EFFECT OF DIFFERENT PLANT MATERIAL BASED CULTURE MEDIA ON GROWTH AND SPORULATION OF *COLLETOTRICHUM CAPSICI* ISOLATED FROM CHILLI

A THESIS

BY

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A Thesis (Course Code: 081108 PLP 6118) Submitted to Agrotechnology Discipline in Partial Fulfillment of the Requirements for the Degree of Master of Science in Plant Pathology

AGROTECHNOLOGY DISCIPLINE

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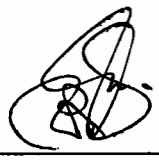

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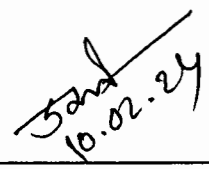

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DEDICATED TO

MY BELOVED PARENTS AND SISTERS

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ABSTRACT

Colletotrichum capsici, causing anthracnose disease of chilli, is a severe disease of chill crops that significantly reduces the commercial output of chilli production worldwide, especially in tropical and sub-tropical countries. Medium for the growth of fungi generally uses Potato Dextrose Agar (PDA) media. Still, it is necessary to find materials for alternative media from organic materials that are easy to obtain and provide better results than PDA. An attempt was made to find the best-supporting medium which may provide profuse mycelium growth and sporulation of *C. capsici*. Here, in this experiment, ten different plant material extracts along with potatoes were used as growth media. Ten different media were tested, among them, there was no significant difference in growth of *C. capsici* on Potato Dextrose Agar (PDA) and Mung Extract Agar (MuEA) media; while least growth was found on Green Chilli Extract Agar (GCEA) media. Sporulation on PDA and MuEA media also showed no significant difference among all media. On the other hand, least sporulation rate was found on Rice Extract Agar (REA) media. Moreover, the colony colour, texture, colony margin of mycelia and size of conidia were also examined. Though, all the formulated media supported the growth of fungi, the effects on the morphology of the fungi varied considerably. Therefore, Mung Extract Agar (MuEA) can be used for the culturing of *C. capsici* instead of Potato Dextrose Agar (PDA) media as the best alternative suitable media for the growth and sporulation of *C. capsici.*

Keywords: *Colletotrichum capsici*, *Capsicum annuum L,* media, growth characteristics.

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Chapter I

Introduction

Chilli (*Capsicum annum L.*) is among the key components of dishes in tropical and subtropical regions. The *Capsicum* genus comprises more than 30 species (Tripodi and Kumar, 2019). Among these, there are five major cultivated species viz., *Capsicum annuum, Capsicum chinense, Capsicum baccatum, Capsicum frutescens* and *Capsicum pubescens* (Thakur et al., 2021). "Carolina Reaper (*Capsicum chinense*)," known as the hottest cultivar, was developed by West Indies grower Ed Currie and can reach a peak spiciness (Saxena et al., 2016).

Chilli is part of the Solanaceae family. While it is generally cultivated as an annual vegetable, this species is a tropical perennial. Among the *Capsicum* genus, *C. annuum* holds the greatest economic importance. This plant, which is Indigenous to the Americas, is believed to have originated in either Northern or Central America and is commonly referred to by various names, such as bell pepper, paprika, chilli pepper, red pepper, sweet pepper, jalapeño, and cayenne. The fruits contain numerous phytochemicals, including vitamin C, vitamin A, flavonoids, anthocyanins, carotenoids, and capsaicinoids, which are the spicy components found in hot peppers (Sottosanti, 2024).

Chilli (*Rabi*) is grown on 188888.78 acres, yielding 507200.88 MT, while Chilli (*Kharif*) is cultivated over 51499.92 acres with a production of 155346.03 MT. Chilli (*Rabi*) is cultivated throughout Bangladesh, and Chilli (*Kharif*) is also grown all across the country, except in Barishal District (BBS 2023). The leading ten chilli-producing countries are China (mainland), Mexico, Indonesia, Turkiye, Spain, Nigeria, Egypt, the United States of America, Algeria, and the Netherlands (FAOSTAT, 2024).

Chilli anthracnose is the most prevalent disease in tropical and subtropical regions, resulting from various species of *Colletotrichum*. Research has identified 24 different *Colletotrichum* species responsible for anthracnose in chilli, documented in numerous countries such as Australia, Brazil, China, India, Indonesia, Korea, Malaysia, Sri Lanka, Thailand, and the USA (De Silva et al., 2019). Among these, *Colltotrichum*

capsici infects both ripe and unripe chilli fruits in the Indian subcontinent (Saxena et al., 2014).

Anthracnose represents a significant economic challenge for chilli cultivation (Garg et al., 2014). Crop diseases like fruit rot or anthracnose, which contribute significantly to crop losses, have been cited as a reason for the decline in chilli production. These diseases inflict serious harm to mature fruits both in the field and during optimal storage conditions, leading to greater yield losses and a reduction in overall production (Saxena et al., 2016).

Warm and humid conditions foster the proliferation of the disease. The seriousness of the disease can result in considerable yield reductions, with some areas reporting losses as high as 89% (Kavya et al., 2024 and Jojy et al., 2024). Among the ten most infamous pathogens worldwide, *Colletotrichum spp.* lead to considerable crop damage globally. The pathogen responsible for chilli anthracnose disease is *C. capsici*, which is classified in the Kingdom-Fungi, Phylum-Ascomycota, Class-Sordariomycetes, Order-Phyllachorales, and Family-Phyllachoraceae (Oo and Oh, 2016). The species belonging to this genus are known to induce anthracnose disease in over 121 genera of plants across 45 distinct plant families. In recent decades, the classification of this group has undergone significant changes (Talhinhas and Baroncelli, 2021). A total of 260 *Colletotrichum* isolates were collected from chilli-growing areas in Indonesia, Malaysia, Sri Lanka, Thailand, and Taiwan. These isolates were associated with necrotic lesions found on chilli leaves and fruits (De Silva et al., 2019).

Anthracnose in chilli fruits, resulting from the fungus *C. capsici*, is typically identified by dark spots and sunken areas of dead tissue featuring concentric rings of acervuli (Kiran et al., 2020). *C. capcisi* is the most adhesive, attaching firmly to the plant surface and remaining dormant until physiological changes in the fruit take place, leading to economic losses for farmers due to reduced fruit quality and marketability. Numerous post-harvest fruit diseases display the characteristic of quiescence, where symptoms only appear as the fruit ripens (Masoodi et al., 2012).

Traditionally, identifying and characterizing *Colletotrichum* species has largely depended on morphological traits including the colour of colonies, dimensions and

shape of conidia, ideal growth temperature, growth rate, and the occurrence of setae. To determine the cultural and morphological traits of any fungal type, it is essential to cultivate them in culture media.

Culture media play a crucial role in microbiology by providing a nutrient-dense environment conducive to the growth and examination of microorganisms. These media consist of a variety of chemical and biological components that facilitate the growth, isolation, and identification of bacteria, fungi, and viruses. The efficacy of culture media is affected by their formulation, which should contain sources of carbon, nitrogen, and other vital elements (Susanti et al., 2022).

In a laboratory setting, Potato Dextrose Agar (PDA) is frequently used as a substrate for cultivating fungi. PDA medium can support the sporulation of various fungal species and features a straightforward formulation. Carbohydrates serve as the energy source that microbes utilize to grow and thrive. Additionally, the presence of protein in the medium is essential for the production of apical hyphal spores (Syamsia et al., 2021).

The objective of this study is to evaluate the growth and sporulation of *Colletotrichum capsici* on different plant materials culture media.

Chapter II

Review of Literature

This chapter presents a review of the literature that is significant to the research, provides context within the research field, identifies research gaps, and establishes the aims and objectives of this study. Many research has been conducted on anthracnose of chilli caused by *Colletotrichum capsici*.

Kavya et al. (2024) noted a considerable occurrence of chilli anthracnose induced by *C. capsici*, recognized by sunken necrotic areas exhibiting concentric rings of acervuli, which indicates the presence of the pathogen.

Jojy et al. (2024) conducted research on the cultural, morphological, and pathogenic differences among *C. capsici* isolates responsible for anthracnose and fruit rot in chilli (*C. annuum L.*). The research also observed a noteworthy occurrence of anthracnose, with symptoms including leaf spots, die-back, and fruit rot.

