERRY (Fragaria ananassa) IN JORHAT (ASSAM)

**A THESIS SUBMITTED TO THE**
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**Master of Science in BOTANY**


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**Academic Year (2020 to 2022)**

**DEDICATED**

**TO**

**MY PARENTS**

**MESAI LAGACHU**

**AND**

**LATE BUDRESWARI LAGACHU**

**PG DEPARTMENT OF BOTANY**

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# CERTIFICATE

This is to certify that this thesis entitled **"Study on fungal diseases of strawberry (*Fragaria ananassa*) in Assam"** submitted to the Assam Science & Technology University, Guwahati, for the award of the degree of Master of Science in Botany is a bonafide research work carried out by the student **Mr Gulap Lagachu, Roll number – 202820047006** under my guidance and supervision during the period between April 2022 to August 2022 in the Department of Botany. I further certify that no part of this thesis has been submitted anywhere else for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

Zakir Malik
21/09/2022

Dated : 21-09-2022

Place – Silapathar, Assam

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This is to certified that this thesis entitled "**Study on Anthracnose disease of strawberry (*Fragaria ananassa*) in Jorhat, Assam**" submitted to the Assam Science & Technoogy University, Guwahati, for the award of the degree of Master of Science in Botany is a bonafide research work carried out by **Gulap Lagachu, Roll No. – 202820047006,** under my guidance and supervision during the period of April 2022 to August 2022 in the Department of Plant Pathology. I further certify that no part of this thesis has been submitted anywhere else for the award of any Degree, Diploma, Associateship, Fellowship or other similar titles.

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# DECLARATION

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I hereby declare that the work embodied in this thesis entitled "**Studies in Anthracnose of Strawberry (*Fragaria ananassa*) in Jorhat, Assam**" is a research work done by me under the supervision and guidance of Dr. Bharat Chandra Nath, Assistant Professor, Department of Plant Pathology, Assam Agricultural University, Jorhat. I further declare that this work has not been submitted earlier in full or in parts to any other university for the award of any other Degree, Diploma, Associateship, Fellowship or other similar tiltles.

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Date : 21/09/2022

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Place- Jorhat, Assam

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# ABBREVIAIONS AND SYMBOL USED

**%** : Per cent

**&** : and

**°C** : Degree centigrade

**AAU** : Assam Agricultural University

**BOD** : Biological Oxygen Demand

**BLAST** : Basic Local Alignment Search Tool

**Cfu** : Colony forming unit

**C.D.** : Critical Difference

**Cv** : Cultivar

**Conc.** : Concentration

**CRD** : Completely Randomized Design

**DI** : Disease Incidence

**e.g** : Exempli gratia (For example)

***et al*** : Et alia (and others)

**Fig** : Figure

**g** : Gram

**h** : Hours

**HRS** : Horticulture Research Station

**ha** : Hectare

**ITS** : Internal Transcribed Spacer

**i.e** : Id est (That is)

**L** : Litre

**MEGA** : **Molecular Evolutionary Genetics Analysis**

**MEA** : **Malt extract media**

**m** : **Meter**

**mg** : **Miligram**

**Mg\l** : **Miligram per litre**

**min** : **Minutes**

**ml** : **Mililitre**

**mm** : **Milimeter**

**MT** : **Metric Tonne**

**MBIT** : **Mycelial Bit Inoculation Technique**

**NA** : **Nutrient Agar**

**NCBI** : **National Centre for Biotechnological Information**

**NCFT** : **National Centre of Fungal Taxonomy**

**NHB** : **National Horticulture Board**

**ng** : **Nanogram**

**nm** : **Nanometer**

**nos.** : **Numbers**

**PDA** : **Potato Dextrose Agar**

**PDI** : **Per Cent Disease Index**

**PDR** : **Per Cent Disease Reduction**

**PPO** : **Poly Phenol oxidase**

**Pvt. Ltd** : **Private limited**

**pH** : **Hydrogen ion concentration**

**µm** : **Micrometer**

# ABSTRACT

Strawberry ***(****Fragaria ananassa****)*** is one of the world's most commercially important fruit crops, and is grown in many countries. Strawberries are often grouped according to their flowering habit. Research published in 2001 showed that strawberries actually occur in three basic flowering habits: short day, long day, and day neutral. These refer to the day length sensitivity of the plant and the type of photoperiod that induces flower formation.

There are many diseases occurred on the strawberry such as Leaf spots, Grey mold, Wilt, Powdery mildew, Anthracnose, Angular leaf spot etc. Anthracnose on strawberry is favoured by warm, humid and wet conditions. In this disease the round black or light gray lesions on leaves appeared. Numerous spots may develop but leaves do not die.

The anthracnose disease is found in very high concentration in strawberry in Jorhat, Assam and the disease sample were taken for further studies. The disease sample is isolated and pure cultured in PDA media and incubated for 28±°C. After 7 days of inoculation the mycelia grow in PDA media. Then the pathogenicity test was done from the isolated sample on the healthy leaves of the strawberry plant by Mycelial Bit Inoculation Technique (MBIT) in in vitro condition. The efficacy of botanicals [Neem (*Azadirachta indica)*, Garlic *(Allium sativum)*, Allamanda (*Allamanda cathartica)*, Tulsi (*Ocimum tenuiflorum)*, and Jetuka (*Lawsonia inermis*)] at different concentration and biocontrol agents (Trichoderma harzianum, T. viride, Bacillus subtillis, B. veleziensis and Pseuodomonas influorescence) were tested against pathogen. *Allium sativum* and *Trichoderma harzianum* were recorded maximum mycelial growth inhibition of 86.66% and 74.44% respectively.

The isolated sample was observed in the microscope and based on symptomatology, cultural, morphological and molecular characterization the isolated fungal pathogen were identified as *Colletotrichum gloeosporiodes,* which were also confirmed by the Bioserve .

# INTRODUCTION

# CHAPTER I

# INTRODUCTION

The genus *Fragaria* is a member of the *Rosaceae* family, *Rosoideae* subfamily, which comprises twenty eight species and several subspecies. *Fragaria x ananassa* is the most important species commercially, being the predominant species cultivated for for strawberry production globally (GAO 2000). The European Union is a major producer of strawberry (accounting for 47% of world output), grown on 165000 ha. The United States produces approximately a 29% of world output) (FAO 2007). China is the most strawberry producing country with 2.7 million tones which is 40% of the total in the world wide, then the US with 1.2 million and Mexico with 0.59 million. In India, the strawberry growing states are Himachal Prades, Uttar Pradesh, Maharashtra, West Bengal, Delhi, Haryana, Punjab and Rajasthan. Sub-tropical areas in Jammu have also the potential to grow the crop under irrigated condition.

In line with the financial and economic importance represented by the cultivation and marketing of this crop, serious financial losses can be incurred due to health problems affecting the strawberry plant. These plants, like other commercial crops, can be damaged by environmental, genetic and biological factors, either directly or through interaction between these factors. Strawberry plants are affected by a large number of diseases caused by fungi, bacteria, viruses, nematodes and arthropods. These pathogens cause damage on the leaves, roots, crowns and fruits. The group of phytopathogenic fungi that attack this crop is especially extensive in terms of the number of genera and species that can produce diseases, and hence in terms of the considerable financial losses, running into hundreds of millions of dollars/euros per year, that this agrarian sector may suffer (FAO 2000). More than 50 different genera can affect *F. ananassa* cultivars, but not all of them have the same commercial importance. The genera *Botrytis, Colletotrichum, Verticillium, and Phytophthora* are included among the most significant. These

pathogens are capable of infecting not only the strawberry crop but more than one hundred other crops cultivated around the world. Although in recent years several genera of strawberry fungal pathogens, such as *Colletotrichum acutatum, Botrytis cinerea, Phytophthora* spp. have been included in the EPPO A2 list (EPPO 2004). Currently, the detection and identification of pathogenic fungi have traditionally been performed using classical mycological methods involving isolation from host material, by plating plant parts, soil or soil extracts onto selective media, followed by morphological, biochemical, chemical and immunological analyses (*Singleton et al*. 1992; *Lievens et al*. 2005). As has been reported by *Garrido et al*. (2008, 2009b), since 1991 several molecular methods for more accurate characterization and differentiation of phytopathogenic fungi have been developed and are being implemented widely.

There are many fungal and bacterial diseases occurred on strawberry plant. Anthracnose is an important disease of strawberry that can affect foliage, runners, crowns and fruit. The disease is caused by several species of fungi in the genus *Colletotrichum: Colletotrichum fragaria, Colletotrichum acutatum,* and *Colletotrichum gloeosporiodes.* They all caused similar or nearly identical symptoms on strawberry. *Colletotrichum* species are anamorphic, necrotrophic fungi that are capable of infecting fruit, buds, blossoms, petioles, runners, crowns, and foliage of strawberry plants. Anthracnose fruit rot is caused primarily by *Colletotrichum acutatum* and is one of the most destructive diseases on strawberry worldwide. *Colletotrichum gloeosporiodes* is less frequently associated with anthracnose fruit rot but occasionally it produces symptoms indistinguishable to those of *C. acutatum* on strawberry fruit. Species of *Colletotrichum* are traditionally identified by conidial morphology, presence or absence of setae, and colony color (Gunnell and Gubler, 1992). In Anthracnose of strawberry, the fungus can attack fruit, runners, petioles, and the crown of the plant. Dark elongated lesions develop on petioles and runner stems. Affected petioles and stems are sometimes girdled by lesions, causing individual leaves or entire daughter plants to wilt and die. Under warm, humid conditions, salmon-colored masses of spores may form on the lesion surface. If the crown tissue is infected, crown rot may develop and the entire plant may wilt and die. When infected crowns are sectioned, internal tissue is firm and reddish-brown to dark-brown in color. Crown tissue may be uniformly discolored or

streaked with brown. Whitish, tan, or light-brown water-soaked lesions up to 3 mm in diameter initially develop on fruit. The lesions eventually turn brown or dark-brown, are sunken, and enlarge within two to three days to cover most of the fruit. Lesions are covered with pale-orange or salmon-colored spore masses. Under moist conditions, the fungus may grow out around the edge of the lesion or through the lesion, giving a fuzzy appearance. Infected fruit eventually dry down to form hard, black, shriveled mummies. Fruit can be infected at any stage of development.

When the pathogens are detected in the field, or to prevent their appearance between crop seasons, disease control has been carried out using chemical treatments. Alternative approaches to disease control, such as the use of biological, cultural and physical methods, must be integrated to reduce the amount of fumigant used for soil and plant treatments. Biological control of fungal plant pathogens with microorganisms has been studied for more than 60 years (Howell *et al*. 2003). This form of biocontrol appears to be an attractive and realistic approach, and numerous microorganisms have been identified as potential biocontrol agents.

Plant pathogens cause severe losses or damage to crops worldwide and thereby significantly reduce the quality and quantity of agricultural commodities. World tendencies are shifting towards reducing the usage of chemically synthesized pesticides, while various biocontrol methods, strategies and approaches are being used in plant disease management. Fungal antagonists play a significant role in controlling plant pathogens and diseases and they are used as Biocontrol Agents (BCAs) throughout the world. This review provides a comprehensive list of fungal BCAs used against fungal plant pathogens according to modern taxonomic concepts, and clarifies their phylogenetic relationships because the wrong names are frequently used in the literature of biocontrol. Details of approximately 300 fungal antagonists belonging to 13 classes and 113 genera are listed together with the target pathogens and corresponding plant diseases. *Trichoderma* is identified as the genus with greatest potential comprising 25 biocontrol agents that have been used against a number of plant fungal diseases. In addition to *Trichoderma*, nine genera are recognized as significant comprising five or more known antagonistic species, namely, *Alternaria, Aspergillus, Candida, Fusarium, Penicillium, Pichia, Pythium, Talaromyces, and Verticillium*. A phylogenetic analysis based on partial sequences of the 28S nrRNA gene (LSU) of fungal antagonists was performed to establish their

phylogenetic relationships. Various methods, strategies, and approaches are used in the management of plant diseases. These encompass the development of resistant varieties through plant breeding, genetically engineered plants, use of agrochemicals and physical methods (i.e., heat treatments, UV irradiation, modified or controlled atmosphere, cold storage, and inducing resistance by applying elicitors), application of biological control agents and good agronomic and horticultural practices (Stevens *et al.,* 1997; Wisniewski *et al*., 2000; Droby, 2006; Singh and Chawla, 2012; Gupta and Sharma, 2014; Singh, 2014; O'Brien, 2017). These approaches have contributed significantly to the remarkable improvements in crop productivity and quality over the past few decades (Punja, 1997; Droby, 2006; Chandrashekara *et al*., 2012). Biological Control: Overview and Significance Biological control approaches of plant diseases include any reduction in the amount or the effect of pathogens (disease producing activity) that is achieved through the induction of biological mechanisms or the action of naturally occurring or introduced antagonists, that occurs by manipulating the microenvironment to favour the activity of antagonists (Baker, 1987; Stirling and Stirling, 1997). Microbial biocontrol agents (BCAs) for plant diseases are usually fungal or bacterial strains isolated from the phyllosphere, endosphere or rhizosphere and they play an important role in controlling plant-pathogenic organisms. Biocontrol agents or microbial antagonists prevent infection of the host plant by the pathogen, or establishment of the pathogen in the host plant. The principal mechanisms for the control have been assumed to be those that act primarily upon the pathogens. The antagonists can exhibit several direct or indirect mechanisms of action involved in biological disease control. These mechanisms include; antibiosis (where an inhibitory metabolite or antibiotic is produced by the antagonist), mycoparasitism (where the antagonist derives some or all of its nutrients from the fungal host), induced resistance (induction of plant defense response against plant pathogens) and growth enhancement (BCAs promote plant growth while the effects of the disease are being reduced and also through microbial hormones such as indoleacetic acidand gibberellic acid). Secretion of extracellular hydrolytic enzymes by the antagonist, competition for space and nutrients between organisms and detoxification of virulence factors are other actions involved in biological disease control (Wilson *et al.,* 1991; Punja, 1997; Heydari and Pessarakli, 2010; Chandrashekara *et al*., 2012; Singh, 2014; Zhang *et al*., 2014; Deketelaere *et al*., 2017). Recent studies have demonstrated that effects such as induced systemic

or localized resistance by microbial BCAs on plants are also crucial. These fungi or bacteria can colonize the root epidermis and outer cortical layers and release bioactive molecules that cause walling-off of the fungal thallus or bacterial colonies (Harman, 2006).

