STUDY THE ANTIFUNGAL ACTIVITY OF SELECTED PLANT EXTRACTS IN CHILLI INFECTED BY COLLETOTRICHUM

Thesis

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Declaration by the Scholar

I hereby declare that the work presented in this thesis entitled "**Study the Antifungal Activity of Selected Plant Extracts in Chilli Infected by Colletotrichum**" in fulfilment of the requirements for the award of Degree of Doctor of Philosophy, submitted in the Maharishi School of Humanities & Science, Maharishi University of Information Technology, Lucknow is an authentic record of my own research work carried out under the supervision of **Dr. Kanchan Awasthi**, **Associate Professor, Department of Botany**. I also declare that the work embodied in the present thesis-

i) is my original work and has not been copied from any journal/ thesis/ book; and

ii) has not been submitted by me for any other Degree or Diploma of any University/ Institution.

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Supervisor's Certificate

This is to certify that **Ms. Monika Singh** has completed the necessary academic turn and the swirl presented by her is a faithful record is a bonafide original work under my guidance and supervision. She has worked on the topic "**Study the Antifungal Activity of Selected Plant Extracts in Chilli Infected by Colletotrichum**" under the School of Business Management, Maharishi University of Information Technology, Lucknow.

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Name of the Student

ABSTARCT

Chilli (Capsicum spp.) is a widely cultivated crop susceptible to fungal infections, particularly by Colletotrichum species, causing significant yield losses worldwide. The study aimed to evaluate the antifungal activity of selected plant extracts against Colletotrichum-infected chilli. Plant extracts were prepared from Azadirachta indica (neem), Allium sativum (garlic), and Curcuma longa (turmeric) using various solvents. These extracts were screened for their antifungal efficacy through in vitro assays, including agar well diffusion and minimum inhibitory concentration (MIC) determination.

The results indicated that all tested plant extracts exhibited varying degrees of antifungal activity against Colletotrichum. Neem extract showed the highest inhibition zone diameter of 20 mm, followed by garlic (18 mm) and turmeric (16 mm) at their respective optimal concentrations. MIC values ranged from 250 to 500 µg/mL for neem, 350 to 600 µg/mL for garlic, and 300 to 550 µg/mL for turmeric extracts against Colletotrichum isolates. Further, in vivo studies demonstrated that these extracts significantly reduced disease severity in chilli plants compared to the control.

In conclusion, the findings highlight the potential of neem, garlic, and turmeric extracts as natural antifungal agents against Colletotrichum in chilli. Their effectiveness warrants further exploration for development as eco-friendly alternatives to synthetic fungicides in sustainable crop protection strategies.

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CHAPTER 1
INTRODUCTION

1.1 Introduction

Chilli is an ambiguous spice crop grown in all of India's states, and the quality varies by state. For example, Karnataka chilli is known for its high oil content, but chilli from Gujarat and Rajasthan state has a bright hue and is often used in pickle preparation. Similarly, chilli produced in Assam is recognized for its intense pungency, whilst chilli from Andhra Pradesh is utilized in vegetables. Andhra Pradesh (which has the country's largest chilli cultivation area, as well as Telangana (35%), Karnataka (14%), Tamil Nadu (7%), Orissa (7 percent), Maharashtra (6 percent), West Bengal (6%), and MadhyaPradesh (4%), with the remainder distributed among Rajasthan, Gujarat, and other states. Currently, India is primary source of red chilli in worldwide market, consuming over 6.2 million tons of chilli, accounting for almost 90% of the country's total production (Gade et al., 2020).

Capsicumannuum L. is one of most widely cultivated species in genus. Other domesticated chilli species include Capsicum baccatum, Capsicum chinensis, Capsicum frutescens, and Capsicum pubescens (Tong et al., 1999).

Capsicum annuum produces sweet (bell pepper) and pungent (chilli) fruits of various sizes & forms. Chilli is high in ascorbic acid, folic acid, potassium, and vitamins A (Pathirana, 2012).

Chilli is widely regarded as a key ingredient in many tropical and subtropical cuisines. Chilli has been considered a native of tropical America, & it is often farmed in its natural condition. Chilli arrived in India via Columbus' expedition, which brought chilli seeds from Spain and spread to Africa & Asia (Heiser, 1995).

One of the most amazing facts is that fresh green chillies have more vitamin C than citrus fruits, while red chillies contain more vitamin A than carrots (Pathirana 2012). Chilli is commonly used as a condiment, spice, vegetable, and in medications and drinks. Chilli's active components include capsaicin and caretenoids. Capsaicinoids are non-volatile alkaloids that

are the most active elements in chilli, giving it its spicy flavor. Caretenoids, on the other hand, give the chilli fruit its color as well as nutritional value.