Rahman et al. (2011) observed that the initial signs of chilli anthracnose on the fruit manifested as water-soaked spots that became soft, slightly indented, and shifted to a tan hue. The lesions could cover a large portion of the fruit's surface, with multiple lesions present. Several black specks appeared on the lesions, which are the asexual fruiting structures known as acervuli. Acervuli were accompanied by numerous setae. The setae were mainly pointed, rarely blunt, elongated, either straight or slightly curved, unbranched, smooth, and dark brown in colour. Conidiophores were short, bearing fusiform and falcate conidia. The conidia were hyaline, with one or both ends curved and pointed, showing either a sudden or gradual taper at both ends. A whitish-grey colony developed on Potato Dextrose Agar (PDA) medium after culturing *C. capsici*.

Joshi et al. (2024) carried out a study on twelve different culture media to determine the most effective one for the growth and development of *C. capsici*, the fungus that causes anthracnose in chillies. Among the twelve media evaluated, *C. capsici* showed the highest growth on Red Chilli Dextrose Agar (90.0 mm), closely followed by Carrot Dextrose Agar and Potato Carrot Agar (89.50 mm). In contrast, the least

amount of growth was observed on Green Chilli Extract Agar (33.50 mm), with Red Chilli Extract Agar (48.00 mm) and Sarbouraud's Agar (48.50 mm) close behind. The media tested included a variety of cultural differences. Red Chilli Dextrose Agar created the most conducive environment for the growth and sporulation of *C. capsici*, resulting in colonies that were greyish-black on the surface with black pigmentation on the underside. Likewise, both Carrot Dextrose Agar and Potato Carrot Agar produced greyish-black surface colonies with black pigmentation on the reverse side. Potato Dextrose Agar (PDA) showed greyish-white surface colonies with black reverse pigmentation. Conversely, Green Chili Extract Agar yielded sparse, creamy-white surface colonies with creamy-black pigmentation on the reverse side. Thus, media derived from red chilli, carrot, and potato carrot were the most effective, while green chilli extract agar was the least suitable.

Falke et al. (2023) performed an experiment to identify the ideal culture media for the growth of the pathogen *C. capsici*. They tested various culture media, which included Richard's agar, Oat Meal Agar, Potato Dextrose Agar, Czapek's Dox Agar, Maize Meal Agar, Yeast Extract Agar, and Yeast Mannitol Agar. In vitro studies were conducted on the radial mycelial growth of *C. capsici* in all tested media. The pathogen was capable of growing in every medium assessed. Among the seven culture media evaluated, Potato Dextrose Agar (PDA) proved to be the most effective, promoting the highest radial mycelial growth at 89.21 mm. Richard's agar (68.45 mm) and Oat Meal Agar (81.75 mm) were also identified as the next most conducive to radial mycelial development.

Al-Khafaji et al. (2024) discovered that when *C. capsici* is grown in media with L-tyrosine, it has the capability to generate melanin, suggesting a potential for pigmentation. The synthesis of melanin, a typical pigment found in fungi, can be affected by the culture medium's composition and the availability of precursors such as L-tyrosine, which has been proven to boost melanin production in pathogenic fungi. This research supports the idea that *C. capsici* can produce pigments in its culture media when certain conditions are met.

Akhtar et al. (2018) experimented to assess the radial expansion of *C. capsici* on five types of media: Potato Dextrose Agar (PDA), Yeast Extract Potato Dextrose Agar (YEPDA), Malt Extract Agar (MEA), Wheat Grain Extract Agar (WEA), and Chilli

Extract Agar (CEA). Among these media, PDA exhibited the quickest growth, achieving complete plate coverage (9 cm) within 9 days. This was closely followed by YEPDA, which took 10 days, CEA at 11 days, WEA at 12 days, and MEA at 13 days. Malt Extract Agar was determined to be the least favourable medium for the mycelial growth of *C. capsici*. Growth of *C. capsici* progressed rapidly in the PDA medium, reaching a full growth of 9 cm in nine days, succeeded by the CEA medium.

Jojy et al. (2024) found that isolates of *C. capsici* displayed growth between 7.20 and 8.60 cm after seven days, characterized by a thinner mycelium and spores that resembled a sickle shape.

Gamit et al. (2023) reviewed the formulation of alternative culture media for microorganisms, highlighting the significance of these media in promoting microbial growth and metabolism while addressing the high costs associated with traditional synthetic media components. The paper discussed various plant-based culture media formulations that can be utilized for microbial growth, including those suitable for fungi like Alternaria. They emphasized on the use of horticultural ingredients and vegetable substrates such as soy, certain beans, maize, and rice as alternative culture media. These formulations had shown satisfactory results in terms of microbial growth efficiency and cost-effectiveness compared to traditional synthetic media, making them viable options for cultivating *Alternaria* and other microorganisms.

Khadka et al. (2023) studied to evaluate eight different culture media to determine their effectiveness in promoting the growth of the pathogen *Alternaria brassicola,* which causes Alternaria leaf spot in cabbage. The experiment was conducted under aseptic conditions for 12 days, with Potato Dextrose Agar yielding the highest radial growth of 88.90mm, followed closely by Malt Extract Agar at 87.45mm. The results indicated varying colony characteristics across the different media, with Carrot Agar showing the lowest growth at 53.12mm. The findings aim to provide insights for future research on the physiology, taxonomy, and management of disease pathogens affecting cabbage.

Prajapati et al. (2020) investigated the cultural and morphological characteristics of *C. capsici*, the pathogen that causes anthracnose in chilli plants. The study identified Oat Meal Agar (OMA) as the most favourable medium for achieving maximum radial growth of *C. capsici*, measuring 90.00 mm, followed closely by Chickpea Flour

Dextrose Agar (CFDA) with a measurement of 88.67 mm. In contrast, Rice Straw Agar (RSA) exhibited the lowest growth, measuring only 69.34 mm. The colony morphology of *C. capsici* grown on OMA, Potato Dextrose Agar (PDA), Corn Dextrose Agar (CDA), Rice Straw Agar (RSA), and Malt Extract Agar (MEA) showed a greyish to white colouration with a fluffy texture and smooth edges. In comparison, colonies cultivated on CFDA displayed colours varying from brown to black, with sparse and thin mycelium. Microscopic examination at a magnification of 40X showed that the mycelium was both dense and filamentous, with septate structures.

Ismail (2023) studied on the effects of culture medium and light durations on the fungal growth of *Bipolaris sorokiniana.* This study investigated that the optimal culture medium and light duration for the growth of *Bipolaris sorokiniana*, a potential bioherbicide for controlling goosegrass in oil palm plantations. Four culture media (PDA, CMA, V8A, and WA) and three light durations (0, 12, and 24 hours) were tested, revealing that PDA was the most effective medium, particularly at 12 hours of light, while the highest colony-forming units (CFU) were produced in the absence of light. Findings indicate that B. sorokiniana can thrive in PDA without light, producing significantly more culturable living cells compared to light-exposed conditions. This suggests its potential for mass production as a bioherbicide, which could reduce reliance on chemical herbicides in agriculture, particularly in oil palm plantations.

Sangdee et al. (2020) studied the morphological, pathological and molecular variability of *C. capsici* causing anthracnose of chilli. They tested ten isolates and found that isolates produced cottony colonies on PDA with a colour of grayish-white to dark grey on the ventral surface. In contrast, the reverse of the colonies was mainly black. The colony diameter ranged from 65 to 80 mm after 7 days of incubation. The colonies produced zigzag cottony to circular colonies. The conidia shape was fusiform with both their ends pointed. The average length and width of conidia varied between 23.5 to 35.0 µm and 2.5 to 3.75 µm, respectively.

Santos et al. (2021) reviewed various formulations of alternative, low-cost culture media derived from products of plant origin and waste from horticulture, highlighting their potential for microbial growth and production of industrially relevant microbial compounds. They emphasized that these alternative media, which utilized vegetable

substrates like soy, beans, corn, and rice, often yield satisfactory results in microbial growth efficiency while being more cost-effective compared to conventional culture media.

Syamsia et al. (2021) performed a study to demonstrate the potential of utilizing organic materials derived from food crops as substrates for endophytic fungi, highlighting their efficiency and cost-effectiveness in fungal cultivation. All three varieties of alternative media - mung bean, corn, and rice—successfully facilitated the growth of six endophytic fungal isolates. The development seen in Mung Bean and Rice media was nearly equivalent to that of the control medium. Potato Dextrose Agar (PDA). Of the media evaluated, Corn contained the highest carbohydrate levels but the lowest protein levels. This difference in nutrient composition had an impact on the growth characteristics of the fungi, including colony size and surface texture. This implies that the nutrient makeup of the media plays a crucial role not just in the growth rate but also in the reproductive success of the fungi.