Insect and disease killers derived from plant extracts are called botanical pesticides or botanicals. Botanical pesticides are derived from plants which have been shown to have pesticidal properties. They are also very closed chemically to those plants from which they are derived, so they are easily decomposed by a variety of microbes common in most soils. Botanical pesticides are good alternatives to chemical pesticides. Botanical pesticides are eco-friendly, economic, target-specific and biodegradable. These are safer to the user and the environment because they break down into harmless compounds within hours or days in the presence of sunlight.

# AIM AND OBJECTIVES

- **1. Isolation and characterization of the pathogen (*Colletotrichum gloeosporiodes*)**
- **2. In vitro evaluation of biocontrol agents (*Trichoderma harzianum, T. viride, Bacillus subtillis, B. veleziensis, Pseudomonas influorescence*) and some botanicals (Tulsi, Neem, Allamanda, Garlic, and Jetuka) against the Pathogen (*Colletotrichum gloeosporiodes*) of strawberry.**

# REVIEW

# OF

# LITERATURE

# CHAPTER II

# REVIEW OF LITERATURE

Literature revealed several pathogens causing foliar, root and floral diseases that have been reported globally from strawberry growing tracts of China, United States, Egypt, Mexico, Poland, and India.

Stephenson *et al* (2000) reported that *C. gloeosporioides* expressed Pel-1 and Pel-2 during infection to host. These genes encodes for pectic lyase activity began at the end of the biotrophic phase and increased in the necrotrophic phase of infection. Initial pH condition and carbon sources affect the expression of Pel-1 and Pel-2. The expression of these genes are higher in MCWE (mallow cell wall extract) broth than in any other broths or MCWE is better inducer for the expression of Pel-1 and pel-2.

Photia *et al* (2001), Promputtha et al (2002), Toofanee and Dulymamode (2002), Kumar and Hyde (2004) reported that *Colletotrichum* species that cause serious plant disease are also commonly isolated as endophytes from healthy plants, and have been identified as saprobes on dead plant material.

Chang *et al*., 2002; Ramji *et al*., 2002; Sharma *et al*., 2009 proposed that a major phenolic compound called hydroxychavicol from aqueous extract of the P. betle leaves, was reported to have antimicrobial activity, antioxidant and anticancer properties in several studies conducted by various researchers.

Cannon and Simmons (2002), Lu *et al.,* (2004), Photita *et al.,* (2004) reported that *C. gloeosporiodes* act as endophytic strains which are isolated form symptomless plant parts.

El Ghaouth *et al.*, (2002), Janisiewicz and Korsten (2002) proposed that a significant amount of harvested fruits and vegetables is lost annually due to

microbial spoilage and this loss can range from 10%–50% depending on the commodity and country.

Eames Sheavly *et al* (2003) reported that common cultural practices treat June-bearing strawberries as a perennial crop, using what is called a 'matted row' system. Rooted plugs of the June-bearing crop are planted in the spring of the first year, known as the establishment year. Flower clusters are removed this entire first season, allowing the plant to invest more energy into crown/branch crown development, root development and runner production.

Chakraborty and Datta (2003) reported that under a high concentration of $CO_2$, there is increase in fecundity (spores produced per lesion area) observed and this may increase the severity and spread of disease.

Barhoom and Sharon (2004) reported that germination in *C. gloeosporiodes* follows two routes: pathogenic and saprophytic.

Xiao *et al* (2004), Amusa *et al* (2005), Nelson *et al* (2008) reported that *C. gloeosporiodes* causes anthracnose disease on a wide variety of fruits, including almond, avocado, apple, Arabica coffee, guava, mango, strawberry, papaya, banana, passion fruit, citrus, grapes and cashews.

Savini *et al* (2005) reported that the strawberry plant is an herbaceous perennial with short internodes forming a modified stem rosette.

Savini *et al* (2005) reported that strawberry leaves present a typical dicot structure with long petioles and foliaceous basal stipules.

Stephenson *et al* (2005) reported that CgDN24 gene encodes cDNA and is induced by nitrogen starvation in axenic culture. The cDNA comprises of 905 bp and predicted a 215 amino acids protein. CgDN24 gene plays no role in pathogenesis and is necessary for normal hyphal development in axenic culture.

CAB international (2005) reported that *C. gloeosporiodes* is more abundant in tropical and subtropical regions than in temperate regions.

Serce & Hancock (2005) reported that strawberry flower cluster anatomy has been thoroughly researched, as possible differences in inflorescence architecture

have been hypothesized to correlate with differences in yield and berry weight among cultivars.

Menzler-Hokkanen, 2006 report thatcommercial products containing microbial BCAs have been successfully exploited in modern agriculture (e.g., Trichoderma based products and biopesticides based on Bacillus thuringiensis).

Harman, (2006) described that recent studies have demonstrated that effects such as induced systemic or localized resistance by microbial BCAs on plants are also crucial. These fungi or bacteria can colonize the root epidermis and outer cortical layers and release bioactive molecules that cause walling-off of the fungal thallus or bacterial colonies.

Brimner and Boland (2003); Menzler-Hokkanen (2006) storage can affect the health of humans and livestock, especially if the pathogen produces toxins in or on consumable products .

Sundravadana *et al* (2006) observed that the Azoxystrobin is one of the strobilurin class fungicide was evaluated both in vitro and in vivo conditions. Azoxystrobin completely inhibit mycelia growth. Azoxystrobin at 1, 2 and 4 ml/l suppressed the development of both panicle and leaf anthracnose and observed total control of mango anthracnose with azoxystrobin.

Mertley *et al* (2007) reported that the diseases that attack strawberries include anthracnose disease caused by *Colletotrichum fragariae* (Brooks), *C. acutatum* (Simmonds), and *C. gloeosporiodes* (Penz). Colletotrichum species cause economically significant disease such as wilt disease, rot, and anthracnose in strawberry production areas.

Li and Zhang (2007) reported that Trichosanthes kirilowii Maxim, a species within the gourd family, is cultivated in China for its edible seeds and medicinal roots. In 2000, there was a heavy loss due to fruit rot caused by *C. gloeosporioides*

Munch *et al* (2008) reported that *C. gloeosporioides* follows the hemibiotrophic mode of infection where, biotrophic and necrotrophic phases are sequentially occur. First of all pathogen establish interaction with host by producing melanized appressorium and then penetrate the host cuticle. After penetration,

infection vesicles and primary hyphae are formed. This stage of infection is called biotrophic phase. Later, necrotrophic secondary hyphae developed and spread to kill the host cell.

Barhoom *et al* (2008) reported that *C. gloeosporioides* requires copper at the initial stages of pathogenesis, germination and the CgCTR2, a putative vacuolar copper transporter involved in regulating cellular copper balance during the process. This transporter is highly expressed in resting spores.

Freeman (2008) mentioned that *Colletotrichum* spp. Are bored-rang pathogens, meaning that species can infect a single host and a single species can infect deserve hosts.

Abril *et al* (2009) studied that the steroidal saponin from cayenne pepper, CAY-1 was tested as potential fungicide in detached leaf assays and field trials. Efficacy of CAY-1 against strawberry anthracnose was compared to the commercial fungicide azoxystrobin.

Arroyo *et al* (2009) recorded that strawberry anthracnose, caused by Colletotrichum acutatum is one of the most important disease of strawberry in south western Spain.

Gupta *et al* (2009) reported that during 2005 and 2006, *C. gloeosporioides* was isolated from diseased samples of bell pepper (Capsicum annuum) collected from various districts of Himachal Pradesh, India. This was the first report of *C. gloeosporioides* on bell pepper from Himachal Pradesh.

Mohamed and Saad (2009) found that the application of specific strains of Pichia anomala was a safe and effective biocontrol agent against Diplodia postharvest rot of guava fruit caused by Lasiodiplodia theobromae (Pat.) Griffon & Maubl. Alvindia and Natsuaki (2008) and Sangeetha et al. (2009)

Mitani *et al* (2009) reported that a patient of 82 years old was suffering from myelodysplastic syndrome and after contract surgery of left eye, patient developed fungal kerratitis. When corneal of patient cultured on media, it grew well and identified as *C. gloeosporioides*

Masyahit *et al* (2009) reported that *C. gloeosporioides* also causes infection on Dragon fruit (Hylocereus spp.) in Peninsular Malaysia.

Srichana *et al*., 2009 proposed that the leaves contain essential oils including chavicol, chavibitol eugenol, carvacrol, aryophyllene and sitosterol, which possess anti fungal properties.

Xie *et al* (2010) reported that in China for many years the strawberry production area was affected by the disease, nearly 50% of the death occurred at the time of seeding and more than 40% yield losses in strawberry production fields caused by anthracnose.

Debode *et al* (2010), Van Hemelrijk et al (2010), and Guidarelli et al (2011) reported that management of anthracnose disease is greatly hindered by the difficulty in detection and control of this fungus during symptomsless infections on strawberry leaves and unripe

Embabv *et al*., (2010) published the first report of *Colletotrichum acutatum* and *C. gloeosporiodes* causing anthracnose diseases on strawberry fields in Kalubia and Ismailia governorate in Egypt.

Stewart and Folta (2010) reported that there is considerable genetic diversity within strawberry germplasm; wild diploid through decaploid plants have been discovered.

Demchak (2011) reported that axillary meristems can differentiate into branch crowns, which stay near and are structurally identical to the original crown, or stolons (also called runners), which give rise to separate daughter plants in strawberry.

Johnny *et al*. (2011) showed that leaf crude extracts of P. betle exhibited the highest antifungal activities overall in inhibiting the mycelial growth of C. capsici among the 15 medicinal plants.

Central Bureau of Statistics. 2011. Several developments Main Socio-Economic Indicators of Indonesia. http://www.bps.go.id, accessed 11 November 2012.

Fan *et al* (2011) & Lewers (2012) porposed that indeed, studies that have shown correlations between strawberry yield and cultural practice have hypothesized soil health as a driving function in the relationship, but this has yet to be empirically quantified.

Jesonbabu *et al*., (2012) stated that betel leaf has a significant antimicrobial activity against broad spectrum of micro-organisms, including fungi.

Poling (2012) reported that leaf lifespan can exceed 3 months in favorable conditions in strawberry.

Poling (2012) reported that crowns typically produce one to two branch crowns in a season, but have been known to produce more than five; from a production standpoint, three to four total crowns per strawberry plant is desirable, as more can result in decreased fruit size.

Chandrashekara *et al*., (2012) proposed that considerable research effort today is focused on seeking safe, eco-friendly and effective alternatives to synthetic, chemical fungicides to reduce the decay loss in harvested commodities and to control crop diseases in the field that lead to significant economic losses (Stirling and Stirling, 1997; Wisniewski et al., 2000; Droby, 2006;

Miyara *et al* (2012) observed that there are several nitrogen-metabolism genes such as: GDH2, GS1, GLT, and MEP are differentially expressed during colonization by *C. gloeosporioides* and induces ammonia accumulation and pathogenicity.

Chi *et al* (2012) reported that Tulip tree (Liriodendron chinense) has been widely cultivated in Korea and infection of *C. gloeosporioides* was detected by mycological characteristics, pathogenicity, internal transcribed spacer sequence. This was the first report on anthracnose disease caused by *C. gloeosporioides* on tulip trees in Korea.

Poling (2012) reported that the plants then overwinter, and flower clusters are left on the plant the following spring for the first harvest. In this system, the number of leaves on each plant at the beginning of overwintering can be correlated with fruit production the following year.

Wu *et al* (2012) reported that a study directly measuring possible differences in SMB between different strawberry production practices would fill this void. The strawberry (Fragaria spp.) is one of the most widely distributed fruit crops in the world. Production of the fruit is present in almost every continent and has exceeded 4 million tonnes per year since.

Alkan *et al* (2013) reported that the transcription factor, pacC, is a regulator of pH-controlled genes and is essential for successful colonization. PacC up-regulates 478 genes and down-regulates 483 genes, comprising 5% of the fungal genome including; transporters, antioxidants and cell wall degrading enzymes.

Reich (2014) reported that the successful production and performance of strawberry, or any food crop, is dependent upon its unique requirements for growth, development, and reproductive maturity (Despite its established role in soil fertility, there is a lack of research examining the effect of organic strawberry cultural practices on soil microbial communities.

A number of biologically based products are being sold worldwide for the control of fungal plant pathogens and generally they are produced as granules, wettable powders, dusts, and aqueous or oilbased liquid products using different mineral and organic carriers (Ardakani *et al.*, 2009; Nega, 2014)

Awad and Al-Shennawy (2015) reported that in vivo experiments, all plant extracts with different concentrations which applied as dipping treatment decreased gray mould disease severity of strawberry fruits especially Galls, Clove and cinnamon extracts.

Wisniewski *et al*., (2000); Droby, (2006); Singh reported that UV irradiation, modified or controlled atmosphere, cold storage, and inducing resistance by applying elicitors, application of biological control agents and good agronomic and horticultural practices. Deketelaere *et al* (2017) O'Brien (2017) reported that the secretion of extracellular hydrolytic enzymes by the antagonist, competition for space and nutrients between organisms and detoxification of virulence factors are other actions involved in biological disease control.

Nabi *et al*., (2017) ; El Ghaouth *et al*., (2002) proposed that developing countries experience greater losses due to inadequate storage and transportation

facilities, and improper handling methods that are employed during harvesting and transit.

Nabi *et al*., (2017); Korsten, (2002) reported that the harvested yield might have been infected by one or several pathogens prior to harvest or they may become infected during transit and storage.

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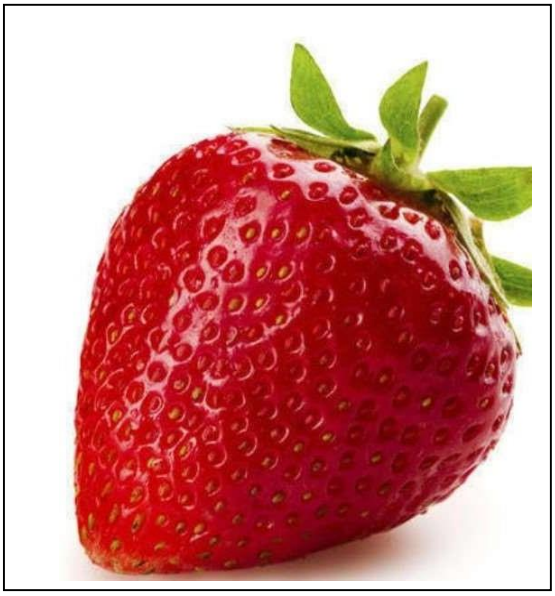
# PLANT

# PROPERTY

## **STRAWBERRY (*Fragaria ananassa*)**

The strawberry is a widely grown species of the genus Fragaria. It was first in Brittany, France, in the 1750s via a cross of Fragaria virginiana from eastern North America and Fragaria chiloensis, which was brought from Chile by Amede-Francois Frezier in 1714.