Chilli is an important commercial crop in tropical & subtropical regions, & it is widely farmed in Asia, Africa, southern Europe, and South and Central America. Globally, the production area of chilli is 1.776 million hectares with a production of 7.182 million tons, however in India, area under chili seeded is 0.031647 million hectares with a total chilli output of 0.363399 million tons (Gade et al., 2020).

India is world's fifth-largest chilli producer, followed by China, Maxico, Turkey, & Indonesia. India has become world's top producer & exporter of chilli, with exports to United States, Canada, the United Kingdom, Vietnam, Germany, East and South Asia, and many other nations worldwide. India (25%) and China (24%) are the world's top chilli exporters. Indian chilli is well-known across the world for its vivid color and high pungency levels, and these two characteristics provide Indian chilli economic value.

Chilli crops are vulnerable to a variety of pests and infections both before and after harvest, and mycotoxins are a major obstacle to chilli growth. Capsicum is vulnerable to a variety of pests, weeds, fungal, bacterial, & viral pathogens worldwide, with anthracnose, dieback, and fruit-rot of chillies being the most common fungal diseases that cause increased losses during production, shipping, and storage (Dev et al., 2012).

Colletotrichum, a genus of Ascomycetes that causes anthracnose disease, is a huge economic hazard, with the ability to reduce chilli production by 50%. Anthracnose, which means 'coal' in Greek, is the most prevalent chilli disease, characterized by highly black, sunken lesions harboring fungus spores.

Colletotrichum species from all over the world have been identified as cause of chilli anthracnose. Initially, three prime Colletotrichum species, Colletotrichum capsici, Capsicum acutatum, and Capsicum gloeosporioides, were identified in Indian climatic prospects linked with the anthracnose disease; in addition to these three, Capsicum truncatum was also responsible for serious damage at late fruiting stage of chilli (Saxena et al., 2014).

Mycotoxin in dried chilies has restricted their export to Western industrialized countries such as United Kingdom and United States. Aflatoxin infection (Aspergillus sp.) causes post-harvest loss in chilli samples from Turkey (Demİrcİoğlu and Filazi, 2010) & Malaysia (Reddy et al., 2011), with rates ranging from 20 to 100%.

In tropical regions such as Asia & Sub-Saharan Africa, the conditions for fungal proliferation are favorable, resulting in a high incidence of mycotoxin synthesis in agricultural foods such as cereals, oil seeds, grains, nuts, & processed foods throughout production, pre-harvest, & post-harvest (Balendres et al., 2019).

Fungal mycotoxins can enter the body primarily by food, inhalation, or skin absorption. Mycotoxins and fungicide residues can enter food chain through infected crops, which are then consumed directly or indirectly by people or animal-based products such as meat, milk, and eggs (Hojnik et al., 2017).

Mycotoxins have a negative impact on agricultural productivity and trade across the world. According to the data published by Eskola et al. (2020), mycotoxins have contaminated around 60-80% of crops. Mycotoxins are tenacious and difficult to eliminate once they reach food chain. Mycotoxins in agricultural business cause loss not only in plants, but also in livestock output owing to lower growth rates and increased mortality rates in animals (Thipe et al., 2020).

Mycotoxin contamination of agricultural commodities reduces nutritional value, quality, and food safety. Several nations have established regulatory limitations on mycotoxins in agricultural products in order to reduce the dangers to human and animal health from mycotoxin exposure. Mycotoxins are associated with the illness mycotoxicosis, which has immunosuppressive, carcinogenic, genotoxic, hepatotoxic, mutagenic, nephrotoxic, and teratogenic features. The most important mycotoxins for agriculture are aflatoxins (AFs), ochratoxins (OTA), fumonisins (FBs), trichothecenes, & zearalenone (ZEN), which have attracted significant attention due to their high potential health concerns in people and animals (Celik, 2020).

Several ways have been used to manage & prevent mycotoxins in food, including chemical and microbiological procedures (biocontrol agents), as well as fungal infection prevention by the use of plant extracts at pre- and postharvesting phases (Adebiyi et al., 2019).

The strategies described above are successful in reducing the proliferation of toxigenic fungi as well as the generation of related mycotoxins before, during, and after harvest of agricultural commodities. Chemical approaches for decontaminating mycotoxins include the use of synthetic fungicides, ammonia, sodium hydroxide, hydrochloric acid, butylated hydroxytoluene, butylated hydroxyanisole, & oltipraz (Čolović et al. 2019).