Vithal et al. (2020) investigated morphological traits including radial mycelial growth, conidial features, setae, and acervuli on Potato Dextrose Agar (PDA). The colonies displayed concentric rings and a light black hue. The highest recorded radial mycelial growth was 82.42 mm, while the lowest was 74.28 mm at 7 days after inoculation (DAI).

Dominguez et al. (2020) studied to evaluate the growth and sporulation of two fungal pathogens, *Bipolaris sorokiniana* and *Drechslera teres*, in various culture media to identify conditions that maximize their vegetative growth and sporulation. The research highlights the importance of selecting appropriate culture media for conducting fungicide sensitivity assays and addresses the scarcity of information on media that enhance vegetative growth. Their result indicated that *B. sorokiniana* exhibited higher growth rates in media such as Rice Agar (RA), Potato Carrot Agar (PCA), and Vegetable Juice Agar (VJA), while *D. teres* showed optimal growth in Commercial Potato Glucose Agar (GPA). The study also noted variations in colony characteristics and sporulation across different media, emphasizing the influence of medium composition on fungal development.

V et al. (2019) reviewed a paper on production of alternative culture medium by using different plant extracts, vegetables and fruit juices. The paper discussed the production of alternative culture media for microbial growth using various plant extracts, vegetables, and fruit juices, highlighting their nutritional benefits and effectiveness in supporting microbial development. It emphasized the use of ingredients such as beetroot, carrot, rye, tomato, soya bean, and natural fruit juices like V8 and V6, which had shown positive results in promoting microbial growth. It also addressed the cost-effectiveness of using kitchen waste and locally available plant materials as alternatives to commercially developed media. The study presented various formulations and their efficacy in supporting the growth of different microorganisms, suggesting that these alternative media can serve as viable substitutes for traditional nutrient agar and broth in laboratory settings.

Gholve et al. (2017) studied to evaluated the growth of Alternaria carthami across eight different culture media. Among the eight culture media tested for the growth of Alternaria carthami, the potato dextrose agar yielded the highest growth at 90.00 mm, followed by potato malt agar at 84.16 mm and yeast mannitol agar at 73.33 mm. These results indicate that potato dextrose agar is the most effective medium for culturing Alternaria carthami, while potato malt agar and yeast mannitol agar can serve as alternative options for growth.

Kumar and Devi (2016) investigated the morphological and cultural traits of *C. capsici*, the pathogen causing blight in Chickpeas in Andhra Pradesh, India. One observed morphological characteristic was the development of white mycelium that shifted from light grey to dark grey over time. Maximum growth of the colony was recorded ten days after incubation, after which growth ceased. The culture exhibited either growing in a fluffy manner above the surface or in a submerged, felt-like form. Incubating on PDA medium for 15 days led to substantial sporulation, exceeding >30 x 10^4 conidia/ml. The developmental patterns of the colonies were diverse and ranged from pale grey to dark grey.

Kumar et al. (2015) observed that various isolates of *C. capsici* showed differences in their radial growth, with the most significant growth recorded in PDA media (77.16 mm) as opposed to Oat Meal Agar (OMA) media (86.67 mm); the average colony diameter was 7.5 mm in PDA and 2.34 mm in OMA. The colour of the colonies

ranged from grey to dark grey or graphite grey in PDA media, whereas they appeared white in OMA. Both media PDA and OMA displayed zonation and concentric rings on the reverse side of the colonies. All isolates were capable of sporulation on both PDA and OMA media; in PDA, the colony margins were irregular with a fluffy mycelium, while some isolates exhibited regular margins on OMA media.

Raj et al. (2014) studied on morphological, pathogenic and genetic variability in *Colletotrichum capsici* causing fruit rot of chilli. They examined on twenty isolates. In culture media, most of the isolates produced cottony, fluffy or suppressed colonies and no significant differences were noticed in shape and size of conidia.

Christopher et al. (2013) conducted an experiment involving 20 isolates of *C. capsici*, discovering that the largest colony diameter on PDA medium reached 9.0 cm. The isolates exhibited significant variation in both colony colour and growth patterns when grown on PDA. They formed fluffy, elevated colonies.

Das et al. (2012) aimed to assess the mycelial growth of *C. capsici* on four different media: Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Sabouraud Dextrose Agar (SDA), and Peptone Yeast Extract Dextrose Agar (PYDA). Among these, PDA promoted the most extensive growth, resulting in blackish-white colonies, while CDA also facilitated substantial growth with pale-white colonies. PDA and CDA were identified as the most effective media, with PDA demonstrating the best overall performance.

Masoodi et al. (2012) found that isolates of the *C. capsici* pathogen displayed a range of colony characteristics when grown on Potato Dextrose Agar (PDA) medium. The colonies showed various colours, including white, off-white, and grey, eventually turning brown or black, with differing margins and concentric patterns.

Roy et al. (2012) examined the growth of *C. capsici* in four distinct culture media: Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Sabouraud Dextrose Agar (SDA), and Peptone Yeast Extract Dextrose Agar (PYDA). Of the media evaluated, PDA was identified as significantly supporting the highest growth of the pathogen in comparison to the other media.

Sangdee et al. (2011) provided an analysis of *C. capsici* isolates, focusing on features such as colony colouration, diameter, and the shape and size of conidia. After a seven-day incubation period, the diameters of the colonies varied from 65 to 80 mm, displaying colours that ranged from greyish-white to dark grey. On Potato Dextrose Agar (PDA), several isolates formed cottony colonies, with a ventral surface colour spanning from greyish-white to dark grey, while the reverse side of the colonies was mostly black. The conidia from the different isolates were fusiform in shape, tapering to a point at both ends.

Singh (2007) investigated the growth rate of *C. capsici* on four distinct types of growth media: Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Sabouraud Dextrose Agar (SDA), and Peptone Yeast Extract Dextrose Agar (PYDA). *C. capsici* produced colonies coloured blackish white on PDA and dark white on CDA, SDA, and PYDA media. The most notable growth was recorded on PDA and CDA based on average colony diameter values, while no significant growth was noted on either SDA or PYDA plates.

Damiri (2007) indicated that *C. capsici* can grow well in various media, including those supplemented with plant extracts, which may also enhance pigment production. Plant extracts present in the media can inhibit fungal growth while potentially affecting the characteristics of the pigments.

Akhtar et al. (2007) studied on the variability in *Colletotrichum capsici* causing chilli anthracnose. They found differences in colony characters such as growth, shape, margin, color, texture and zonation. The radial growth of the isolates on culture media differed significantly from each other. The size, shape, septation and pigmentation of conidia of five isolates grown on five culture media, differed significantly from each other. On PDA media colony shape was circular to irregular; Margin was smooth to wavy; Color was greyish to blackish; Texture was thick. Conidia length was found 25.27-26.15µm and width 3.17-3.55 µm.

Sharma et al. (2005) examined thirty-seven isolates of *C. capsici*, which they classified into five distinct types based on colony features such as pigmentation, growth patterns, and size, thereby uncovering morphological and acervuli development variations. The colonies exhibited a variety of textures, ranging from cottony to fluffy, and displayed colours from gray to black, measuring between 74

and 85 mm after a ten-day growth period. Some groups showed distinctive colony shapes, like zigzag or ringed patterns, while others appeared more circular with zonation.

Chapter III

Materials and Methods

The current study was performed in the Plant Protection Laboratory of the Agrotechnology Discipline at Khulna University, Khulna.

3.1 Collection of Disease Samples

Samples of chilli affected by anthracnose symptoms were gathered from the local market.

3.2 Cleaning and Sterilization of Glassware

The glassware used in this laboratory procedure (including Petri dishes, conical flasks, watch glasses, culture tubes, beakers, pipettes, etc.) was thoroughly cleaned with detergent and left to air dry. Petri dishes, flasks, and test tubes were sterilized in a hot air oven set to 170°C for 30 minutes, as described by Alkadhim (2018).

3.3 Surface Sterilization

Initially, hands were disinfected using absolute alcohol, specifically ethanol. The inoculating needle, forceps, razor, and the laminar flow surface where the inoculation took place were all treated with absolute alcohol for sterilization. Small 2-3 mm pieces of diseased leaf, with approximately half being healthy, were sterilized by soaking them in a 0.1 percent mercuric chloride (HgCl2) solution for 30 seconds, followed by three washes in distilled water to eliminate saprophytic microorganisms adhering to the diseased samples. To achieve optimal results, extra care was taken to limit the immersion time in the mercuric chloride solution, preventing the destruction of any microorganisms that might be surviving intracellularly or intercellularly for isolation. The inoculating needle was also sterilized by dipping it in absolute alcohol and then flaming it using a spirit lamp until it reached a bright red color. This process was repeated two to three times to ensure that all microorganisms were completely eradicated.