# Nutrients value per 100 g (3.5 oz)

| Energy | 136 kJ (33 kcal) |
|---|---|
| Carbohydrates | 7.68 g |
| Sugars | 4.89 g |
| Dietary fiber | 2 g |
| Fat | 0.3 g |
| Protein | 0.67 g |

| Vitamins | Quantity | %DV+ |
|---|---|---|
| Thiamine (B1) | 0.024 mg | 2% |
| Riboflavin (B2) | 0.022 mg | 2% |
| Niacin (B3) | 0.386 mg | 3% |
| Pantothenic acid (B5) | 0.125 mg | 3% |
| Vitamin (B6) | 0.047 mg | 4% |
| Folate (B9) | 24 µg | 6% |
| Choline | 5.7 mg | 1% |
| Vitamin C | 58.8 mg | 71% |
| Vitamin E | 0.29 mg | 2% |
| Vitamin K | 2.2 µg | 2% |

| Minerals | Quantity | %DV+ |
|---|---|---|
| Calcium | 16 mg | 2% |
| Iron | 0.41 mg | 3% |
| Magnesium | 13 mg | 4% |
| Manganese | 0.386 mg | 18% |
| Phosphorus | 24 mg | 3% |
| Potassium | 154 mg | 3% |
| Sodium | 1 mg | 0% |
| Zinc | 0.14 mg | 1% |

**Other constituents = Water = 90.95 g**

# Strawberry growth, reproduction and commercial management

## A. Vegetative Growth

The strawberry plant is an herbaceous perennial with short internodes forming a modified stem rosette (Savini *et al*. 2005). This modified stem is commonly known as a crown, where long-petiole trifoliate leaves and axillary meristems converge spirally around its axis, ending in a terminal inflorescence (White 1927). Strawberry leaves present a typical dicotyledonous structure with long petioles and foliaceous basal stipules (Savini *et al*. 2005). Leaf lifespan can exceed 3 months in favorable conditions (Poling 2012). Axillary meristems can differentiate into branch crowns, which stay near and are structurally identical to the original crown, or stolons (also called runners), which give rise to separate daughter plants (Demchak 2011). Crowns typically produce one to two branch crowns in a season, but have been known to produce more than five; from a production standpoint, three to four total crowns per plant is desirable, as more can result in decreased fruit size (Poling 2012).

## B. Flower Structure

Inflorescences have two internodes and develop terminally on the crown or branch crown of the plant in a structure known as a dichasial cyme (Savini *et al*. 2005). Dichasial cymes have a terminal, primary flower branch with opposite secondary branches beneath the terminal bud, leading to secondary flowers. In strawberry, the inflorescence is commonly known as a flower cluster, and the primary flower, known as the "king flower", typically bears the largest fruit. (Thomson 2008). Secondary branches begin at the juncture of the first and second internodes; some inflorescences have tertiary and quaternary branches and flowers as well. Strawberry flowers have five sepals; fleshy green structures beneath the petals which enclose the flower at bud stage and eventually become the 'calyx', or cap of the berry. Stamens discharge pollen and fertilize the pistils, which are secured on a conical stem known as the receptacle. This receptacle becomes the full, fleshy "berry" at fruit maturity. Despite this plant's common name, the fruit itself is not botanically classified as a berry. The seed-like organs embedded on the epidermis of

the receptacle are actually modified dry fruits known as achenes. Each achene is connected to the interior of the receptacle by fibrovascular strands, and hold the true seed within their pericarp (Fait *et al*. 2008). In Fragaria vesca auxin and gibberellin biosynthesis occurs in the endosperm and seed coat of the developing achenes, which in turn triggers the maturity of the surrounding receptacle (Kang *et al*., 2013). Because the strawberry fruit contains multiple achenes and is comprised of a receptacle in addition to its ovaries it can be classified as both an aggregate and accessory fruit.

## C. Flower Induction, Initiation and Development

Flower induction, initiation and development are highly variable by cultivar, and dependent on genotypic responses to temperature and photoperiod (Savini *et al* 2005) Stewart and Folta 2010). These responses are commonly grouped into three flowering categories: June-bearing, everbearing and day-neutral. Strawberry cultivars are typically classified under one of these three categories based on their photoperiodic flowering habits, and it was originally assumed these habits remained constant over a wide range of temperatures (Darrow and Waldo 1933). However, further research led to the discovery that the photoperiod response of many cultivars would be altered if temperatures were either sub- or supraoptimal (Guttridge 1985; Nishiyama and Kanahama 2000; Sonsteby and Heide 2007). This interaction of temperature with photoperiod, known as thermo photoperiod, adds a quantitative factor to the original categorical classifications. Indeed some believe it improper to assign broad flower habit categories to strawberry at all, as photoperiod responses appear to be cultivar specific (Durner, 2015). However as the vast majority of strawberry publications use these classifications, this review will utilize them as well with the implicit understanding of variance and interaction even within each flowering type. This section will discuss photoperiod response and common cultural practices of the three groups assuming optimal temperature conditions, and then explore how the responses have been observed to change under different temperature ranges.

## 1. June-bearing

Natural flowering patterns of cultivated octoploid strawberry, *F. x ananassa*, are of the June-bearing type (Darrow 1966). June-bearing cultivars are predominantly grown for commercial purposes in the Upper Midwestern United States, where other flowering types have historically performed poorly (Durner *et al*. 1984; Luby *et al*. 1987, Luby 1989). June-bearing cultivars induce flowers under shortening day lengths, optimally from 9.5 to 13 hour days, depending on cultivar (Darrow 1933). The change in day length over time in the United States Upper Midwest (specifically using Minneapolis, MN 44.9833° N as a representative point) compared to a more southern latitude where strawberries are also grown (specifically using Santa Maria, CA 34.5914° N as a representative. The figure implies that flower induction would typically occur in mid-September for June-bearing cultivars in the Minneapolis area, until temperatures drive plants into dormancy. Savini *et al*. (2005) notes that June-bearing cultivars will also have flower initials before they enter dormancy. For many June-bearing cultivars the dormancy inducing temperature is a high of 10° C (Kronenberg *et al*. 1976). On average this threshold temperature will be reached in early November in the United States Upper Midwest. As daylength and temperatures increase the following spring, June-bearing plants stop flower induction and divert resources into flower development (Salisbury and Ross 1992; Nishizawa & Shishido, 1998). This induction-to-development shift leads to Junebearing plants bearing high fruit yields until the induced flower buds are depleted, typically in late June or early July. Thus, June-bearing strawberry plants can be considered to have short day induction requirements and long day development requirements. Under high temperatures (>30°C), June-bearing plants will experience severely reduced flower development even in optimal photoperiods (Serce and Hancock 2005). Savini *et al*. (2005) also noted that the morphology and differentiation time of inflorescences is based on the thermo-photoperiod the plant is exposed to; June-bearing plants growing in warmer, short day conditions tend to have faster and more prolific flower differentiation and shorter petiole lengths than plants exposed to long day, cooler conditions. Common cultural practices treat June-bearing strawberries as a perennial crop, typically using a 'matted row' system. Rooted plugs of the June-bearing crop are planted in the spring of the first year (the "establishment" year). Flower clusters are typically removed during this entire first

season, allowing the plant to divert more reserves into crown/branch crown development, root development and runner production (Eames Sheavly et al. 2003). June-bearing cultivars rarely establish runners during early season flower development. However, both flowering and runnering period take place as day length increases, and finally runners alone are developed during the hottest, longest photoperiods of the summer (Stewart and Folta 2010). Growers often arrange runners spatially from the crown to eventually root themselves, creating a thick, matted row of plants (Archbold and MacKown 1995). The plants then overwinter, and flower clusters induced during the short daylengths of fall are left on the plant the following spring for the first harvest. In this system, the number of leaves on each plant at the beginning of overwintering can be correlated with fruit production the following year (Poling 2012).

## 2. Everbearing and Day-neutral

The second and third flowering types, everbearing and day-neutral, are sometimes considered synonymous, likely due to crossover in pedigrees. Everbearing cultivars include the diploid alpine strawberry *Fragaria vesca*, along with various more common octoploids (Duchesne 1766; Fletcher 1917). Cultivars categorized as everbearing cultivars both induce and develop flowers under longer photoperiods, typically 12 hours or more. Sironval and El Tannir-Lomba (1960) found that flower induction and development of F. vesca var. semperflorens was inhibited when plants were exposed to short day treatments. Octoploid everbearing cultivars initiate most of their flowers on unrooted or recently rooted runners during the long days of summer, leading to fall harvests (Stewart and Folta 2010). The origin of the everbearing trait appears to have occurred separately in North America and Europe, as little crossbreeding occurred between European everbearing *F. vesca* and North American everbearing F. virginiana cultivars (Stewart and Folta 2010). The North American everbearing phenotype is due to a single, unstable locus within the typical June-bearing genome, while the origin of the European everbearing trait is older and more difficult to identify (Darrow 1966). The first recorded instance of a day-neutral phenotype was F. virginiana sub. glauca, and was used as a parent in commercial everbearing breeding programs in the 1930s and 1940s (Darrow 1966).

F. vesca may display day-neutrality as well (Iwata *et al.* 2012). Many everbearing cultivars such as 'Arapahoe' and 'Ogallala' have day-neutral parents present in their pedigrees, which may contribute to why everbearing and day neutral cultivars are sometimes thought to be the same (Hildreth and Powers 1941). However, true day-neutral cultivars often exhibit flowering habits that are phenotypically distinct from their everbearing relatives. The crowns of all day-neutral genotypes have a strong tendency to fruit proliferously in their first year, as opposed to most everbearing genotypes (Ahmadi and Bringhurst 1991). Day-neutral runners can also develop inflorescences before rooting occurs. Just as important, day-neutral cultivars are historically documented as insensitive to changing photoperiods, fruiting at the same rate throughout a growing season of dynamic daylength (Durner *et al.*1984). This photoperiodism for flower induction and development. These traits, in addition to increased heat tolerance (Stewart and Folta 2010), have contributed to abundant strawberry production in California, where day-neutral cultivars performed well. Other areas of the United States, such as the Upper Midwest, did not observe the same success, as day-neutrals yielded poorly in Midwestern climates and were difficult to propagate (Durner *et al*. 1984; Luby *et al*. 1987, Luby 1989). This day-neutral market advantage allows California to account for 44% of the total national strawberry acreage and almost 90% of total yields, leading to a total revenue of $2.12 billion in 2012 (California Agric. Statistics Review 2014; National Ag. Statistics Service 2014). In environments where they are commercially viable, day-neutral phenotypes are typically managed as annual plants in raised-bed systems with drip-tape irrigation and combinations, with the consensus being that yearly and environmental variances complicate the development of a single, optimal cultural practice for day-neutral production (Hughes *et al*. 2013). Recently, high tunnels structures that increase air and soil temperatures offer season extension potential and have been shown to increase total and marketable yields in day-neutral strawberry cultivars without inhibiting pollination (Kadir *et al.* 2006). However there has been a documented increase in fungal disease incidence in high tunnel systems due to reduced air circulation (Kennedy *et al.* 2013). It is often considered good horticultural practice to remove flower clusters from June-bearing plants for the first 4-6 weeks after initial planting (Eames-Sheavly *et al.* 2003); this forces the plants to partition more metabolites into vegetative growth and runner production, making the perennial crop more productive in subsequent years. The practice of flower cluster

removal is also practiced in day-neutral production, even though day-neutral cultivars are often only grown as annuals. Interestingly Lantz *et al*. (2009), conducting a study in Garrett County, Maryland (39.2833° N) demonstrated no significant difference in total yield when day-neutral 'Seascape' plants did not have flower clusters removed compared to treatments where flower clusters were removed two and four weeks after planting.

## 3. Thermophotoperiod and temperature effects

There is still some uncertainty regarding the photoperiodic nature of June-bearing, everbearing and day-neutral flowering habits. While the common consensus is that June bearing cultivars display short day flower induction, everbearing cultivars display long day flower induction and day-neutral cultivars are truly photoperiod insensitive, additional research has led many to believe that the photoperiodic tendencies of strawberry cultivars can be altered with temperature (Sonsteby and Heide 2007). In many cases, cultivars classified under photoperiodic categories only display their classified flowering response in moderate temperature conditions; once a certain threshold temperature is exceeded, their photoperiodic nature changes. For example, Guttridge (1985) found that flower induction of certain June bearing cultivars can occur under any photoperiod if temperatures are <15°C. Nishiyama and Kanahama (2000) demonstrated that day-neutral cultivar 'Hecker' had inhibited flowering at high temperatures (30°/26°C) when long day lengths were not present (>14 hr). This implies that someday-neutral cultivars may display long day flowering habits under high temperature conditions. Indeed, Sonsteby and Heide (2007) found similar results when testing the cultivar 'Elan', leading them to conclude that everbearing strawberry cultivars, in general, whether of the older European-type or the modern Californian-type originating from crosses with selections of Fragaria virginiana ssp. glauca, are qualitative (obligatory) LD plants at high temperature (27°C), and quantitative LD plants at intermediate temperatures. Only at temperatures below 10°C are these cultivars day-neutral." Such general statements should be avoided, however, since there is considerable variability in strawberry flowering and fruiting response to temperature, even within the June-bearing, everbearing and day-neutral categories (Wagstaffe 2009). For example, Bradford *et al*. (2010) discovered that plants of the day-neutral cultivar 'Tribute'

required long photoperiods for flowering after a threshold temperature of 26°C was exceeded, while plants of the day-neutral cultivar 'RH-30' required short photoperiods for flowering once temperature exceeded 23°. This variance of thermo-photoperiod within a flowering category suggests that study is merited on all cultivars of commercial significance, even if research has already been conducted on similar cultivars within their traditional photoperiod classification. Temperatures can also affect fruit production in ways that are not related to photoperiod. Kumakira and Shishido (1995) observed that strawberry flower buds of everbearing cultivars aborted during periods of high temperature (30°C), and Karapatzak *et al*. (2012) found that everbearing cultivars exposed to supraoptimal temperatures (30°C/20°C) experienced severely reduced pollen viability leading to significantly reduced yields. Similar supraoptimal temperature effects were observed with June bearing cultivars (Ito and Saito 1962; Durner *et al*. 1984). Yield reductions likely manifest as a result of unviable pollen contributing to poor fertilization and misshapen fruit (Ariza *et al*. 2011). These reductions in pollen viability appear to be dependent on high night temperatures, as supraoptimal day temperatures with cool night temperatures did not result in reduced viability (Wagstaffe 2009). The effect of supraoptimal temperatures on flowering and yield in day-neutral cultivars is less thoroughly researched, though day-neutral cultivars have previously been regarded as being more heat tolerant (Stewart and Folta 2010). Suboptimal temperatures can affect fruit development as well. Ariza *et al*. (2015) conducted a thorough analysis of cold temperature on differentiating inflorescences and observed that chilling events (24h at 2°C) can reduce pollen grain production and viability as early as 20 days before anthesis, and increase ovule abortion 3-6 days before anthesis. These events would be especially deleterious for June-bearing plants, as all June-bearing flower buds are developing in the spring when chilling events are more likely to occur. A chilling event on day-neutral plants may inhibit fruit production on developing inflorescences as well, but since they tend to produce inflorescences throughout the growing season it likely would not have as large as an effect on cumulative yields. In the diploid *Fragaria vesca*, Davik *et al*. (2013) observed the accumulation of alcohol dehydrogenase, dehydrins and galactinol as biomarkers associated with cold tolerance.