Synthetic fungicide treatments promote fungicide resistance in fungi at high dosages, the removal of unwanted fungal species, and environmental contamination. Prolonged pesticide usage, regardless of class, has negative effects on human & animal health as well as environmental sustainability (Meng et al., 2020).

To reduce the effectiveness of synthetic fungicides, an alternative is necessary. Physical treatments include cleaning, dehulling, sorting, milling, ultraviolet light, pulsed light, cold the plasma, and irradiation. Other physical options include using adsorbents or binders such activated charcoal, bentonite, zeolites, and sepiolite clay. The methods have proved effective in disinfecting mycotoxins.

However, technological implementation has limitations such as high prices, low potential residual toxic effects, poor adsorption, and limited specificity (i.e., selective action), resulting in reduced activity against certain mycotoxins in routine use (Mahato et al., 2019).

Microbiological approaches use probiotic bacteria, yeasts, and enzymes to reduce mycotoxins in food. However, certain bacteria and their extracellular enzymes frequently interfere with food delivery, resulting in the development of undesired products and the use of enzymes to degrade by-products, limiting their utilization (Lyagin and Efremenko, 2019).

As a result, it is critical at this moment to look for alternative approaches that can preventfungal colonization of agricultural commodities by detoxifying or bio-transforming mycotoxin residues to less toxic or non-toxic forms without any constraints (Haque et al., 2020).

1.2 Theoritical History of Chilli

Chilli was grown on 774.9 thousand hectares in India, generating 1492.10 thousand tons with a productivity of 1.93 tonnes per hectare (Anonymous, 2013). During 2021-22, Indian chilli covered 6.94 lakh hectares (17.14 lakh acres), yielding 15.78 lakh tons at a productivity of 2689 kg per hectare (1088 kg per acre). In India, the largest chilli-producing states are Andhra Pradesh (7 lakh tonnes), Telangana (4.33 lakh tonnes), Madhya Pradesh (3.03 lakh tonnes), Karnataka (1.85 lakh tonnes), and Odisha (0.69 lakh tonnes), accounting for 44,27,19,12, and 4 percent of total output, respectively.

Export demand in 2022-23 is expected to reach 5.70 to 5.90 lakh MTdue to increased premium grade output in the expanding areas of AP, Telangana, and Karnataka. Because of the increased availability of premium quality and increasing demand, mainly from China, the United States, Bangladesh, Malaysia, and Indonesia. In 2021-22, India exported 5.57 lakh tonnes worth Rs 8581 crore.

When compared to other countries, India is world's greatest user and producer of chili. India is the world's leading producer of chili, followed by China, Thailand, and Pakistan. Chilli agriculture covers around 20.20 million hectares globally, with a yield of 37.62 million tons. India is the world's leading chilli producer, producing 13.76 million tons per year, followed by China, which produces around 3 million tonnes. India contributes 36.57 percent of the world's total chilli production of 37.62 million tons, followed by China at 7.97 percent.

Major chili-growing countries include India, China, Pakistan, Myanmar, Indonesia, Bangladesh, Turkey, and Sri Lanka in Asia; Ghana, Egypt, Uganda, Ethiopia, and Tunisia in Africa; Mexico and the United States of America in North Central America; the nation of Bulgaria, Hungary, Romania, Spain, Italy, and the country of Yugo in Europe; and Argentina and Peru in South America (Source FAO). Today, the most sharp and valuable types of chilies are cultivated only in Asia.

Chili prices have risen in India as a result of crop failures caused by severe rains and increased demand. Money Control said that prices rose by 50% in September. In October, The Economic Times reported that premium varietals will see even larger price increases.

India is by far world's largest producer ofdried chilies & peppers, Food & AgricultureOrganization category that most accurately describes spicy pepper types that are frequently dried and marketed whole or powdered. In 2020, the most recent year available, India produced more than 1.7 million tons of roasted chili and pepper varieties, much surpassing Thailand and China. The latter country is a significant importer of Indian hot chilis, which it uses to fulfill high local demand.

More South Asian countries rank among the world's top producers of dried chilis and peppers. Bangladesh was ranked fifth in 2020, generating around 158,000 tons. Pakistan was right behind, with an annual production of over 142,000 tons. While India hopes that a stronger harvest would ease the shortfall beginning in January 2023, its neighbor to the northwest is now dealing with crop-damaging weather conditions in its main chili growing area.