The chamber equipped with the laminar flow was sealed, and UV light was activated for 30 minutes to sterilize the entire area. Prior to this, a pre-filter and an absolute filter were operated for 10 minutes to remove dust and dirt particles. As a precaution during the inoculation, the UV light was switched off before entering the room.

3.4 Isolation of Diseased Parts

A small segment (3-5 mm) from the infected fruit was obtained using a surface-sterilized razor, which had been rinsed in 70% ethanol for 1 minute and 1% NaClO for another minute, followed by three washes with distilled water prior to inoculating onto the media. The inoculation was performed near a spirit lamp flame to reduce the risk of contamination. The samples were then dried in an aseptic environment using filter paper and subsequently placed onto the solid PDA medium that had solidified. The plates were incubated at 27±°C for 2-3 days until mycelial growth began to develop on and around the piece of diseased tissue.

3.5 Identification of Pathogen

On the fourth day, mycelial growth was noted, appearing greyish-white in the Petri dishes with media. A small section was taken from the eight-day-old colony and placed on a slide, which was prepared using cotton blue and lactophenol. The slide was examined under a microscope for pathogen identification, which was verified by consulting pertinent literature and monographs (Singh, 2009). Various structures of the pathogen, such as mycelium and conidia, were also observed.

3.6 Preparation of plant material based media

Ten distinct types of solid media were selected for examination. The ingredients for creating these media, including potato, bean, carrot, green chili, masoor, mung, red dry chili, rice, sweet pumpkin, taro, and tomato, were acquired from the local market.

3.6.1 Potato Dextrose Agar (PDA)

The potatoes were cleaned to eliminate any dirt. After washing, the potatoes were peeled and chopped into small pieces. One hundred grams of the chopped potatoes were placed in an Erlenmeyer flask with 500 ml of distilled water and then cooked in a pressure cooker. The cooker was set to release steam three times to ensure boiling. Following the boiling, the potatoes were filtered and transferred to an Erlenmeyer flask, where 10 g of agar, 10 g of dextrose, and distilled water were added until reaching a final volume of 500 ml. The mixture was stirred on a hot plate for 20-25 minutes until it became homogeneous and boiled. This solution was then sterilized in an autoclave at 121° C for 15 minutes at a pressure of 2 atm. It was subsequently poured into sterile petri dishes under a laminar airflow hood. Once cooled, the dishes were covered with plastic wrap. The medium was prepared to serve as a control for this investigation.

3.6.2 Mung Extract Agar (MuEA)

Mung beans were rinsed and allowed to drain. One hundred grams of mung beans were placed in an Erlenmeyer flask with 500 ml of distilled water and then cooked in a pressure cooker. Steam was released three times to achieve boiling. After boiling, the mung was filtered and placed in an Erlenmeyer flask, followed by the addition of 10 g of agar and distilled water to fill a total volume of 500 ml. The mixture was stirred on a hot plate for 20-25 minutes until it became homogeneous and boiled. The final solution was sterilized in an autoclave at 121° C for 15 minutes at a pressure of 2 atm.

3.6.3 Masoor Extract Agar (MaEA)

Masoor was rinsed and drained. One hundred grams of masoor were placed in an Erlenmeyer flask with 500 ml of distilled water and subsequently cooked in a pressure cooker. The cooker was set to release steam three times to ensure boiling. The boiled masoor was filtered and transferred to an Erlenmeyer flask, and then 10 g of agar was added along with distilled water to a total volume of 500 ml. The mixture was stirred for 20-25 minutes on a hot plate until it became homogeneous and boiled. The solution was sterilized by autoclaving at 121° C for 15 minutes at a pressure of 2 atm.

3.6.4 Carrot Extract Agar (CEA)

The carrots were washed to remove any dirt. After washing, the carrots were peeled, chopped into small pieces, and then rinsed and drained. One hundred grams of the diced carrots were placed in an Erlenmeyer flask with 500 ml of distilled water and cooked in a pressure cooker. Steam was released three times to ensure boiling. The boiled carrots were filtered into an Erlenmeyer flask, and 10 g of agar was added along with distilled water to achieve a total volume of 500 ml. The mixture was stirred on a hot plate for 20-25 minutes until homogeneous and boiled. The solution was then sterilized in an autoclave at 121° C for 15 minutes at a pressure of 2 atm.

3.6.5 Tomato Extract Agar (ToEA)

The tomatoes were rinsed and drained before being chopped into small pieces. One hundred grams of diced tomatoes were placed in an Erlenmeyer flask with 500 ml of distilled water and then cooked in a pressure cooker. Two whistles were given in the pressure cooker to ensure boiling. The boiled tomatoes were filtered and placed in an Erlenmeyer flask, to which 10 g of agar and distilled water were added to reach a final volume of 500 ml. The mixture was stirred on a hot plate for 20-25 minutes until it

became homogeneous and boiled. The solution was sterilized by autoclaving at 121° C for 15 minutes at a pressure of 2 atm.

3.6.6 Taro Extract Agar (TaEA)

Remove the taro skin and chop it into small pieces, then rinse and drain the pieces. 100 grams of the finely chopped taro were placed in an Erlenmeyer flask and mixed with 500 ml of distilled water before being cooked in a pressure cooker. The cooker was allowed to whistle twice to ensure boiling. The cooked taro was then filtered, transferred to another Erlenmeyer flask, to which 10 g of agar was added, along with distilled water to achieve a total volume of 500 ml. The mixture was stirred on a hot plate for 20-25 minutes until it became homogeneous and heated until boiling. The resulting solution was sterilized by autoclaving at 121° C for 15 minutes at a pressure of 2 atm.

3.6.7 Rice Extract Agar (REA)

The rice was rinsed and drained. 100 grams of rice were added to an Erlenmeyer flask and combined with 500 ml of distilled water, then cooked in a pressure cooker. The cooker was allowed to whistle four times to facilitate boiling. The boiled rice was filtered and transferred to an Erlenmeyer flask, to which 10 g of agar was added along with distilled water to a total volume of 500 ml. The mixture was stirred on a hot plate for 20-25 minutes until it was homogeneous and heated until boiling. The solution was sterilized by autoclaving at 121° C for 15 minutes at a pressure of 2 atm.

3.6.8 Sweet Pumpkin Extract Agar (SPEA)

The outer skin and inner flesh of the sweet pumpkin were discarded and cut into small pieces, then rinsed and drained. 100 grams of the diced sweet pumpkin were placed in an Erlenmeyer flask with 500 ml of distilled water and cooked in a pressure cooker. The cooker was allowed to whistle twice to achieve boiling. The boiled pumpkin was filtered and transferred to an Erlenmeyer flask, where 10 g of agar was added along with distilled water to make the total volume 500 ml. The dough was stirred for 20-25 minutes on a hot plate until it became homogeneous and cooked until it boiled. The solution was sterilized by autoclaving at 121° C for 15 minutes at a pressure of 2 atm.

3.6.9 Red Dry Chilli Extract Agar (RDCEA)

The stems of the red dry chilies were removed. 100 grams of red dry chilies were placed in an Erlenmeyer flask, combined with 500 ml of distilled water, and cooked in a pressure cooker. The cooker was allowed to whistle six times to boil the contents. The extracts were then filtered and transferred to an Erlenmeyer flask, where 10 g of

agar was added along with distilled water to achieve a total volume of 500 ml. The dough was stirred for 20-25 minutes on a hot plate until homogenized and heated until boiling. The solution was sterilized by autoclaving at 121° C for 15 minutes at a pressure of 2 atm.

3.6.10 Green Chilli Extract Agar (GCEA)

Green chilies were rinsed and drained before being chopped into small pieces. 100 grams of the finely chopped green chilies were placed in an Erlenmeyer flask with 500 ml of distilled water and cooked in a pressure cooker. The cooker was allowed to whistle three times to ensure boiling. The boiled mixture was filtered and transferred to an Erlenmeyer flask, where 10 g of agar was added with distilled water to reach a total volume of 500 ml. The dough was stirred on a hot plate for 20-25 minutes until it became homogeneous and heated until boiling. The solution was sterilized by autoclaving at 121° C for 15 minutes at a pressure of 2 atm.