## Inflorescence architecture

Strawberry flower cluster anatomy has been thoroughly researched, as possible differences in inflorescence architecture have been hypothesized to correlate with differences in yield and berry weight among cultivars (Webb *et al*. 1978). Savini *et al*. (2005) documents the most common flower cluster and inflorescence anatomy in an architectural model, with primary, secondary and tertiary flowers. Inflorescences that follow this architectural pattern appear to display two primary internodes leading to the primary flower, secondary branch internodes that form opposite the primary node and lead to secondary flowers, and tertiary internodes that form at the node of secondary branches, leading to tertiary flowers. Unlike the Savini diagram, tertiary internodes can grow much longer than secondary internodes, making tertiary flowers appear "ahead" of secondary flowers. Thus, the best way to distinguish secondary flowers from tertiary flowers is to compare differences in flower development; secondary flowers should be further advanced along the development path to mature fruit than tertiary flowers. Interestingly, the formation of inflorescences from new branch crowns after planting follows the same architecture as flowers forming on individual inflorescences, with secondary branch crowns branching from the primary crown, and tertiary branch crowns branching from secondary branch crowns. There is, however, observable variability from this typical inflorescence pattern, and of the day-neutral cultivars only 'Seascape' inflorescences have been formally documented (Hancock 1999; Savini *et al*. 2005). June-bearing cultivar 'Annapolis' displaying the most typical inflorescence architecture, with day-neutral cultivars 'Albion' and 'Seascape' displaying similar habits. 'Monterey' and 'San Andreas' inflorescences will sometimes only form a single secondary branch. Occasionally, more developed inflorescences displaying this habit will create additional secondary branches, but these branches display an alternate growth habit, as opposed to the opposite secondary branching pattern of the more documented habit typical in 'Seascape'. The inflorescences of 'Evie-2' and 'Portola' sometimes appear to form two separate primary branches, forking off the first node. Interestingly, 'Monterey' and 'San Andreas', whose inflorescences typically only form a single secondary branch, were also the two lowest yielding cultivars in 2013 University of Minnesota trials of day-neutral cultivars, while 'Evie-2' and 'Portola', which seem to produce two primary

branches, were the highest yielding (Petran *et al.* 2015). While causation cannot be applied, these findings do raise the question of inflorescence architecture/yield relationships for further research.

## Genetics of flower induction

The underlying genetics that promote or inhibit flowering is complex and debated, and in orderto appreciate that complexity in strawberry, the history of genetic flowering research in general can provide some background. The idea of florigen, a plant hormone (or family of hormones) responsible for flower initiation and development in all flowering plant species was proposed by Chailakhyan (1936) after a series of grafting experiments. A quest to isolate and identify the florigen hormone took place thereafter, spanning the rest of the 20th century (Zeevaart, 2006). The existence of florigen as a universal floral initiator was doubted after genetic research discovered multiple distinct flowering pathways in different species, but this dissonance was resolved after it was seen that the each separate pathway converged to a shared set of flower promoting genes, the most well known being FLOWERING LOCUS T (FT, Koornneef *et al*. 1991; Samach *et al*. 2000; Simpson and Dean 2002; Putterill *et al.* 2004). Thus florigens are indeed understood to be universal flowering inducers, but the production of florigen hormones are regulated by single genes in certain species and are polygenic in others. The protein produced by FT, now considered to be a florigen hormone, travels through the phloem to the shoot apical meristem and interacts with other proteins already present in the meristem to induce flower differentiation (Abe *et al.* 2005; Notaguchi *et al.* 2008). Lifschitz *et al*. (2014) emphasizes that, in addition to florigen, there are agents that act antagonistically to this pathway, known as anti-florigens. The protein of TERMINATION FLOWER 1 (TFL1) in the model plant Arabidopsis thaliana is an antiflorigen that suppresses termination of the inflorescence and maintains vegetative growth in shoot meristems (Bradley *et al*. 1997). Lifschtiz *et al*. (2014) propose that not just florigens but the florigen:anti-florigen ratio determines flowering times in short day, long day and day-neutral flowering plants. Gene pathways that regulate florigen and antiflorigen production in strawberry must be discovered and understood before breeding efforts can be refined to accurately select for day-neutral habits. While previous reviews have stated that no genes involved

in strawberry flowering have been identified (Darnell *et al*. 2003), subsequent research has proposed specific floral promoters and suppressors within the strawberry genome. These discoveries are first reviewed within the diploid *F. vesca* genome, followed by the octoploid genome of commercial *F. x ananassa.*

# MATERIAL

# AND

# METHODS

# CHAPTER III

# MATERIAL AND METHODS

The present investigation was made to study the prevelant fungal diseases of Strawberry, identification of their causal organisms and to evaluate efficacy of different botanicals, biocontrol agents including against the most severely occurring fungal diseases. Details of the methodologies adopted during the course of experimentation and materials used during the investigation for recording various observation and analysis are presented in this chapter.

## 3.1 Geographical location

### 3.1.1 Survey on the occurrence of fungal diseases of strawberry

A random survey was conducted in jorhat district (Horticulture), the major agro-climatic zone of Assam viz., located at the upper Brahmaputra Valley Zone, during the March to May in 2022.

During the survey incidence of different kinds of foliar diseases symptoms on Strawberry was observed in surveyed areas. Strawberry leaves showing symptoms like Anthracnose (black spot) were collected for isolation of pathogens. The details of the site of the survey and collection of various disease samples of strawberry plants are presented in Table1.1

**Table 1.1 Details of the site of the survey and collection of disease sample of strawberry from Jorhat (Horticulture orchard) district of Jorhat, Assam.**

| Site of the survey | GPS Coordinates |
|---|---|
| **Horticulture Orchard, AAU, Jorhat** | **26°43´35´´ N 94°12´05´´ E** |

### 3.1.2 Assesment of disease incidence and percent disease index

The intensity of the per cent disease incidence was assessed by recording the number of plants showing disease symptoms and the total number of plants observed. In surveyed location, plants were selected randomly and the number of plants showing typical symptoms and the total number of plants were recorded. Disease incidence (DI%) was calculated using the formula given by Wheeler (1969).

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

The Per cent Disease Index (PDI) of the leaf spot of gerbera was calculated by using 0-5 scale as per the formula given by Wheeler (1969) (Table 3.2). The lower four leaves of each plant were considered to calculate the PDI in each surveyed plot.

**Table 3.2. Score chart for per cent disease index on leaves**

| Grades | Description |
|---|---|
| 0 | No symptoms on leaf |
| 1 | 1% leaf area infected |
| 2 | 1-10% leaf area infected |
| 3 | 11-25% leaf area infected |
| 4 | 26-50% leaf area infected |
| 5 | 50-100% leaf area infected |

$$PDI = \frac{\text{Sum of individual disease rating}}{\text{Total number of leaves observed x maximum disease grade}} X\ 100$$

For wilt disease,

$$PDI = \frac{\text{No. of plants showing wilt symptoms}}{\text{Total no. of plants observed}} X\ 100$$

## 3.2 Site of the experiment

The site of the experiment is Horticultre Orchard in Assam Agricultural University, Jorhat which is a premier establishment for study in Agricultural Science is located in 26°44´ N latitude and 94°10´ E longitude with an elevation of 91 m above the mean sea level in the Upper Brahmaputra Valley Zone. The experiment was carried out in the Department of Plant Pathology and in the experimental farm

of the Department of Horticulture, Assam Agricultural University, Jorhat during the year 2022.

### 3.2.1 Weather and climate condition

The Jorhat district is located in the upper Brahmaputra Valley Zone of Assam which served as a site for survey and collection of diseases sample and field experiments. The area of Jorhat is experiences subtropical hot and humid climate with summer temperature ranging from 25-35° C from May to August and winter temperature ranging from 10 to 22 C from November to February with high rainfall (>2200 mm per annum) and high average humidity (85-90%).

## 3.3 Cleaning of Glassware

Glassware like Petri dishes, Erlenmeyer flasks, measuring cylinder, test tubes, etc. used for the experiments were washed with potassium dichromate, rinsed with water, and air-dried before use.

### 3.3.1 Sterilization of glassware

The glassware's were sterilized in a hot air oven (OMNI, DTC 72) at 180°C for 2 hours.

## Preparation of media

### 3.3.2 Potato Dextrose Agar (PDA) medium

Potato Dextrose Agar (PDA) medium was prepared by suspending 39.9 g of readymade PDA powder (Hi- Media Laboratories Ltd.) in 1000 ml distilled water. Mild heating was done to dissolve the medium completely and pH was measured and maintained at 7.0 by adding either Hydrochloric acid (HCI) or Sodium Hydroxide (NaOH). The medium was poured in culture tubes and Erlenmeyer flasks, plugged with non-absorbent cotton, and sterilized in an autoclave (IK-104) at 121℃ (15lb pressure per square inch) for 20 minutes.

## 3.4 Isolation and Purification of the pathogen

The pathogen was isolated by the tissue isolation technique as described by Ricker and Ricker (1936). The leaves sample was washed with distilled water to remove the soil and other unwanted materials. Small thin section of the diseased tissue along with some healthy portions cut with the help of a sterilized blade and disinfected with 1% sodium hypochlorite for 30 seconds followed by three subsequent washing in sterilized distilled water to remove the traces of sodium hypochlorite the method described by Johnston and Booth (1983). The cut samples were dried by sterilized blotting paper. Three to four pre-sterilized sections were inoculated in sterilized Petri plate containing potato dextrose agar (PDA) media with the help of sterilized forceps. All the above-mentioned procedure were done under the aseptic condition inside the laminar airflow and kept for incubation at room temperature 28±1° C for up to full growth of the fungus into the plate.

## 3.5 Maintenance of the culture

The culture of the pathogen was maintained throughout the period of investigation on PDA slants by routine sub-culturing at regular intervals and storing at 4° C in refrigerator.

## 3.6 Single spore isolation

The fungal culture was purified by a single spore isolation technique (Johnston and Booth, 1983). 10 ml of clear filtered two per cent water agar solutions were poured into sterile Petri plates and allowed to solidify. The dilute spore suspension was prepared in sterile distilled water from ten days old culture. 2 ml of spore suspension was spread uniformly on water agar plates. The excess suspension was aseptically drained off. After 4 hours of incubation at 28±1° C, the plates were examined to locate the germinated conidia. Single isolated germinated conidium was marked with link on the lower glass surface of the Petri plate, which was cut and transferred to PDA slants in such a way that the conidium bearing surface was in contact with the PDA surface and incubated at 28±1°C. The pure culture obtained was preserved in a refrigerator and sub cultured once in a month.

## 3.7 Characterization and identification of the pathogens

Among the various foliar diseases in strawberry studied, the pathogens that are endemic in nature causing severe diseases were selected for its characterized and further study. Identification of the fungus was done by the following methods.

## 3.7 Cultural characteristics

The isolated fungus was grown in sterilized Petri plates with PDA media by transferring 5 mm diameter mycelia disc of 7 days old pure culture using the cork borer to the center of Petri plates under aseptic condition. The inoculated plates were then incubated at 28±1°C temperature in the BOD incubator in an inverted position. Identification and characterization of the fungal pathogen was carried out according to reference materials (Ellis 2972; Barnett and Hunter, 1972; Mathur and Kongsdal, 2003; Agrios, 2005). The color, shape and branching habit of the fungus were studied. The colony color of the fungus was judged by using the quality of the Methuen handbook of color (Kornerup and Wanscher, 1967)

## 3.7.2 Morphological characteristics

Morphological characteristics of the isolated pathogen were studied by microscopic study. A portion of the fungal mass was taken from the PDA plates with an aseptic needle. The fungal mass was placed on a drop of lactophenol cotton blue previously placed on a grease-free glass slide. A cover slip was put over it and was observed under a compound microscope. Observation on morphological characters of mycelium, conidiophores, conidia were noted as per the method of Chowdhry and Varshney, 2000. Measurement of the conidia and micro-photographed done with the help of a computer-generated micrometer. The pathogens were identified by comparing with the available relevant literature a keys and monograph (Thom and Raper, 1945; Raper and Thom, 1949; Ellis, 1971; Barnet and Hunter, 1972; Riley *et al*. 2002; Carlile *et al*., 2001; Mathur and Kongsdal, 2003).

The pure cultures of the fungus wee also sent to the National Centre of Fungal Taxonomy (NCFT), New Delhi for confirmation and identification up to species level.

### 3.7.3 Molecular Characteristics

Two fungal pathogens were selected based on the severity of infection and subjected to molecular characterization. The molecular characterization was carried out at Bioreserve Biotechnologies India Pvt. Ltd by ITS sequencing to identify at the species level.