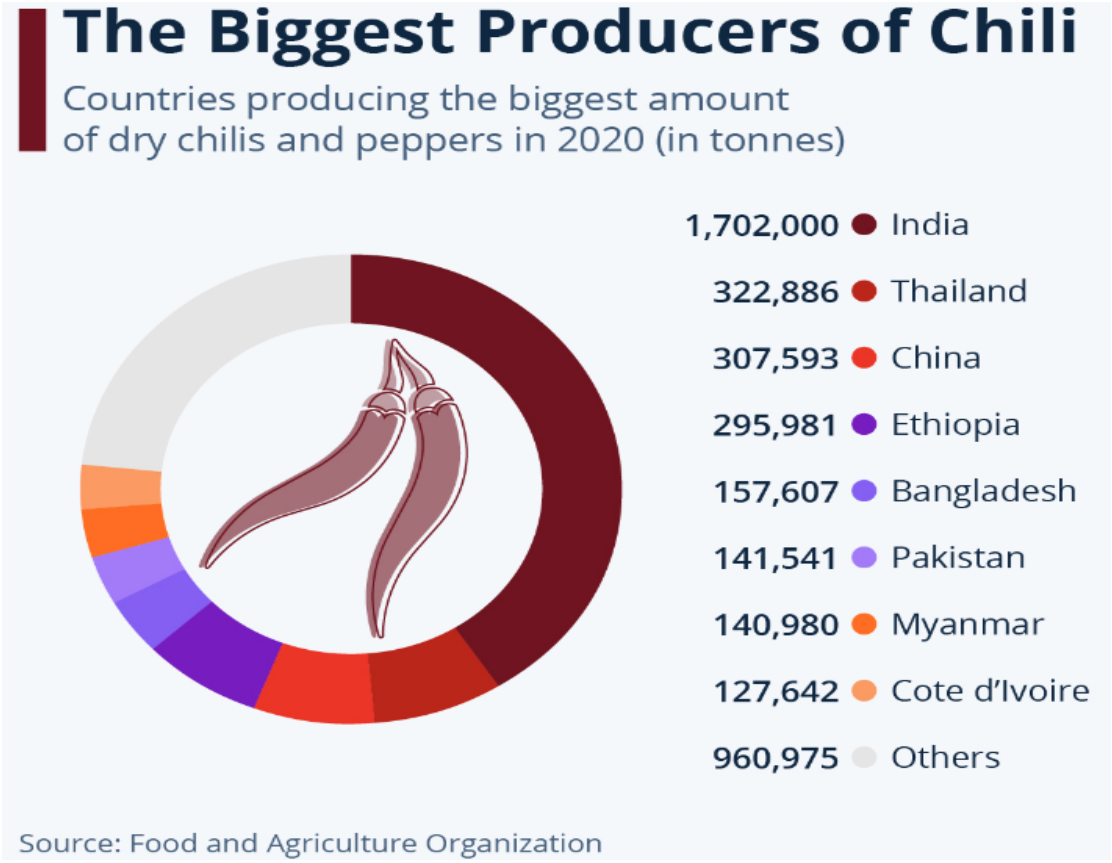


Figure 1.1: Country-wise share in chilli production

Indian chilli is well-known for 2 significant commercial qualities: its color & pungency levels. Some types of chilli are known for their red color due to pigment, while otherquality factors inchilli include skin thickness, length, & breadth. India, China, Thailand, Mexico,

United Kingdom, Sweden, & Germany are among countries that consume most chilli. However, India is the only supplier of spicy chillies.

India provides 36% of global chilli output and remains the leader in international commerce, exporting approximately 30% of its entire production. Chilli is planted in practically every Indian state. Andhra Pradesh, Odisha, Maharashtra, which West Bengal, Bangalore, Rajasthan, and Tamil Nadu are the states with the most chili production.

In India, chilli crops are cultivated on 774.9 thousand hectares, with a yield of 1492.10 thousand tonnes & a productivity of 1.93 tonnes pehectare. Andhra Pradesh has the greatest chilli crop area in India, with around 131.3 thousand hectares cultivated, a total production of 602 thousand tonnes, & a yield of 4.58 tonnes per hectare, followed by Telangana, Karnataka, West Bengal, Gujarat, & Maharashtra.

Andhra Pradesh leads India in dried chilli output in 2021-22, with 4.07 lakh tons grown on 2.25 lakh hectares and an 1809 kg/ha yield, followed by Telangana, Madhya Pradesh, Karnataka, and West Bengal. Figure 1.2 depicts the output of chillies by state in India.