PDA media was utilized for isolating, growing, and maintaining the pathogen (*C. capsici*). The media solutions from all preparations were poured into conical flasks, which were then plugged with non-absorbent cotton in preparation for media plates. The conical flasks were sterilized in an autoclave at 15 lbs/sq. inch for 15 minutes. After autoclaving, the flasks containing the media were kept in a laminar flow hood until warm for media plate preparation; approximately 15-20 ml of autoclaved media (in the melted state) was poured into sterilized Petri plates under aseptic conditions and left to solidify. The medium was then ready for use in this study.

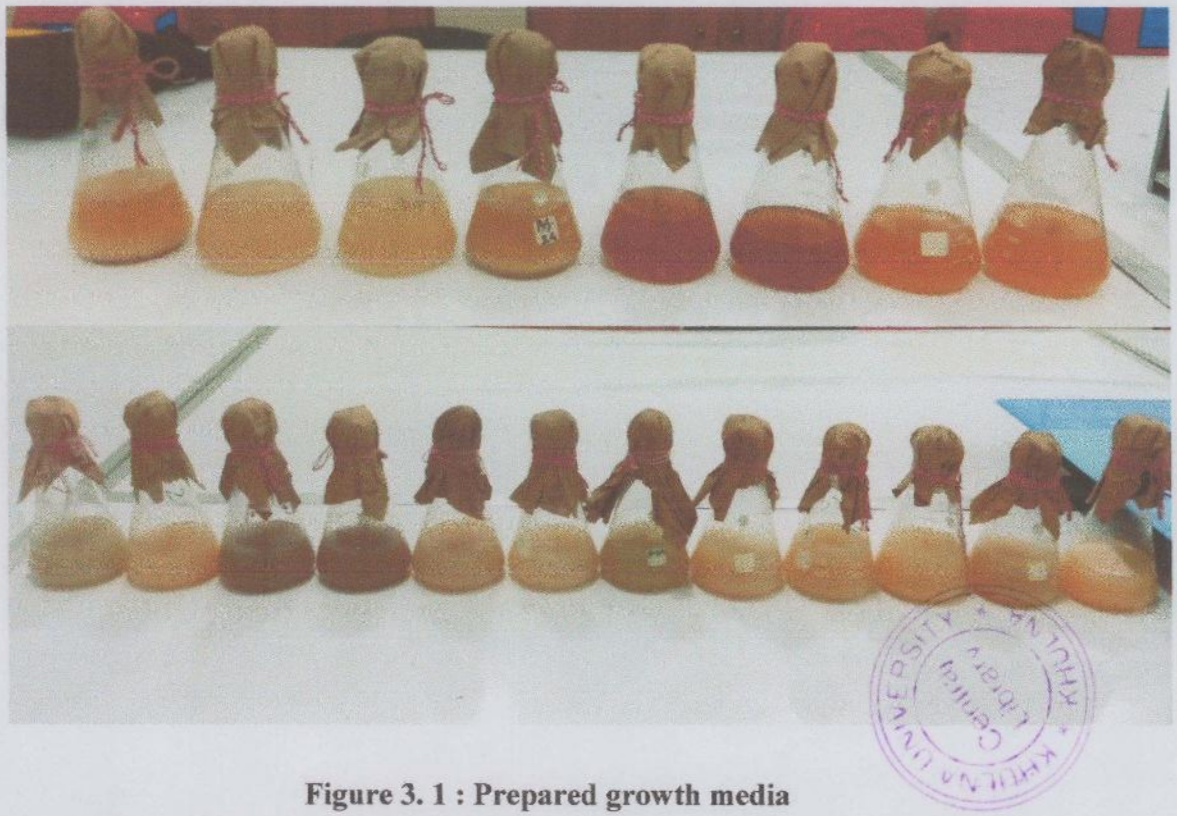

Figure 3. 1 : Prepared growth media

Figure 3. 2 : Prepared petriplate for inocultion of isolated *C. Cpasici*

3.7 Inoculation of the Petri Plates Containing Media

The pure culture of seven days old fungus was so obtained on PDA and was used for inoculation of Petri plates. A mycelial disc of 5 mm diameter was scooped out with a sterilized cork borer from the advancing margin of the colony and transferred at the centre of the Petri plates aseptically. Plates were incubated at 28°C within the growth chamber for 15 days.

3.8 Fungus Growth Measurement Technique

Radial growth of the fungus was determined directly by measuring the diameter of colonies from the underside of the culture plate against light in the perpendicular axis at 24hrs intervals up to the 9^{th} day of incubation. The radial growth was measured using slide callipers.

3.9 Observation of growth characteristics

The colour, margin and topography of the colony were observed by the naked eye.

3.10 Sporulation

To assess sporulation on different media, the culture plates were thoroughly washed with 20 ml of sterile distilled water to create a spore suspension. This suspension was filtered through filter paper to eliminate the mycelium. Following that, 3 drops of twin and 4 drops of lactophenol blue were added. A micro pipette was used to take 20µl of the suspension and place it on a slide. Finally, the number of spores was counted under a microscope at 40X magnification.

3.11 Size of conidia

For measuring the size of conidia, image of conidia was taken with microscope at 40X magnification and measured with ImageJ software.

3.12 Expeimental design and data analysis

The experiment was laide out in Completely Randomized Design (CRD) with four replication. The statistical analysis of collected data was carried out by using of Statistix 10 computer program. Tukey's HSD tests at 5% significance level were conducted to detect differences among treatments.

Chapter IV

Results and Discussion

4.1 Disease Identification

4.1.1 Symptological identification of *C. capsici* on chilli fruit

Spots on chilli fruit were dark sunken lesions with concentric rings with dirty white conidial masses on decaying tissues. The presence of black acervuli, which are fruiting bodies of the fungus, was observed on the surface of affected fruits as shown in Figure 4.1.

The result agreed with Kavya et al. (2024), who reported that infected fruits showed sunken necrotic tissues featuring concentric rings of acervuli. The result also agreed with Sawant et al. (2023), who reported that Symptoms of *C. capsici* on chilli fruit include dark, sunken lesions that may expand and lead to fruit rot.

4.1.2 Morphological identification of *C. capsici* on chilli fruit

Cultures were examined for fungal growth and identified based on cultural and microscopic characteristics. The mycelium was hyaline. Conidia were sickle-shaped and hyaline with one or both ends curved and pointed as well as sudden or gradual tapering towards both ends. The asexual fruiting bodies/structures called acervuli were present with abundant setae. Brownish black rounded acervuli, and long needle-like setae. Conidia are hyaline, single-celled, sickle-shaped. The setae were mostly pointed, elongate, straight or slightly curved, aseptate, smooth and dark brown (Figure 4.2).

The morphological features of the causal organism including seate, conidiophores and conidia were similar to the findings of Rahman et al. (2011) and Azad et al. (2005).

Figure 4.1 : Symptological characteristics of *C. capsici*
A. Initial symptom, B-C, severe symptom on mature fruit