### 3.7.4 Phylogenetic analysis and sequence similarity.

Phylogenetic analysis has been used on comparative genomics and proteomics to depict evolutionary relationship. The obtained sequences were aligned using multiple sequence alignment in MEGA 6.0 (Tamura *et al.,* 2013). A phylogenetic tree was constructed using maximum-Likelihood method included in MEGA 6.0 by taking 500 boostrap.

## 3.8 Pathogenicity test

Pathogenecity test prove the association of the causal agents were done by following the method Mycelial Bit Inoculation Technique (MBIT). Pathogenicity tests were carried out to determine the parasitic nature of pathogen. Healthy strawberry saplings (30 days old) of highly susceptible variety (Red Gem) were selected for proving the pathogenicity test. The healthy leaves of strawberry (*F. ananassa*) were surface sterilized by HgCl2 (1%) or NaOCl (5%) and followed by washing with sterilized distilled water. Bit of mycelia of the pathogen are made with the help of sterile cork borer and bit are placed upper surface of the leaves. Inoculated leaves were placed in a sterilized Petri plate. Small amount of absorbent cotton were placed around the leaves to avoid dryness. When typical symptoms were developed on inoculated leaves, re isolation of the causal agents was done to establish Koch's postulates. The isolates of the pathogenic fungi thus obtained were transferred on fresh PDA slants for comparison with the original culture. The fungal pathogen thus obtained was maintained on PDA slants in refrigerator at 4° C for further study.

## 3.9 In vitro efficacy of different botanicals and biocontrol agents against pathogen.

### 3.9.1 In vitro efficacy of different botanicals against pathogen

#### 3.9.1.1 Collection of botanicals

Different plant parts (fresh leaves, rhizome and bulbs) of the botanicals (Table 3.3) were collected from various localities of Assam Agricultural University, Jorhat Campus. Selection of the botanicals was done based on their medicinal properties. These botanicals were evaluated for their efficacy against the pathogens (Table 3.3)

**Table 3.3 List of botanicals tested against*C.gloeosporiodes.***

| Sl No | Scientific Name | CommonName | Family | Plants parts Used |
|---|---|---|---|---|
| 1 | Allium sativum L. | Garlic | Amarylidaceae | Bulb |
| 2 | Azadirachta indicaA.Jus. | Neem | Meliaceae | Leaf |
| 3 | Ocimum tenuiflorum | Tulsi | Lamiaceae | Leaf and bulb |
| 4 | Lawsonia inermis | Jetuka | Lythraceae | Leaf |
| 5 | Allamanda cathartica | Allamanda | Apocynaceae | Leaf |

## 3.10 Preparation of plant extracts with cold water

Collected fresh leaves, bulbs of *A. indica, A. sativum, O. tenuiflorum, L. inermis* and *A. cathartica*, respectively were washed thoroughly in sterile distilled water. 100 g of washed plant parts were grind in pre-chilled mortar and pestle by adding an equal amount of (100ml) of sterilized water (1:1 W\V). The extract was filtered through muslin cloth and centrifuged at 10,000 rpm for 20 minutes at room temperature. The supernatant was taken as 100% basic stock solution. The plant extract, thus obtained was further filtered through a bacterial membrane filter (RanDisc, PVDF 0.22 µm) under aseptic condition and at 10% concentration the efficacy was tested against pathogen by 'poisoned food technique' (Nene and Thapliyal, 2000).

### 3.10.1.1 Screening of botanicals against pathogen.

Selected botanicals were initially screened for their anti fungal efficacy against both the pathogen at different concentration (5%, 10%, 15% and 20% concentration). For the screening test, 5 ml of 100 % aqueous plant extracts of each botanical was aseptically added 95 ml molten PDA, 90 ml molten PDA for 10 ml of aqueous plant extracts, 85 ml molten PDA for 15 ml of aqueous plant extracts and 80 ml for 20 ml aqueous plant extracts, in Erlenmeyer flasks respectively to get the final concentration of 5, 10, 15 and 20 per cent respectively of the extract medium. PDA without any extract served as control. The media was poured in 90 mm Petri plates at the rate of 20 ml per plate. The fungal culture disc using a cork borer (5 mm diameter) from the tip obtained from a 7 days old culture was taken and inoculated in the center of Petri plates aseptically after solidification the medium and incubated at 28±1°C for 7 to 10 days.

## Treatment combinations for botanical as were followed:

T1 : Control (Pathogen only)

T2 : *Allium sativum* (5%, 10%, 15% and 20% conc.) + Pathogen

T3 : *Azadirachta indica* (5%, 10%, 15% and 20% conc.) + Pathogen

T4 *: Ocimum tenuiflorum* (5%, 10%, 15% and 20% conc.) + Pathogen

T5 : *Lawsonia innermis* (5%, 10%, 15% and 20% conc.) + Pathogen

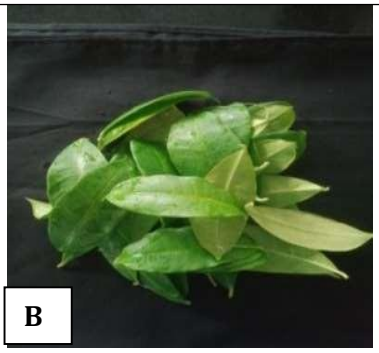
T6 : *Allamanda cathartica (*5%, 10%, 15% and 20% conc.) + Pathogen.

The colony diameter of the pathogen was measured when the mycelium fully covered the Petri plates in control plates. The per cent inhibition of the mycelial growth was calculated by the following formula Vincent (1927)

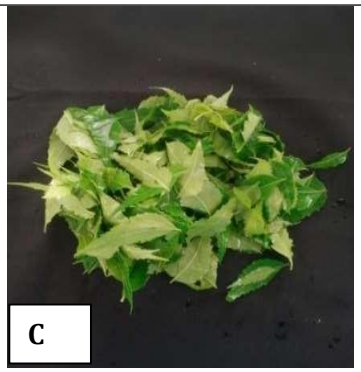
$$I = \frac{C-T}{C} \times 100$$

Where, I= Inhibition of mycelia growth, C= Growth in control and T= Growth in treatment.


*Allium sativum* (Garlic)




*Allamanda cathartica* (Allamanda)




*Azadirachta indica*(Neem)


*Ocimum tenuiflorum*(Tulsi)


*Lawsonia inermis*(Jetuka)

**Different botanicals used against *Colletotrichum gloeosporiodes.***

## 3.10.2 In vitro efficacy of different biocontrol agents against pathogen.

In this study, five different biocontrol agents viz, *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescence, Bacillus subtillis, Bacillus velezensis* were evaluated against both the pathogen by dual culture technique (Dennis and Webster, 1971). For this study, a culture disc (5mm diameter) of each tested fungus was inoculated at one end of each Petri plates and another culture disc (5mm diameter) was placed equidistantly exactly opposite end of the same Petri plate. But for the bacterial antagonist P. fluorescence, a loopful of 48 hours old inoculums was streaked at one end of the Petri plate, and control was maintained without biocontrol agent. Each of the treatments was replicated thrice and these Petri plates were incubated in BOD incubator at 28±1°C till full growth observed in the control.

### Treatment combinations for biocontrol agents as were followed :

T1 : Control (Pathogen only)

T2 : *T. harzianum* + Pathogen

T3 : *T. viride* + Pathogen

T4 : *P. fluorescence* + Pathogen

T5 : *B. subtillis* + Pathogen

T6 : *B. velezensis* + Pathogen

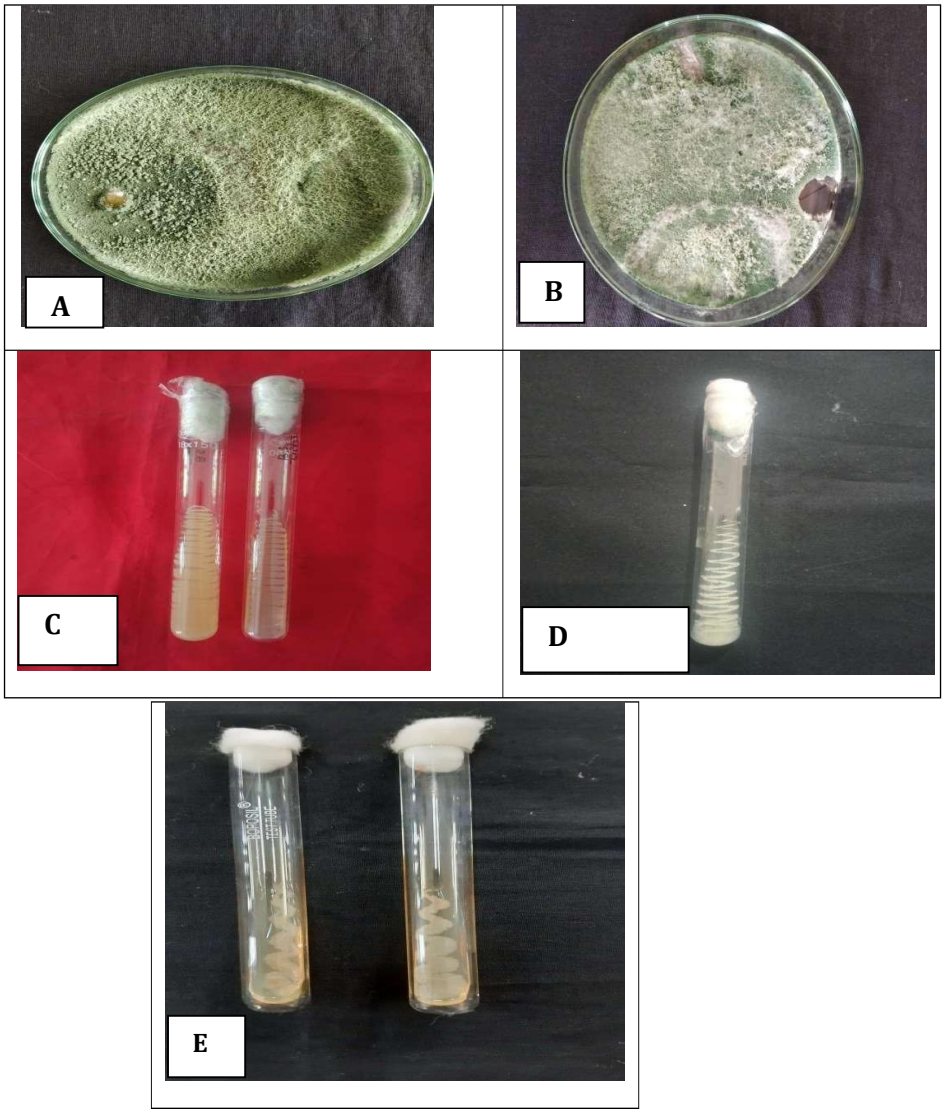



**Plate 3.2 (A-E). Biocontrol agents used against *C. gloeosporiodes*.**

**A :** ***Trichoderma harzianum***

**B :** ***Trichoderma viride***

**C :** ***Bacillus subtilis***

**D :** ***Bacillus veleziensis***

**E :** ***Pseudomonas influorescence***

# EXPERIMENTAL FINDINGS

# CHAPTER IV

# EXPERIMENTAL FINDINGS

The results of different experiments of the present studies entitled "Study on Anthracnose disease of Strawberry (*Fragaria ananassa*) in Jorhat, Assam" are presented below:

## 4.1 Survey on the occurrence of fungal diseases of strawberry

Survey was conducted to observe the incidence of different disease of strawberry grown both open and protected conditions in Horticulture Orchard, Assam Agricultural University in Jorhat, Assam. The details of the site of the survey and collection of disease samples of strawberry plants are presented in Table 3.1. The disease specimens were collected from these surveyed location and the pathogens were isolated. The Anthracnose (black spot) were recorded and based on the Per cent disease incidence and Percent disease index of the diseases Anthracnose (black spot) with highest disease incidence and disease index were selected for further studies.

### 4.1.1 Assessment of Per cent disease incidence and Per cent disease index.

Per cent disease incidence (DI%) and Per cent disease index (PDI) were recorded for distinct types of symptoms of fungal disease developed at the location during the survey [Plate 4.2 (A-D)]. The details of disease incidence and per cent disease index of the location of jorhat are furnished in Table 4.1.

## 4.2 Symptomatology

During the period of survey and sample collection, different types of symptoms were observed caused by various pathogens in strawberry. Out of the pathogens, Anthracnose (black spot) on leaf were observed in most of the strawberry growing area Jorhat, Assam and found to produce different types of symptoms.

**Table 4.1. Per cent disease incidence and per cent disease index of fungal diseases of strawberry grown in Jorhat, Assam under both protected and open field condition.**

| SL N0 | District | Location | Per cent disease incidence | | | |
|---|---|---|---|---|---|---|
| | | | Anthr-Acnose | Leaf spot | Wilt | Grey mold |
| 1 | JORHAT | Horticulture orchard | 58.33 | 47.33 | 50.00 | 41.66 |


**Plate 4.1(A-B). Survey and collection of disease sample from strawberry field.**

### 4.2.1 Anthracnose on leaf

Anthracnose caused by *Colletotrichum* species is a serious threat to strawberry production, especially in warm and humid climates. Anthracnose on strawberry is favoured by warm, humid and wet conditions. In this disease the round black or light gray lesions on leaves appeared. Numerous spots may develop but leaves do not die.

## 4.3 Characterization and identification of the pathogens

The fungal pathogens isolated from different diseased samples from different locations were subjected to cultural, morphological and molecular studies for characterization and identification of the isolates. A detailed description of each isolate is presented below.

### 4.3.1 Cultural and morphological characteristics

#### *4.3.1.1 Colletotrichum gloeosporiodes*

The colony color of the mycelium was whitish in color with profuse aerial mycelium on PDA medium. Reverse side of the culture in the Petri dish was found as light brown in color. Microscopic identification results showed that the colony was white to pink and dark gray, conidia were aseptate, hyaline and one-celled, oval shape with a blunt edge with length of 10.1 um x 4.02 um, hyphae was hyaline and septated, seta was straight, dark brown, with the size of 62-112 um. The results showed similarity with the reference identification of Lubbe *et al*. (2004) and Xie *et al*. (2010) and identified as *Colletotrichum gloeosporioides* (Penz.)