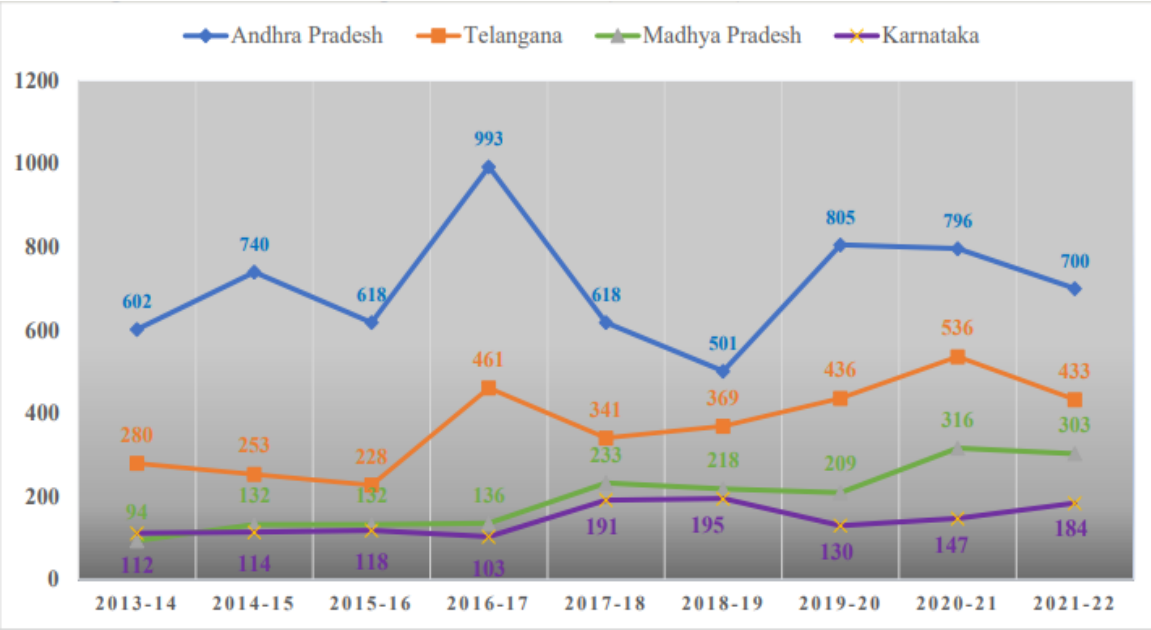


FIGURE 1.2: STATE WISE CHILLI PRODUCTION IN INDIA

Guntur chilli yard, Asia's largest chilli market, impacts both domestic and international chilli pricing. Guntur district in Andhra Pradesh alone generates 15% of all chiles produced in India, while the state as a whole provides 26% (des.ap.gov.in, 2021). There are around 400

distinct types of chiles found throughout the world. Guntur, Prakasam, Kurnool, and Krishna are the four largest chili farming districts in Andhra Pradesh. Teja, Byadgi, DD Best, 341, 273, and 334 are among the top types that exporters favor.

According to the Government of Andhra Pradesh's final estimations, chilli production would be 4.07 lakh tons cultivated on 2.25 lakh hectares with a productivity of 1809 kg/ha in 2021-22. According to the first advance projections for 2022-23, chilies were produced on 1.69 lakh hectares with a yield of 6.94 lakh tons & a productivity of 4109 kg/ha.

Andhra Pradesh, with its lush soils and skilled growers, is known as the Chilli Capital of India, accounting for an amazing 44% of total national output. From the bustling chilli markets of Guntur to the lush fields of Prakasam, Andhra Pradesh's devotion to chilli production has not only solidified its status as a leader, but has also greatly contributed to the state's economic success. Join us as we explore the top ten states influencing India's spice history, delving into district-specific production specifics, historical beginnings, and the unique nomenclature of chilli in various Indian languages. Discover the spice secrets that have made India a global leader in chilli manufacturing.

Andhra Pradesh is uncontested monarch of chili production in India, accounting for a whopping 44% of the entire output in 2024. Its lush soils, ideal climate, and competent farmers have resulted in a booming chili industry, which contributes greatly to state's economy & livelihoods. From bustling chilli markets of Guntur to the green plains of Prakasam, Andhra Pradesh's commitment to chilli farming has won it the well-deserved distinction of "India's Chilli Capital."