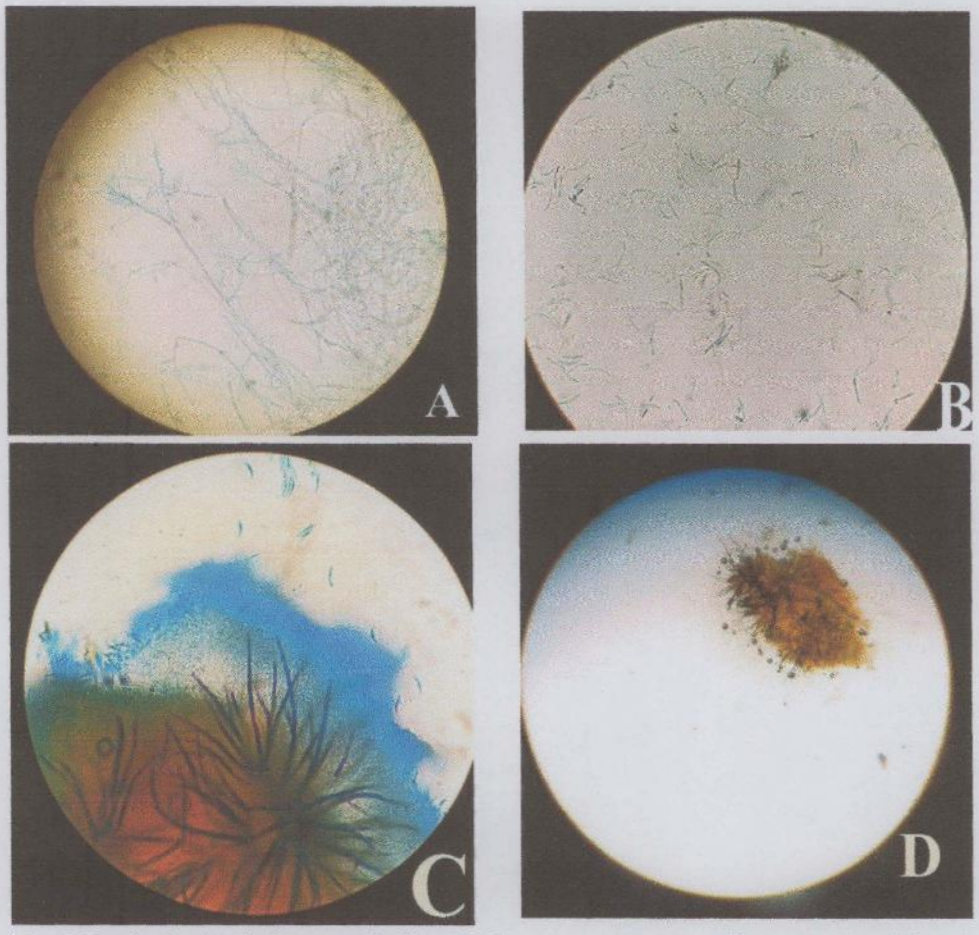

Figure 4.2 : Morphological characteristics of *C. capsici* A. Mycelium, B. Conidia, C. Setae, D. Acervuli

4.2 Radial mycelial growth of *C. capsici* on different plant materials extract based culture media

The radial mycelial growth rate of *C. capsici* was significantly influenced by various plant material-based culture media. The observations and results recorded are detailed in Table 4.1.

Table 4.1: Evaluation of different culture media for radial growth of *C. capsici*

SL No.	Media	Radial Growth Per Day						
		3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	9th Day
1	PDA	21.07a	31.23a	41.59b	50.16b	59.39b	70.49a	82.79a
2	MuEA	18.52b	28.17bc	44.85a	54.51a	63.70a	71.04a	79.93ab
3	MaEA	17.24c	24.60d	33.71e	41.18d	49.91d	58.89c	72.76d
4	CEA	20.72a	29.67ab	38.70cd	47.23c	58.83b	69.07ab	78.12bc
5	ToEA	14.86d	21.83e	28.43g	35.78f	42.39e	49.36e	55.14f
6	TaEA	17.37c	26.83c	37.46cd	52.75a	59.65b	67.29b	75.37cd
7	REA	14.72d	23.05de	31.88ef	38.60e	46.83d	53.53d	60.29e
8	SPEA	17.55bc	27.03c	37.96cd	42.48d	49.10c	55.12d	61.26e
9	RDCEA	15.19d	23.30de	30.96f	38.66e	45.39d	52.65d	59.98e
10	GCEA	14.38d	17.95f	24.10h	30.69g	36.74f	43.65f	50.65g
CV		3.94	4.57	3.92	3.71	2.92	3.01	2.99

Values with different letters are significantly differed at 5% level of significance according to Tukey's HSD test

PDA= Potato Dextrose Agar, MuEA= Mung Extract Agar, MaEA= Masoor Extract Agar, CEA= Carrot Extract Agar, ToEA= Tomato Extract Agar, TaEA= Taro Extract Agar, REA= Rice Extract Agar, SPEA= Sweet Pumpkin Extract Agar, RDCEA= Red Dry Chilli Extract Agar, GCEA= Green Chilli Extract Agar

Potato Dextrose Agar (PDA) facilitated the highest radial growth of *C. capsici* (21.078 mm), closely followed by Carrot Extract Agar (CEA). During the initial three days, these two media exhibited no significant difference in mycelial growth. Additionally, Green Chilli Extract Agar (GCEA) notably resulted in the least growth of *C. capsici* (14.383 mm), with Rice Extract Agar (REA) (14.725 mm) following closely behind as shown in Table 4.1.

In the subsequent 24 hours, *C. capsici* once again displayed the greatest radial growth on PDA media (31.238 mm), with Mung Extract Agar (MuEA) coming in second. Remarkably, the growth rate on GCEA medium was only 3.57 mm during that period (Table 4.1).

Over the next three days, *C. capsici* exhibited the most significant growth on MuEA medium, which was statistically different from PDA. At this stage, MuEA displayed the highest growth across all media (Table 4.1).

The growth rate on MuEA media decreased compared to the earlier rate, and by the 8th day, no significant difference in total mycelial growth of *C. capsici* was observed between MuEA and PDA media. In that 24-hour period, PDA media recorded the highest growth rate, while GCEA media showed merely 5 mm (Table 4.1).

On the final day of observation, it was found that *C. capsici* exhibited the greatest radial growth on PDA media (82.793 mm), followed by MuEA (79.93 mm) and CEA (78.12 mm) media. Eventually, mycelial growth on both PDA and MuEA media completely covered the plates. Conversely, *C. capsici* demonstrated significantly lower radial growth on GCEA (50.65 mm), which was followed by Tomato Extract Agar (ToEA) media. Thus, there was no significant difference between PDA and MuEA media regarding the growth of *C. capsici* as shown in Table 4.1.

A similar study focusing on this pathogen (*C. capsici*) across different media was conducted by Joshi et al. (2024). Their findings indicated the highest growth on Red Chilli Dextrose Agar (90.0 mm), followed closely by Carrot Dextrose Agar and Potato Carrot Agar (89.50 mm). Conversely, the least growth was noted on Green Chilli Extract Agar (33.50 mm), with Red Chilli Extract Agar (48.00 mm) and Sarbouraud's Agar (48.50 mm) showing similar low growth rates.

An investigation aimed at identifying the optimal culture media for *C. capsici* was carried out by Falke et al. (2023). They experimented with seven culture media,

including Richard's agar, Oat Meal Agar, Potato Dextrose Agar, Czapek's Dox Agar, Maize Meal Agar, Yeast Extract Agar, and Yeast Mannitol Agar. The results showed that Potato Dextrose Agar (PDA) was the most effective in promoting the highest radial mycelial growth, measuring 89.21 mm. Richard's agar (68.45 mm) and Oat Meal Agar (81.75 mm) also proved to be the next best media for radial mycelial development.

Prajapati et al. (2020) conducted a study on the cultural and morphological characteristics of *C. capsici*, the pathogen responsible for anthracnose in chilli plants. They determined that Oat Meal Agar (OMA) was the optimal medium for maximal mycelial growth of the pathogen (90.00 mm), followed by Chickpea Flour Dextrose Agar (CFDA), while Rice Straw Agar (RSA) recorded the lowest mycelial growth (69.34 mm).

An experiment to evaluate the radial growth of *C. capsici* using five different media: Potato Dextrose Agar (PDA), Yeast Extract Potato Dextrose Agar (YEPDA), Malt Extract Agar (MEA), Wheat Grain Extract Agar (WEA), and Chilli Extract Agar (CEA) was performed by Akhtar et al. (2018). Their results indicated that PDA achieved the fastest growth, covering the entire plate (9 cm) in just 9 days. YEPDA was closely behind, taking 10 days, followed by CEA at 11 days, WEA at 12 days, and MEA at 13 days. In another study, S. Kumar et al. (2015) noted that different isolates of *C. capsici* displayed variations in their radial growth, with the highest growth recorded in PDA media (77.16 mm) compared to Oat Meal Agar (OMA) media (86.67 mm).

4.3 Growth rate of *C. capsici* on different media

The daily growth rate of *C. capsici* on various media is illustrated in Figure 4.3. Among all the media tested, PDA exhibited the highest growth rate at 8.82 mm/day. Additionally, MuEA demonstrated a growth rate very similar to PDA at 8.77 mm/day. Moreover, both CEA and TaEA showed an identical growth rate of 8.29 mm/day, which is comparable to that of PDA and MuEA. Following this, MaEA had a slightly lower growth rate of 7.93 mm/day. ToEA, REA, SPEA, and RDCEA displayed growth rates that were relatively close, measuring at 6.75 mm/day, 6.51 mm/day, 6.24 mm/day, and 6.39 mm/day, respectively. These growth rates were significantly lower than the highest recorded value. With the exception of GCEA, all other media exhibited moderate to high daily growth rates. The lowest growth rate observed for *C. capsici* was on GCEA, which was only 5.18 mm/day.