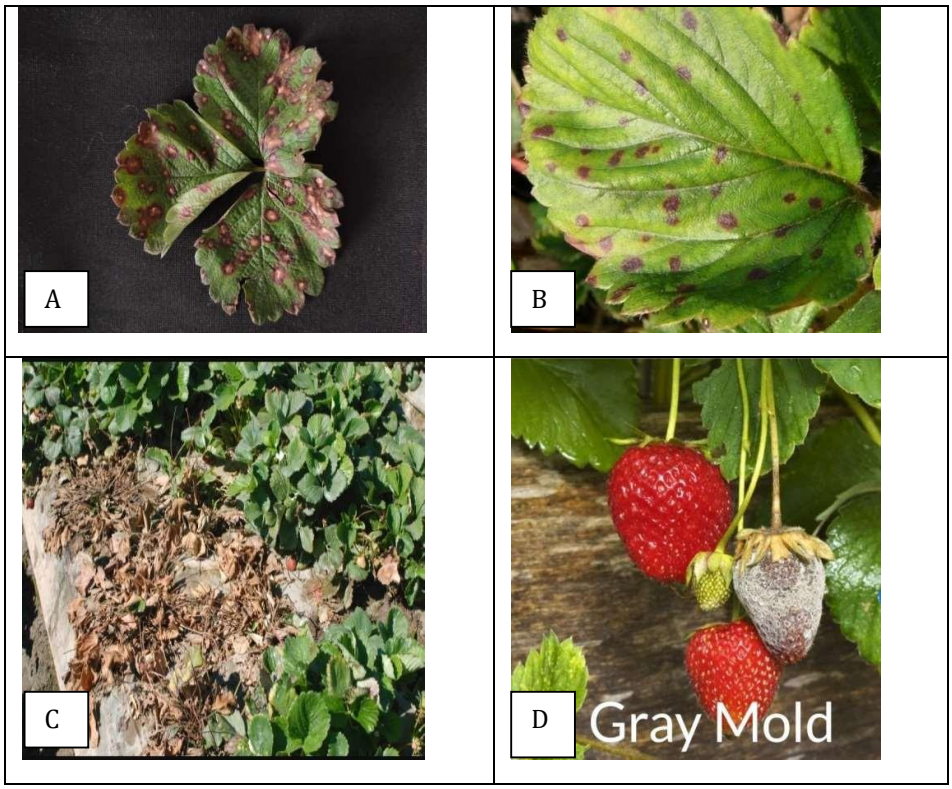



**Plate 4.2 (A-D). Different typical symptoms of fungal diseases of strawberry.**

**A : Anthracnose**

**B : Leaf spot**

**C : Wilt**

**D : Grey mold**

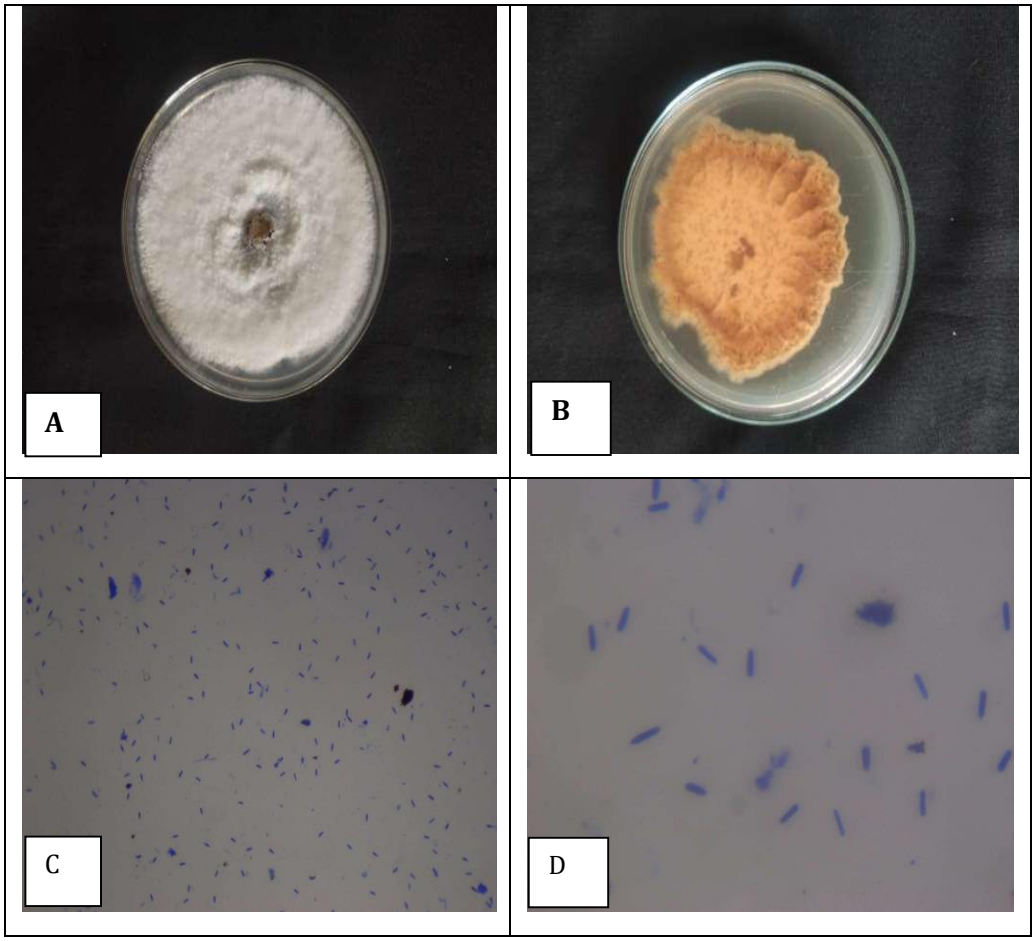



**Plate 4.3 (A-E). Cultural and morphological characteristics of *Colletotrichum gloeosporiodes***

**A : Pure culture of *Colletotrichum gloeosporiodes* in PDA (7 days old culture)**

**B : Reverse view of *C. gloeosporiodes* in petri plate**

**C : Microscopic view of conidia (10X)**

**D : Microscopic view of conidia (40X)**

## 4.3.1.2 Molecular characterization of the pathogens

The identification of the fungus *C. gloeosporiodes* up to generic level was done tentatively based on cultural and morphological characteristics. The species level identification of the fungus was done by molecular characterization.

## 4.3.2.1 Sequencing and Phylogenetic analysis

The fungal culture was sent to Bio serve Biotechnologies India Pvt. Ltd., Hyderabad for partial sequencing of 18 s RNA gene. Sequence has been converted to FASTA sequence using MEGA 6 software followed by n BLAST using NCBI link. Obtained sequence was analyzed for homology using n BLAST (NCBI) to obtained possible identities. BLAST results revealed that isolate A1t01 having 91.98% homology with *Colletotrichum gloeosporiodes* (hypothetical protein partial m RNA, USA) and catergorized as *C. gloeosporiodes.*

## 4.4 Pathogenecity test

The healthy strawberry leaves were put inside the big Petri plate with the blotting paper. For bit inoculation method bit are made with the help of sterile cork borer and bit are placed upper surface of the leaves. The pathogenecity test of isolated fungus was done by Mycelial Bit inoculation Method (MBIM) (Rocha *et al*., 1998). The inoculated leaves showed the initial symptoms 7 days after inoculation and the typical symptom appeared after 10 days of inoculation. The control plates did not show any symptoms. Appearance of minute circular black spots at the tip and margins of the leaflets was the initial symptoms of *C. gloeosporiodes.*

On re-isolation of the pathogens from such leaves revealed identical morphological characteristics when compared with the original cultures of the pathogens. Hence, the causal agents of the diseases were confirmed as *Colletotrichum gloeosporiodes*.

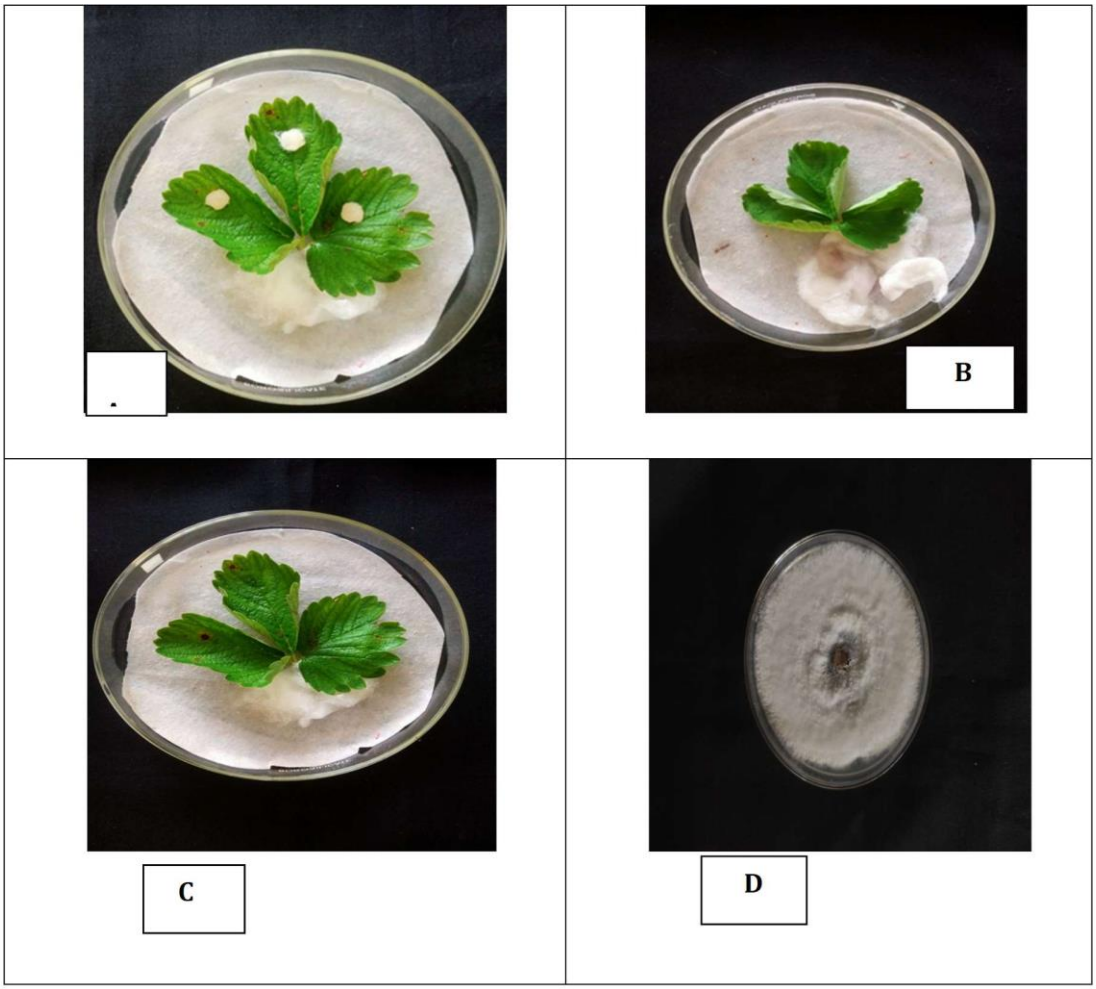



**Plate 4.4 (A-E). Pathogenicity test of *C. gloeosporiodes* on healthy leaves of strawberry plant.**

**A : Pathogenicity test by Mycelial Bit Inoculation Technique (MBIT)**

**B : Control leaf plate**

**C : 3 days after inoculation of *C. gloeosporiodes* developed initial symptoms**

**D : Re isolation of *C. gloeosporiodes* from infected leaf.**

## 4.5 In vitro efficacy of different botanicals and biocontrol agents against *Colletotrichum gloeosporiodes*.

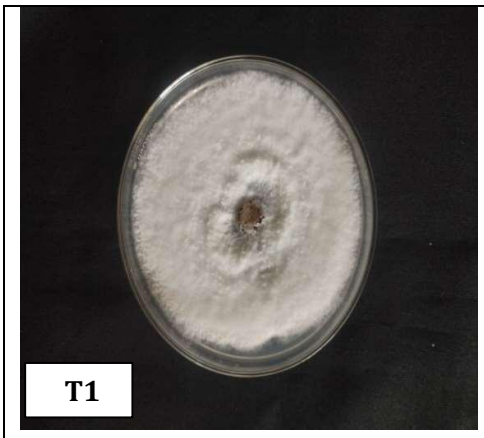
### 4.5.1 In vitro efficacy of different botanicals against *Colletotrichum gloeosporiodes*.

Five different botanicals viz., *Allium sativum, Azadirachta indica, Ocimum tenuiflorum, Lawsonia inermis and Allamanda cathartica* at different concentration (5%, 10%, 15% and 20%) were tested against *C. gloeosporiodes* by poison food technique. The results presented in Table 4.2, Fig. 4.3 and Plate 4.6 (A-F) showed that all the botanicals significantly reduced the mycelia growth of the pathogen over control. Among the botanicals tested, treatment T2 (*Allium sativum*) recorded highest inhibition (86.66) mycelia growth of *C. gloeosporiodes* which was followed by T3 *Allamanda cathartica*, T4 *Azadirachta indica*, T5 *Ocimum tenuiflorum*, T6 *Lawsonia inermis* with the mycelia growth inhibition of 77.22%, 76.33%, 75.44% and 67.44% respectively. The per cent inhibition for the rest of the treatment on mycelial growth of the pathogen ranged from 67.44% to 86.66% respectively. The lowest (67.44%) inhibition of mycelial growth of the pathogen was recorded in T6 (*Lawsonia inermis*).

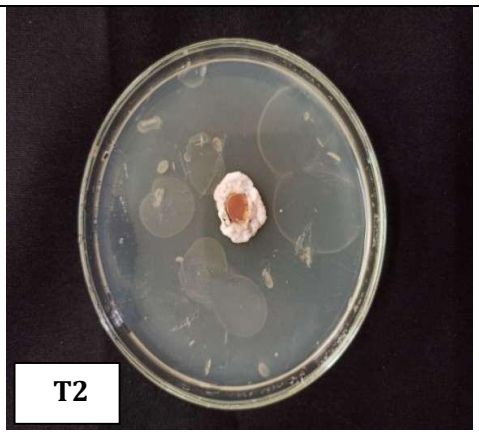
Based on the results of this preliminary screening, botanical *Allium sativum* that exhibited 86.66% inhibition over control was selected for further evaluation of their efficacy at different concentrations against *C. gloeosporiodes* for in vitro condition.