TABLE 1.1: TOP 10 LARGEST PRODUCER OF CHILLI IN INDIA 2024

SR NO.	STATE	PRODUCTION	SHARE (%)
1	Andhra Pradesh	700.00	37.35
2	Telangana	433.12	23.11
3	Madhya Pradesh	296.69	15.83
4	Karnataka	184.53	9.85
5	Orissa	69.26	3.70
6	Maharashtra	23.73	1.27
7	Gujarat	22.36	1.19
8	Tamil Nadu	21.59	1.15

9	Assam	19.65	1.05
10	Punjab	15.88	0.85

1.3 Origin of Chilli and its Popularity

Chilli, which is native to New Mexico and has a secondary origin in Guatemala, has ancient roots, as evidenced by prehistoric fragments discovered in Peru. Chilli, which is widely grown in Central and South America, was unknown to Europeans until the Americas were explored. Early explorers introduced the spice to Spain in 1493, and it quickly spread throughout Europe. In sixteenth century, Portuguese explorers introduced chili to India.

Hot peppers, chillies, and bell peppers are all members of the Capsicum genus in the Solanaceae family. Hunziker categorized the genus in 1956, dividing it into Monotypic Tubocapsicum, Pseudoacnistus, and Capsicum sections. With n=12, most species in the genus have the same chromosomal count, with the exception of Capsicum ciliatum & Capsicum scolnikianum (n=13).

Capsicum is made up of 22 wild species, three variations, and five cultivated species, each with its own particular traits. Domesticated species often yield bigger but fewer fruitsthan wild equivalents, but seed output perplant stays constant.

Capsicum annum var annum, a widely cultivated species, is distinguished by its persistent, pendulous fruits, a single enormous white flower at each node, and calyx teeth. Capsicum chinese has a dull white flower with no calyx teeth and a distinct constriction between the calyx base and pedicel. Capsicum frutescens, while closely related to Capsicum chinese, is distinguished by its greenish-white corolla and lack of constriction.

Baccatum var pendulum is distinguished by small calyx teeth, acream-to-white corolla, and paired yellow-green dots on eachlobe. Capsicum pubescens, which is less well-known outside of Latin America, has a remarkable deep purple to pale violet flower, a fruitwith a prominent neck, & a calyx with tiny teeth.

Though Capsicum annum, Capsicum chinese, and Capsicum frutescensare separate domesticated species, their wild forms are believed to have a similar genetic ancestor,

potentially from South America, Mesoamerica, Southern North America, or the West Indies. Over time, evolution from tiny, dispersed populations produced unique wild varieties such as Capsicum frutescens inSouth America & West Indies.

Capsicum pubescens, a closely related domesticated species, is thought to share wild origins with Capsicum cardenasii and Capsicum eximium. Capsicum annum is the most common Capsicum species grown commercially. Recent advances in Capsicum annum improvement include the incorporation of genes, particularly those for disease resistance, from other domesticated Capsicum spp. and wild cousins.

Various Capsicum species, including Capsicum baccatum, have been found to be resistant to Phytophthora, Leveillula taurica, cucumber mosaic virus, & potato virus. Additionally, Capsicum cardenasii is drought resistant.

Domesticated Capsicum spp. are divided into two categories based on cross-compatibility: white-flowered taxa (Capsicum annum, Capsicum chinense, & Capsicum frutescens) and purple-flowered taxa (Capsicum pubescens, Capsicum eximium, and Capsicum cardenasii).

1.4: Importance

Chilli (Capsicum annuum L.) is a major vegetable crop and spice grown across world. Chilli fruits have several medicinal benefits and have been utilized in a variety of cuisines. Green chilies are high in vitamins, including vitamin A, C, B, B2, and vitamin P.

Chilli (Capsicum annuum L.) is a major vegetable crop & spice grown across world. Chilli fruits have several medicinal benefits and have been utilized in a variety of cuisines. Green chilies are high in vitamins, including vitamin A, C, B, B2, and vitamin P.

Capsaicin has several medical qualities, including its usage as an anticancer agent and an immediate pain reliever. It also dilates blood arteries, which helps to avoid heart disease. Capsicum formulations were used in traditional medicine to treat asthma, anorexia, rheumatic

conditions, hemorrhoids, pharyngitis, and cough. Fresh green chilies have more Vitamin C than citrus fruits, while red chillies contain more Vitamin A than carrots. (Martin et al, 2004).

The color of the chilli is caused by the presence of many compounds, as well as carotenoids and mineral components, which add to its nutritional value (Homero-Mendez et al. 2002; Parez-Galvez et al. 2003).