Figure 4. 3 : Growth rate of *C. capsici* on different media

PDA= Potato Dextrose Agar, MuEA= Mung Extract Agar, MaEA= Masoor Extract Agar, CEA= Carrot Extract Agar, ToEA= Tomato Extract Agar, TaEA= Taro Extract Agar, REA= Rice Extract Agar, SPEA= Sweet Pumpkin Extract Agar, RDCEA= Red Dry Chilli Extract Agar, GCEA= Green Chilli Extract Agar

4.4 Sporulation on a different media

When the colonies fully developed, sporulation of *C. capsici* became apparent. The conidia were carefully harvested and measured, with results shown in Figure 4.4. Significant differences in sporulation were observed among the tested media, with no notable difference between Potato Dextrose Agar (PDA) and Mung Extract Agar (MuEA). Maximum sporulation occurred on MuEA media, which was comparable to PDA, while the minimal sporulation was observed on Rice Extract Agar (REA) and Taro Extract Agar (TaEA).

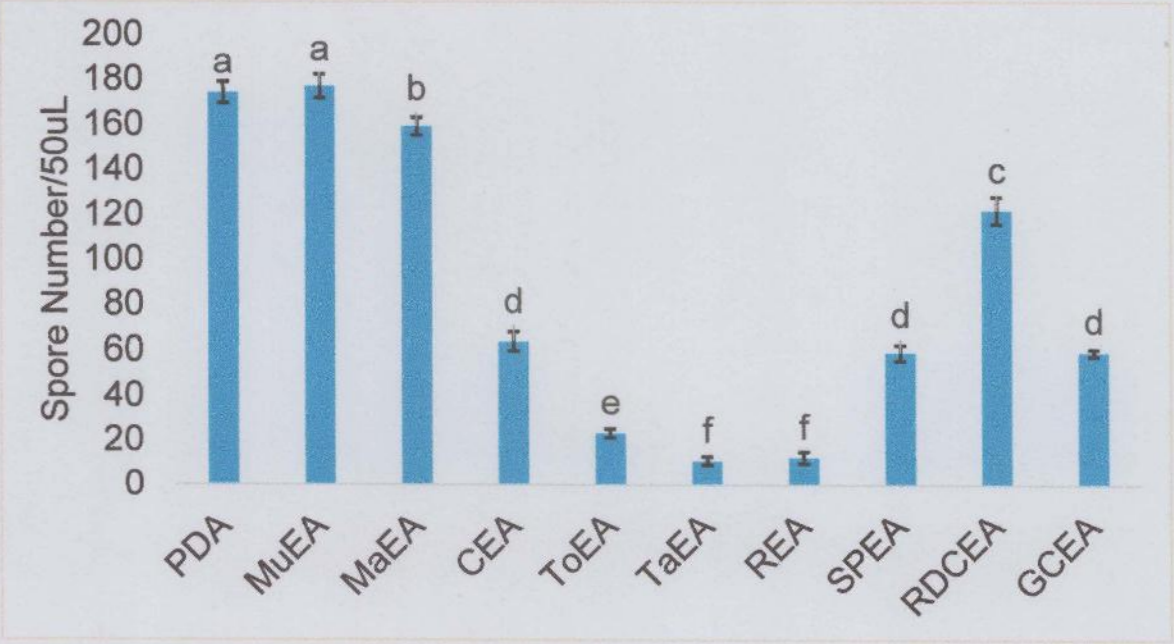

Figure 4. 4 : Sporulation on different media

Values with different letters are significantly differed at 5% level of significance according to Tukey's HSD test

PDA= Potato Dextrose Agar, MuEA= Mung Extract Agar, MaEA= Masoor Extract Agar, CEA= Carrot Extract Agar, ToEA= Tomato Extract Agar, TaEA= Taro Extract Agar, REA= Rice Extract Agar, SPEA= Sweet Pumpkin Extract Agar, RDCEA= Red Dry Chilli Extract Agar, GCEA= Green Chilli Extract Agar

4.5 Colony characteristics of *C. capsici* in different media

Table 4.2: Colony characteristics of *C. capsici* in different media

SL. No.	Media	Colony Characteristics					
		Color		Texture	Colony shape	Colony margin	Mycelium growth pattern
		Converse	Reverse				
1	PDA	Grayish white	Grayish black	Cottony	Circular	Smooth	Flattened and unifrom, concentric
2	MuEA	Grayish white	Grayish black	Cottony	Circular	Smooth	Flattened and uniform, concentric
3	MaEA	Whitish grey	Yellowish black	Fluffy	Circular	Serrate	Raised and concentric
4	CEA	Grey	Grayish black	Cottony	Circular	Irregular	Dense at centre and concentric
5	ToEA	Black centre turn into dark beige towards periphery	Black turn into dull brown towards periphery	Sparse	Circular	Filiform	Very thin aerial mycelium, Concentric
6	TaEA	Grey	Dark brown to dark beige	Cottony	Circular	Irregular	Flattened but not uniform, concentric
7	REA	Dull brown	Grey brown	Sparse	Circular	Filiform	Suppressed and no concentric ring
8	SPEA	Dark greyish green	Greyish brown	Sparse	Circular	Filiform	Suppressed with very thin aerial mycelium and Concentric
9	RDCEA	White	Rust brown	Fluffy	Circular	Serrate	Raised and Concentric
10	GCEA	Grey centre Surroundied by dark brownish pigmentation	Reddish brown to pale brown	Fluffy and sparse	Circular	Serrate	Dense at centre, Raised and concentric

PDA= Potato Dextrose Agar, MuEA= Mung Extract Agar, MaEA= Masoor Extract Agar, CEA= Carrot Extract Agar, ToEA= Tomato Extract Agar, TaEA= Taro Extract Agar, REA= Rice Extract Agar, SPEA= Sweet Pumpkin Extract Agar, RDCEA= Red Dry Chilli Extract Agar, GCEA= Green Chilli Extract Agar