**Table 4.2 In vitro efficacy of different botanicals at different concentration (5%, 10%, 15% and 20%) against mycelial growth of *Colletotrichum gloeosporiodes***

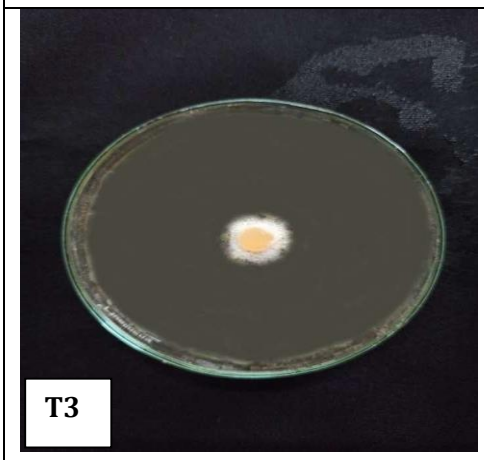
| Treatments | Mycelial growth* (cm) | Per cent inhibition Over control |
|---|---|---|
| T1 Control (*C.gloeosporiodes*) | 9.00 | |
| T2 *Allium sativum* | 1.20 | 86.66 |
| T3 *Allamanda cathartica* | 2.05 | 77.22 |
| T4 *Azadirachta indica* | 2.13 | 76.33 |
| T5 *Ocimum tenuiflorum* | 2.21 | 75.44 |
| T6 *Lawsonia inermis* | 2.93 | 67.44 |
| S.Ed(±) | | |
| CD (P=0.05) | | |



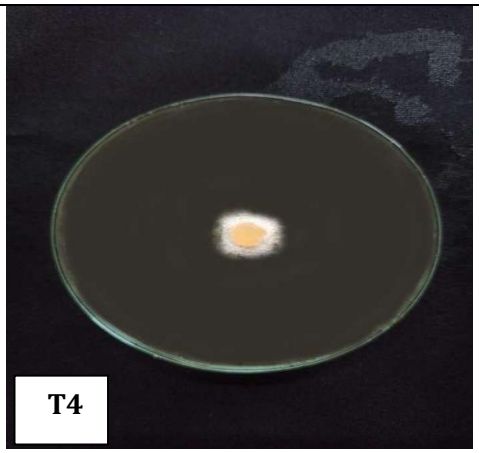

A



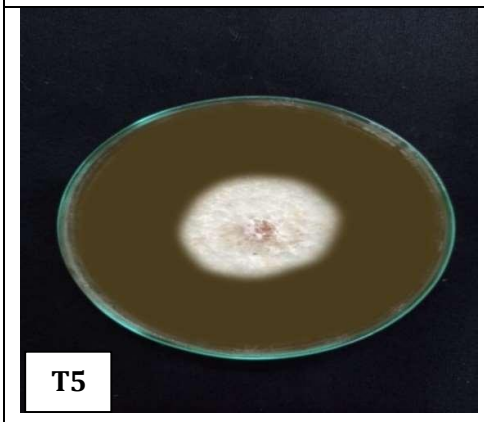

B



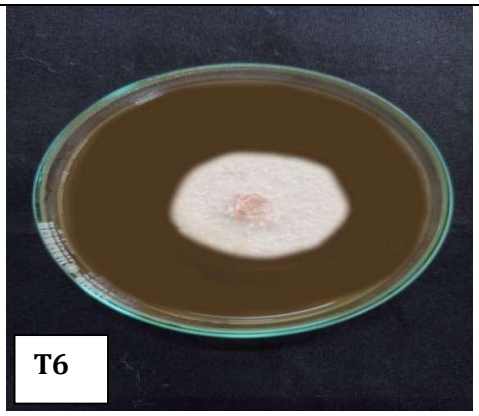

C




D




E




F

## Plate 4.5 (A-F). In vitro efficacy of different botanicals against *Colletotrichum gloeosporiodes*.

**A : T1 [Control (*C. gloeosporiodes*)]**

**B : T2 [*Allium sativum* (5% concentration)]**

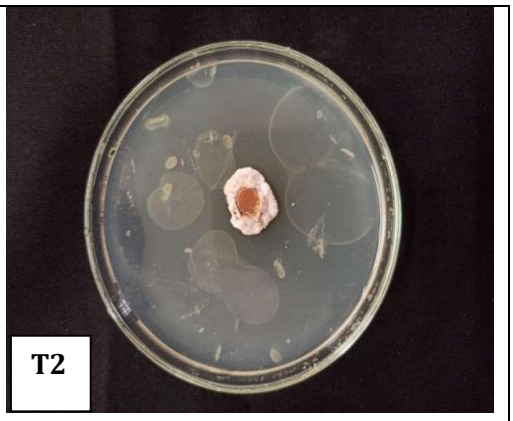
**C : T3 [*Allamanda cathartica* (5% concentration)]**

**D : T4 [*Azadirachta indica* (5% concentration)]**

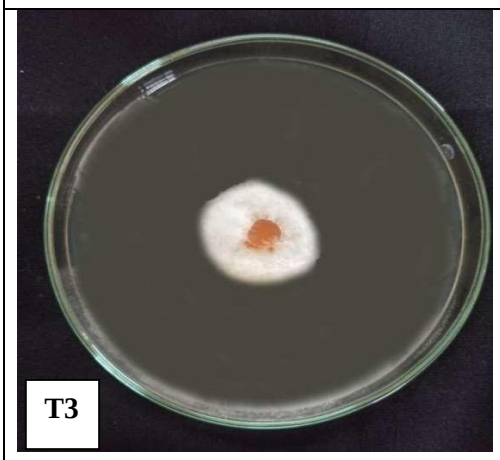
**E : T5 [*Ocimum tenuiflorum* (5% concentration)]**

**F : T6 [*Lawsonia inermis* (5% concentration)]**


A



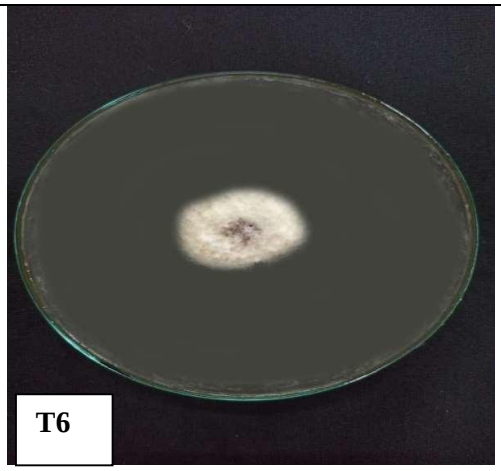

B




C


D


E




F

## Plate 4.6 (A-F). In vitro efficacy of different botanicals against *Colletotrichum gloeosporiodes*.

**A : T1 [Control (*C. gloeosporiodes*)]**

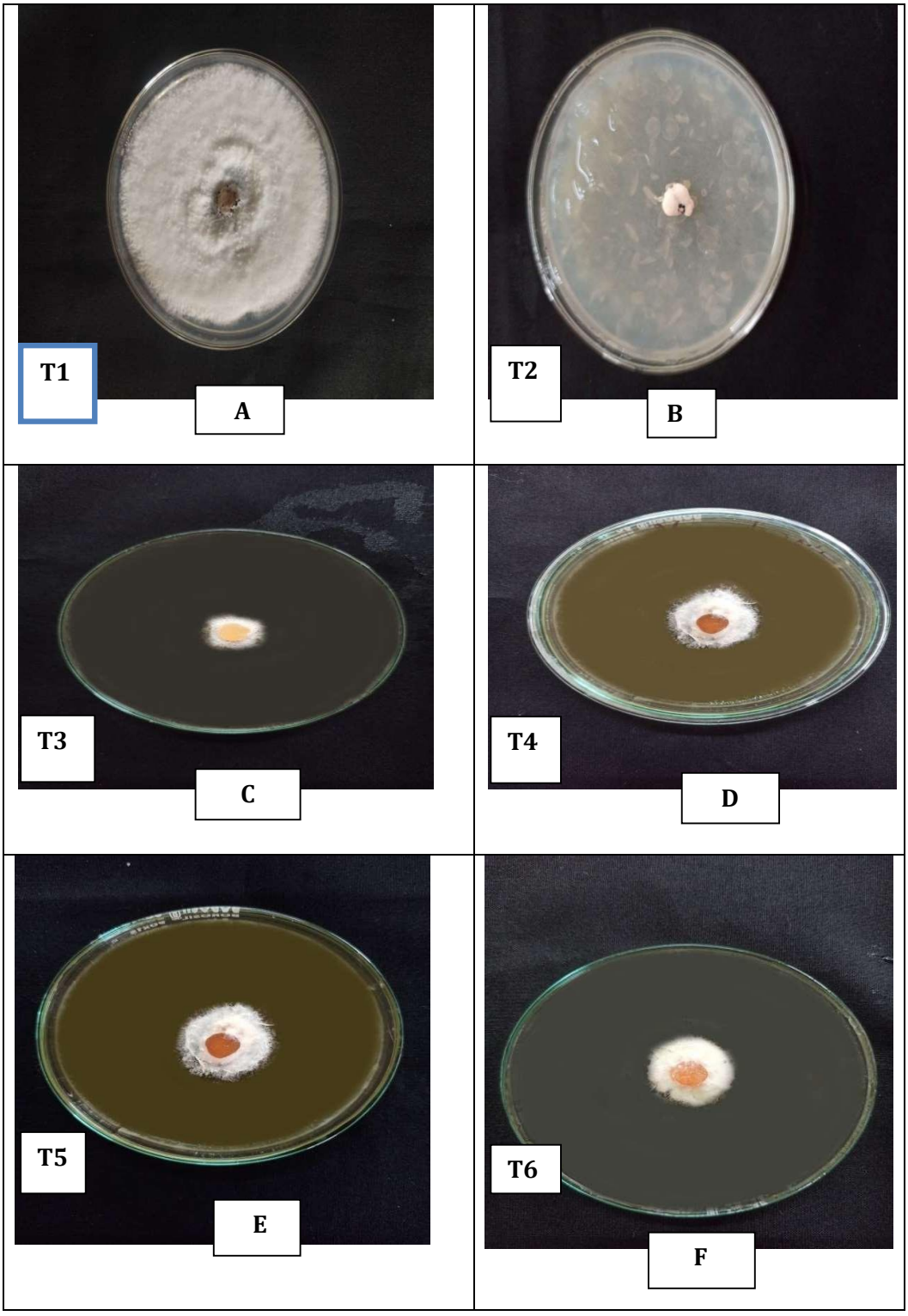
**B : T2 [*Allium sativum* at (10% concentration)]**

**C : T3 [*Allamanda cathartica* at (10% concentration)]**

**D : T4 [*Azadirachta indica* at (10% concentration)]**

**E : T5 [*Ocimum tenuiflorum* at (10% concentration)]**

**F : T6 [*Lawsonia inermis* at (10% concentration)]**


T1
A
T2
B
T3
C
T4
D
T5
E
T6
F

## Plate 4.8 (A-F). In vitro efficacy of different botanicals against *C. gloeosporiodes.*

**A : T1 [Control (*C. gloeosporiodes*)]**

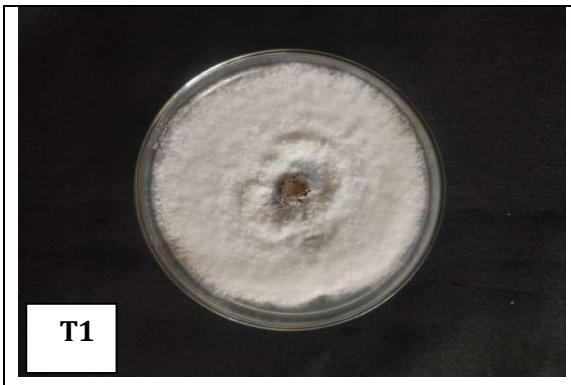
**B : T2 [*Allium sativum* (15% concentration)]**

**C : T3 [*Allamanda cathartica* (15% concentration)]**

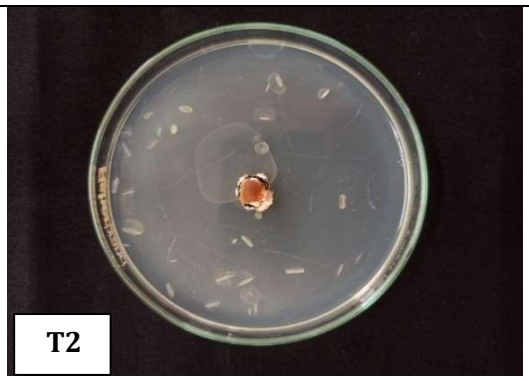
**D : T4 [*Azadirachta indica* (15% concentration)]**

**E : T5 [*Ocimum tenuiflorum* (15% concentration)]**

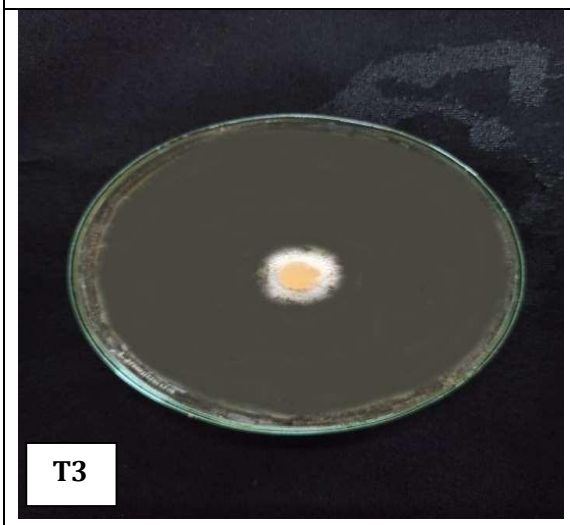
**F : T6 [*Lawsonia inermis* (15% concentration)]**