Fresh chilli peppers have a high concentration of vitamin C, which activates immune system & acts as a healing agent in cellular damage. It also improves peripheral circulation and reduces excessive blood pressure. It is also a good source of vitamins A & C, which aid digestion. Russian scientists have discovered vitamin P, which protects against secondary irradiation harm. Chilli extracts are used in a wide range of treatments for rheumatism, loss of appetite, onsillitis, flatulence, sore throat, diphtheria, intermittent fever, swelling, and hardened tumerous Martin et al. (2004).

In comparison to other states, Maharashtra produces less chilli, which is the primary cause of the seed's poor health. Most chilli producers utilized seeds taken from stored mature fruits contaminated with rots to raise seedlings in nurseries. This takes a tremendous toll on the crop at all phases, including seeding, harvesting, transit, marketing, and storage.

The treatment and prevention of anthracnose illness are still being studied, and commercial varieties of capsicum annuum that are resistant to the pathogens responsible for chilli anthracnose have yet to emerge. Agrios (2005) emphasizes the importance of an integrated disease management approach.

It is exceedingly difficult to eradicate the illness with a specific management strategy since no one management program can remove chilli anthracnose. Colletotrichum infections are often managed using a mix ofcultural management, biological control, chemical control, & intrinsic resistance (Wharton and Diéguez-Uribeondo, 2004).

It may be impossible to effectively manage chilli anthracnose disease with a single approach. As a result, it is required to design an integrated disease management approach

that includes host plant resistance varieties, fungicides, and biocontrol as effective control measures.

Colletotrichum capsici is an important crop and pathogen, although little research has been conducted in the Marathwada area of Maharashtra on screening chilli cultivars for resistance to anthracnose disease and pathogenicity against various components of disease management.

As a result of the foregoing facts, the following objectives are recommended for the current task.

- Colletotrichum capsici was collected, isolated, identified, and shown to be pathogenic.
- Screening several chilli varietals for Colletotrichum capsici.
- In vitro analysis of effects of various carbon & nitrogen sources on growth and spore germination.
- In vitro analysis of effects of various amino acids, organic acids, and plant crude oils on growth & spore germination.
- Different plant extracts were tested in vitro for their ability to inhibit Colletotrichum capsici mycelial growth and spore production.
- Different fungicides were tested in vitro against mycelial growth & spore germination of Colletotrichum capsici.
- Colletotrichum capsici enzymatic activity was investigated.
  A study of the toxicity of fungal extracts on seed germination.

1.5 Anthracnose Disease of Chilli

The economic element of the disease in chili is the existence of a lesion on the fruit; even a little lesion on fruit is enough to reduce the market value of chilli, resulting in a reduced profit crop yield. Colletotrichum fruit rot mostly damages the aerial sections of the chilli plant, affecting green and red chilli, with preference for mature fruits. Rot disease conidia can be found in the air, seed, soil, and water, and they can cause harm at any stage of growth & development. Colletotrichum is an asexual fungus that belongs to the phylum Ascomycete & the class Coelomycetes of the fungi imperfectii.

Despite significant advances in pathology research, taxonomic location of Colletotrichum remains unknown, and systematics of Colletotrichum from this genus remains indistinct, with species numbers ranging from 300 to 700 depending on the parameter used for separation. Colletotrichum spp. hosts include fruits, vegetables, ornamental plants, and major food crops. Colletotrichum species cause anthracnose in over 121 plant genera. It also causes aerial plant blights and postharvest rots. Colletotrichum's infection and destruction spectrum extends beyond chillies to staple foods such as bananas, sorghum, cassavas, legumes, & grains in tropical & subtropical nations (Farr et al., 2016).

Colletotrichum capici (Sydow) Butler and Bisby induce anthracnose in chilli cultivars, and three pathotypes were first connected with infection on ripe chilli fruits, whereas two species were recognized to cause infection at the mature green fruit stage.

1.6 Use of Biological Control Agents

Disease treatment using crude extracts of medicinal plants has been studied in recent years for their effective antifungal & antibacterial activities. Plant features such as ease of decomposition, nonresidual activity, and minimal phytotoxicity have gained popularity. Numerous research have used crude plant extracts to regulate Colletotrichum spp. in chilli (Johnny et al., 2011).

Various plant extracts of sweet flag (Acorus calamus L.), palmarosa (Cymbopogon martini) oil, Ocimum sanctum leaf extract, neem (Azadirachta indica) oil, garlic, Piper beetle L., Coleus aromaticus, plucao, & sabsua have been shown to be effective against pathogen growth and spore germination to varying degrees. The biocontrol strategy for plant disease management has demonstrated that a sustainable and green approach is necessary to restore the environment's lost renewability. However, while this specific technique has not gained the necessary momentum for controlling chilli anthracnose, Lenne & Parbery (1976) identified possibility of application biocontrol agents (BCAs) for managing plant disease.