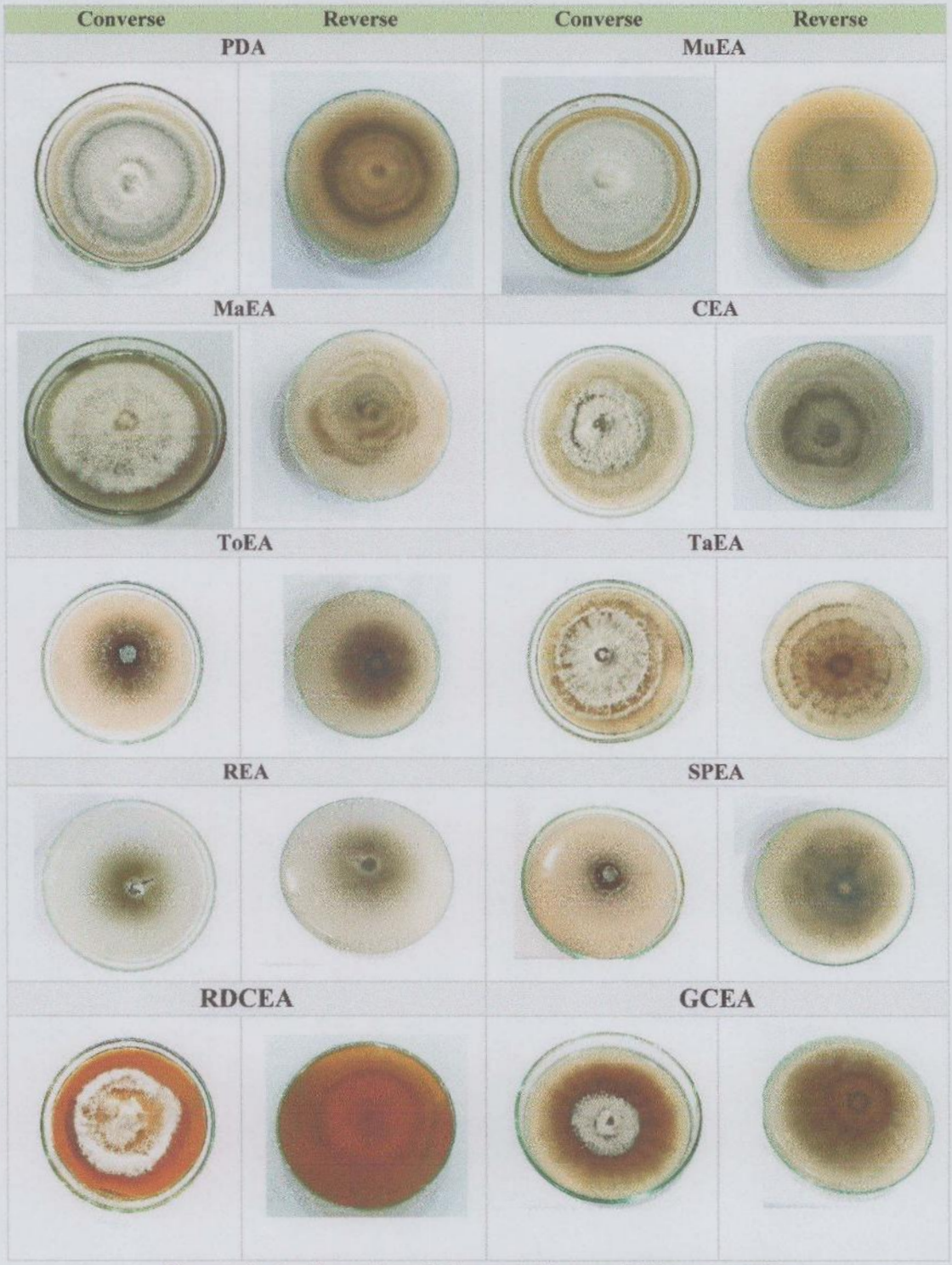

Figure 4. 5 : Growth of *C. capsici* in ten different media

PDA= Potato Dextrose Agar, MuEA= Mung Extract Agar, MaEA= Masoor Extract Agar, CEA= Carrot Extract Agar, ToEA= Tomato Extract Agar, TaEA= Taro Extract Agar, REA= Rice Extract Agar, SPEA= Sweet Pumpkin Extract Agar, RDCEA= Red Dry Chilli Extract Agar, GCEA= Green Chilli Extract Agar

4.4.1 Variation in Colony Color

The colony color results are displayed in Figure 4.5. The mycelium colony of *C. capsici* exhibited a greyish-white hue on the upper surface, which gradually transitioned to a blackish-grey at the edges when cultured on PDA and MuEA media. Joshi et al. (2024), Prajapati et al. (2020) and Sangdee et al. (2020) also reported a grayish-white appearance on the upper side and a black color on the underside. The reverse side showed a blackish-grey color. On MaEA and CEA media, the colony color was whitish and grey, respectively. The black center changed to dark beige at the edges on the upper surface of ToEA media, while it transitioned to dull brown at the periphery on the reverse side. On TaEA, a grey color was observed on the upper surface, and a dark brown to dark beige color appeared on the reverse side. The upper surface of REA presented a dull brown color, while the reverse showed grey-brown. On SPEA, the upper surface showed a dark greyish-green color, with greyish-brown on the reverse. The upper surface of RDCEA displayed white, matching the findings of Joshi et al. (2024), while the reverse side showed rust brown. In GCEA, the upper surface exhibited a grey center surrounded by dark brownish pigmentation, and the reverse side ranged from reddish-brown to pale brown.

4.4.2 Variation in Colony Texture

Table 4.2 presents the results concerning colony texture. A cottony texture was observed on PDA, MuEA, CEA, and TaEA media. In contrast, MaEA and RDCEA displayed a fluffy texture. However, ToEA, REA, and SPEA had a sparse appearance. GCEA exhibited a fluffy texture in the center and sparse at the edges. The texture may be categorized as cottony, fluffy, or sparse, which is consistent with the findings of Raj et al. (2014), who noted cottony, fluffy, or suppressed colonies of various *C. capsici* isolates in culture media. This aligns with the results of Sharma et al. (2005), who found mycelium varying from cottony to fluffy in different *C. capsici* isolates.

4.4.3 Variation in Mycelial Growth Pattern

The mycelial growth patterns are detailed in Table 4.2. A flattened and consistent growth pattern was observed in both PDA and MuEA media. TaEA exhibited a flattened yet inconsistent mycelium. RDCEA showed raised mycelium that was dense in the center with concentric rings, whereas GCEA presented a dense center with sparse growth at the edges. ToEA displayed very thin aerial mycelium. Suppressed mycelium was noted in REA and SPEA media. Nearly all the media displayed

concentric growth patterns, with the exception of REA media. (Figure 4.5) Research conducted by Vithal et al. (2020) and Kumar et al. (2015) also identified concentric rings in various media.

4.4.4 Variation in colony shapes and margin

Across all media, *C. capsici* displayed a circular shape. PDA and MuEA media exhibited smooth margins. MaEA, RDEA, and GCEA media demonstrated serrate margins, while ToEA, REA, and SPEA media displayed filiform margins. Irregular margins were noted on CEA and TaEA (Table 4.2). The findings indicate that the colony shape is circular, consistent with the observations of Sangdee et al. (2020). In another study, Akhtar et al. (2007) found the colony shape on PDA media to range from circular to irregular.

4.6 Effect of culture media on size of conidia (µm) of *C. capsici*

The observations regarding the size of conidia (µm) of *C. capsici* across the ten culture media are summarized in Table 4.3. This result shows that there is no significant difference in length of conidia between PDA, MuEA and RDCEA and width of MuEA, PDA, CEA, TaEA, SPEA, RDCEA and GCEA media. Moreover on other media , there was very negligible difference. Generally, the length of conidia ranged from 23.46 to 27.15 µm, while the width ranged from 3.12 to 3.65 µm. Minimal variations in conidia size were noted for *C. capsici* grown on different media. The present results closely resemble those of Akhtar and Singh (2007), who reported conidia lengths of 25.27 to 26.15 µm and widths of 3.17 to 3.55 µm. Sangdee et al. (2020) also found average conidia lengths and widths to range from 23.5 to 35.0 µm and 2.5 to 3.75 µm, respectively.

Table 4.3: Size of conidia (µm) of *C. capsici* in different media

SL. No.	Name of Media	Size of Conidia (µm)	
		Length	Width
1	PDA	26.52ab	3.55a-c
2	MuEA	27.15a	3.65a
3	MaEA	25.62bc	3.17cd
4	CEA	25.43bc	3.58a-c
5	ToEA	23.46d	3.12d
6	TaEA	25.39bc	3.62ab
7	REA	24.52cd	3.22b-d
8	SPEA	25.30bc	3.41a-d
9	RDCEA	26.34ab	3.40a-d
10	GCEA	25.71bc	3.25a-d

Values with different letters are significantly differed at 5% level of significance according to Tukey's HSD test

PDA= Potato Dextrose Agar, MuEA= Mung Extract Agar, MaEA= Masoor Extract Agar, CEA= Carrot Extract Agar, ToEA= Tomato Extract Agar, TaEA= Taro Extract Agar, REA= Rice Extract Agar, SPEA= Sweet Pumpkin Extract Agar, RDCEA= Red Dry Chilli Extract Agar, GCEA= Green Chilli Extract Agar

Chapter V

Summary and Conclusion

5.1 Summary

C. capsici exhibited highest rates of mycelial growth and sporulation on Potato Dextrose Agar (PDA) and Mung Extract Agar (MuEA) media. Besidea, there was no significant variation in the radial growth rate on PDA and MuEA media. However, when tested on Green Chilli Extract Agar (GCEA), it demonstrated the least growth. In terms of sporulation rates, PDA and MuEA media showed the highest values for *C. capsici*, while the Rice Extract Agar (REA) media showed the lowest sporulation rate among all the media tested. Additionally, on Taro Extract Agar (TaEA) and Sweet Pumpkin Extract Agar (SPEA), the sporulation was also poor. The mycelial colony colors ranged from white to whitish grey and dark brown to blackish, depending on the media. Most of the media displayed concentric rings. The colony texture was predominantly cottony to fluffy, although on some media, such as REA and SPEA, exhibited a more suppressed and sparse texture. *C. capsici* demonstrated abundant mycelial growth with circular and smooth margins on PDA and MuEA media. The size of the conidia did not exhibit any noticeable differences across various media. Moreover, on Carrot Extract Agar (CEA), *C. capsici* also showed favorable results concerning mycelial growth, while Masoor Extract Agar (MaEA) and Red Dry Chilli Agar (RDCEA) media demonstrated good sporulation.

5.2 Conclusion

Based on the observed growth rates and sporulation, this research demonstrate that *Colletotrichum capsici* has grown best in Potato Dextrose Agar (PDA) and Mung Extract Agar (MuEA) media. These two media were found to be the best for *C. capsici* to improve the radial growth and sporulation. According to this research, Mung Extract Agar (MuEA) proves to be a suitable substitute for Potato Dextrose Agar (PDA) regarding the growth and sporulation of *C. capsici*. So, the result of this research can be useful for future researchers and cultivators focused on studying or managing this pathogen in chilli cultivation. This analysis is crucial for laboratory investigations.

Chapter VI

References

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