A

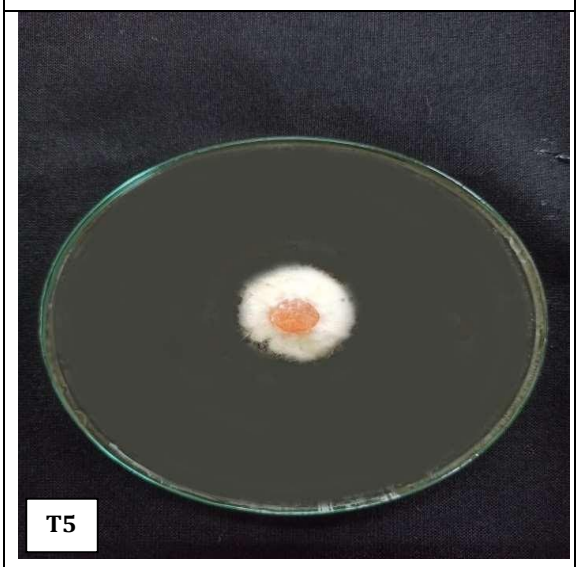



B

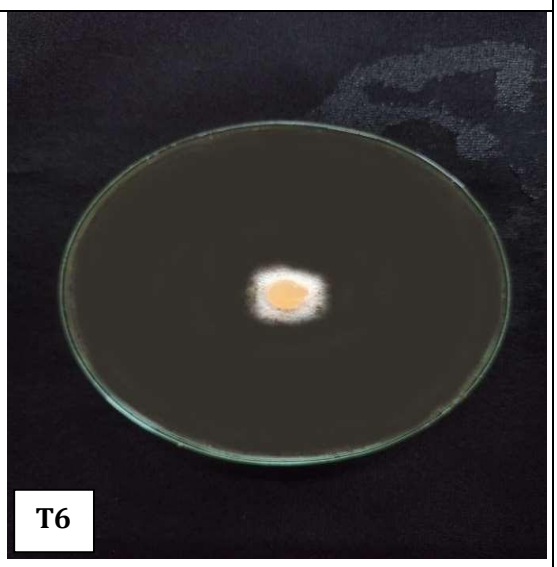



C


D




E




F

## Plate 4.8 (A-F). In vitro efficacy of different botanicals against *C. gloeosporiodes.*

**A : T1 [Control (*C. gloeosporiodes*)]**

**B : T2 [*Allium sativum* (20% concentration)]**

**C : T3 [*Allamanda cathartica* (20% concentration)]**

**D : T4 [*Azadirachta indica* (20% concentration)]**

**E : T5 [*Ocimum tenuiflorum* (20% concentration)]**

**F : T6 [*Lawsonia inermis* (20% concentration)]**

### 4.5.2 In vitro efficacy of different biocontrol agents against *Colletotrichum gloeosporiodes*.

Biocontrol agents viz., *Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescence, Bacillus subtillis* and *Bacillus velezensis* were evaluated singly against C. gloeosporiodes through dual culture technique and control were maintained without biocontrol agent. The results presented in Table 4.4, Fig 4.5 showed that all the biocontrol agents significantly inhibited the mycelial growth of the pathogen over control. Among the biocontrol agents, treatment T2 (*Trichoderma harzianum*) showed highest inhibition (74.44%) on mycelial growth of the pathogen. This was followed by T2 (*Trichoderma viride*), T3 (*Bacillus subtillis*), T4 (*B. velezensis*) and T5 (*Pseudomonas fluorescence*) which recorded inhibition of 73.88%, 71.11%, 66.11%, 62.22% respectively. The per cent inhibition on mycelial growth of the pathogen exhibited by the rest of the treatments (T1, T2, T3, T4 and T5) in the range of 66.41 to 74.44. The lowest inhibition (66.41%) on mycelial growth of the pathogen was recorded in T6 (*Pseudomonas fluorescence*).

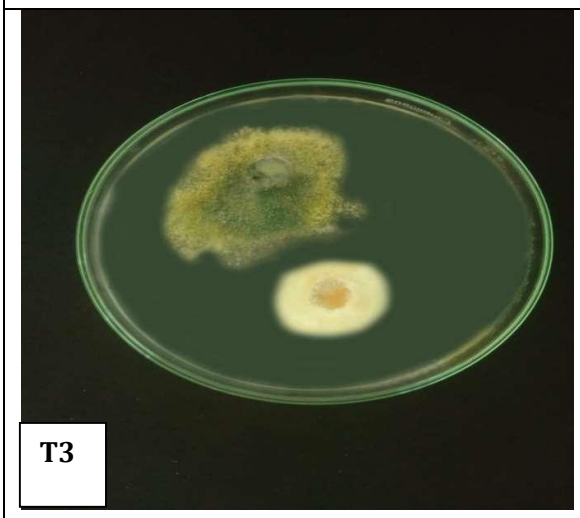
Based on the results of this in vitro efficacy of the biocontrol agents, the most effective biocontrol agent *Trichoderma harzianum* which exhibited 74.44% inhibition over control was selected for further evaluation of their efficacy against *C. gloeosporiodes* for in vivo condition.

**Table 4.3 In vitro efficacy of different biocontrol agents against mycelia growth of *Colletotrichum gloeosporiodes***

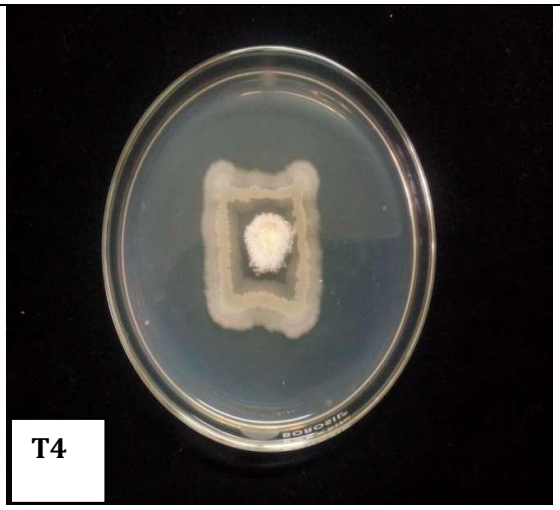
| Treatments | Mycelial growth* (cm) | Per cent inhibition Over control |
|---|---|---|
| **T1 Control (*C. gloeosporiodes*)** | **9.00** | **--** |
| **T2 *Trichoderma harzianum*** | **2.30** | **74.44** |
| **T3 *Trichoderma viride*** | **2.35** | **73.88** |
| **T4 *Bacillus subtillis*** | **2.60** | **71.11** |
| **T5 *Bacillus velezensis*** | **3.05** | **66.11** |
| **T6 *Psudomonas influorescence*** | **3.40** | **62.22** |
| **S.Ed(±)** <br> **CD (P=0.05)** | | |


A

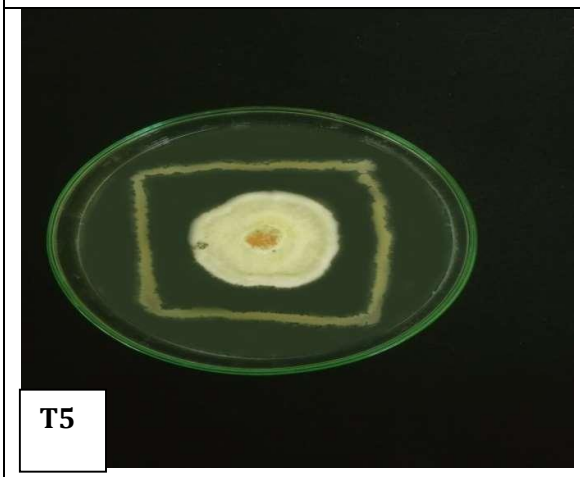

B




C




D




E


F

**Plate 4.9 (A-F). In vitro efficacy of different biocontrol agents against *C. gloeosporiodes*.**

**A : T1 [Control (*C. gloeosporiodes*)]**

**B : T2 (*Trichoderma harzianum* + *C. gloeosporiodes*)**

**C : T3 (*Trichoderma viride* + *C. gloeosporiodes*)**

**D : T4 (*Bacillus subtillis* + *C. gleosporiodes*)**

**E : T5 (*Bacillus veleziensis* + *C. gloeosporiodes*)**

**F : T6 (*Pseudomonas influorescence* + *C. gloeosporiodes*)**

# RESULTS

# AND

# DISCUSSION

# CHAPTER V

# RESULTS AND DISCUSSION

Strawberry is one of the most fruit crops grown in all over the world including India. Owing to diversity in topography, altitude, congenial agro-climatic condition coupled with fertile soil, well distributed rainfall and assured domestic and national market, the importance of commercial floriculture is gaining momentum in Assam like other Indian states. Diseases are a major constraint in economic crop production as they inflict heavy losses. A large number of pathogens have been reported in Strawberry, among the various diseases affecting strawberry, Anthracnose (black spot) caused by *Colletotrichum gloeosporiodes*, has become a severe threat to the successful and profitable cultivation of strawberry in India. In the present investigation, Anthracnose was found in the majority of strawberry growing locations of India. Hence, it is pertinent to have a detailed investigation to identify various fungal pathogens infecting different botanicals and biocontrol agents against the pathogens in in vitro condition. With this background, the present study was made to record the incidence of major fungal diseases of strawberry, identification of causal organism and also explore the potential management of severe fungal diseases of strawberry grown in India. The findings of the present investigation are discussed in this chapter.

During the survey incidence of the different fungal diseases of strawberry was observed in the Horticulture Orchard, Jorhat. Anthracnose (black spot), common leaf spots are commonly observed in strawberry grown both under open and protected conditions. Highest *Colletotrichum* spot (*Colletotrichum gloeosporiodes)* incidence of 60% with maximum per cent disease index were observed in the site. Sporadic incidence of grey mold, wilt and Angular leaf spot was also observed in both open and protected condition during the investigation. Based on the severity of the diseases, the frequently occurring Anthracnose caused by *C. gloeosporiodes* were selected to carry out for further works. Similar types of observation were reported by the reference identification of Lubbe *et al*. (2004) and Xie *et al*. (2010) and identified as *Colletotrichum gloeosporioides* (Penz.)

Strawberry leaves affected by *Colletotrichum gloeosporiodes* produced light brown, small scattered dots that gradually enlarged and coalesced to crate large, oval, circular, or irregular brown to black lesions with concentric rings. In severe infection, several lesions merged together enlarged quickly to the blighted appearance of the leaves.

The fungal pathogens were identified based on morphological, cultural and molecular characters. Further confirmation of the pathogen was assured by National Centre of Fungal Taxonomy (NCFT), New Delhi.

Cultural and morphological characters characteristics of one of the frequently occurring fungal pathogens isolated revealed that the colony color of the mycelium was whitish in color with profuse aerial mycelium on PDA medium. Reverse side of the culture in the Petri dish was found as light brown in color. Microscopic identification results showed that the colony was white to pink and dark gray, conidia were aseptate, hyaline and one-celled, oval shape with a blunt edge with length of 10.1 µm x 4.02 µm, hyphae was hyaline and septate, seta was straight, dark brown, with the size of 62-112 µm. The results showed similarity with the reference identification of Lubbe *et al*. (2004) and Xie *et al*. (2010) and identified as *Colletotrichum gloeosporioides* (Penz.) The identity of the fungus was also confirmed by the bioserve as *Colletotrichum gloeosporiodes*.

In the present investigation, results of the pathogenicity test of the isolated fungus *C. gloeosporiodes* causing diseases in the strawberry plant were proved by Koch's postulate under polyhouse condition. Pathogenecity test of the isolated fungus was tested by the Mycelial Bit Inoculation Technique (MBIT) (Rocha *et al.*, 1998). The inoculated leaves showed the initial symptoms 7 days after inoculation and the typical symptom of leaf spot appeared after 10 days of inoculation. The disease symptoms produced by *C. gloeosporiodes* on pathogenecity test are in agreement with the findings of Farhood and Hadian (2012) and nagrale *et al* (2012).

Five different botanicals at different concentration (5%, 10%, 15% and 20%) were tested singly against *C. gloeosporiodes* by poison food technique. All the botanicals significantly reduced the mycelial growth of the pathogen over control. Among the botanicals tested against *C. gloeosporiodes*, T2 (*Allium sativum*) resulted highest inhibition (86.66%) on mycelial growth of the pathogen which was followed

by T3 (*Allamanda cathartica)*, T4 (*Azadirachta indica)*, T5 (*Ocimum tenuiflorum*), T6 (*Lawsonia inermis)* with the mycelia growth inhibition of 77.22%, 76.33%, 75.44%, 67.44% respectively. The lowest inhibition on mycelial growth of the pathogen was recorded in T6 (*Lawsonia inermis*).

Five biocontrol agents tested against *C. gloeosporiodes* and among the all biocontrol agents tested, T2 (*T. harzianum*) was found to record highest inhibition (74.44%) on mycelial growth of *C. gloeosporiodes* followed by T1 (*Trichoderma viride*), T3 (*Bacillus subtillis*), T4 (*B. velezensis*) and T5 (*Pseudomonas fluorescence)* with the mycelia inhibition of 73.88%, 71.11%, 66.11% and 62.22% respectively. The lowest inhibition on mycelial growth (62.22%) of the pathogen was recorded in Pseudomonas fluorescence.

# SUMMARY

# AND

# CONCLUSION

# CHAPTER VI

# SUMMARY AND CONCLUSION

The findings of the present investigation entitled "**Study on fungal diseases of strawberry (*Fragaria ananassa*) in Jorhat, Assam"** are summarized below:

Random survey was conducted in district of Assam viz., Jorhat, to record the disease incidence (%) of fungal diseases of strawberry.

Four diseases of strawberry viz., Anthracnose (black spot), Common leaf spot (brown spot), wilt, grey mold were recorded. Based on the per cent disease incidence and per cent disease index of the diseases, frequently occurring disease Anthracnose (black spot) of strawberry with highest disease incidence and disease index were selected for further studies.

Based on the symptomatology, cultural, morphological and molecular characterization the isolated fungal pathogens were identified as *Colletotrichum gloeosporiodes,* which were further confirmed by the National Centre of Fungal Taxonomy, New Delhi.

Pathogenecity test was conducted by Mycelial Bit Inoculation Technique (MBIT) under in vitro condition and it was confirmed with

In vitro efficacy of different botanicals singly against C. gloeosporides at different concentration(5%, 10%, 15% and 20%) showed that *Allium sativum* inhibited highest mycelial growth of 86.66%

In vitro efficacy of five different biocontrol agents evaluated singly against *C. gloeosporiodes* employing dual culture technique revealed *Trichoderma harzianum* recorded highest mycelial growth inhibition of 74.44%.

# Conclusion

From the present study, it can be concluded that among the fungal diseases of strawberry studied, the incidence of Anthracnose (black spot) caused by *Colletotrichum gloeosporiodes* was found to be most common in the site Horticulture, Jorhat, Assam condition. *Trichoderma harzianum* was found to be most effective biocontrol agents and *Allium sativum* found to be most effective botanicals against *Colletotrichum gloeosporiodes*.

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# REFERRENCES


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