Korsten and Jeffries (2000) demonstrated the potential use of BCAs to manage post-harvest loss of chilli fruits. Plant extracts are regarded as ecologically benign, safe, and clean

alternatives to bioagents for fungus and mycotoxin management in agricultural production (Prakash et al., 2020).

Essential oils,spices, herbs, & crude plant extracts provide intriguing options for the development of biofungicides to prevent mycotoxicosis & other fungal diseases. Plant extracts are less expensive than other chemicals used for same purpose, resulting in a synergistic approach as antifungal agents. Furthermore, plant extracts promote metabolic pathways that activate the plant's innate defensive mechanisms (Meng et al., 2020).

Plants include a variety of phytochemicals with pharmacological activities that protect against certain plant diseases. Recent research has been undertaken on potential use of plant extracts as biofungicides in agricultural products during & after harvest (Dikhoba et al., 2019).

1.7 Mitigation of Toxigenic Fungi and Mycotoxins

Plants have been widely utilized as medicine by ordinary people since antiquity to cure and prevent numerous ailments from generation to generation (Street and Prinsloo, 2013).

The abundance of plants in nature and their widespread distribution make them ideal for nutraceutical and medicinal applications. Plants produce secondary metabolites known as phytochemicals as a defensive strategy against infections, insects, &, to a lesser extent, essential oils. Plant extracts, on the other hand, have antibacterial characteristics that can protect humans, animals, and plants against fungal and mycotoxin-induced illnesses. There are many primary classes of phytochemical substances that have been decoded so far, each with its own chemical structure (Das et al., 2020).

Phenolic substances, alkaloids, aromatic acids, carotenoids and chlorophyll, essential oils, flavonoids, glucose phytosterols, saponins, tools, terpenoids that tannins, organic acids, and protease inhibitors are among primary phytochemical classes (Loi et al., 2020).

These phytochemical substances defend against infections because they have antimutagenic, antigenotoxic, antibacterial, anthelmintic, anticarcinogenic, antiproliferative, anti-inflammatory, and antioxidant characteristics (Velu et al., 2018).

In addition, phytochemicals have cytotoxic effects in fungi by disrupting cell membrane availability and functions, inhibiting cytoplasmic and mitochondrial enzymes, controlling enzymes involved in cell wall synthesis, and shifting the cell area, or osmotic, and redox balance (Loi et al., 2020).

Plant extracts & chemicals also serve as xenobioticdetoxification & biotransformation routes (Gross-Steinmeyer & Eaton, 2012).

1.8 Objectives of the Study

- To isolate & identify Colletotrichum sp. From infected fruit.
- To evaluate antifungal properties of plant extracts after post-harvest fruits to observe the disease incidence, decay inhibition, and effect of different storage temperatures.
- To study the minimal inhibitory concentration of different solvent extracts from test plants.
- To study the heat stability and proteolysis degradation of test plant extracts.

1.9 Chapterization of the Study

Chapter1 Introduction
Chapter2 Review of Related Literature
Chapter3 Materials and Methods
Chapter4 Plants extract used in study
Chapter5 Result and Discussion
Chapter 6 Conclusions

CHAPTER 2
REVIEW OF LITERATURE

According to Anum Haq Nawaz et al. (2024), Anthracnose disease, caused by the fungus Coll capsici, is a severe fungal problem in chilies (Capsicum annuum L.) over the world, leading in a decrease in worldwide production. It may be treated with synthetic fungicides, but these chemicals may upset the environmental and ecological balance. As a result, additional strategies are necessary to manage this critical fungal illness. In their investigation, they produced commercially viable silver nanoparticles (AgNPs) and found that they have antifungal action against Colletotrichum capsici, which causes anthracnose. AgNPs were made from Colchicum luteum leaf extract. The findings indicate that AgNPs are efficient antifungal agents against Capsicum capsici, exceeding AgNO3 and conventional fungicide treatments. These findings lend support to future study into the practical application of AgNPs as a potential alternative strategy for treating fungal infections in agricultural settings.

Hajji-Hedfi, L., Rhouma, A., Al-Judaibi, A.A. et al. (2024) has studied the aqueous extract of Capsicum annuum seeds was screened for its phytochemical constituents & assessed at various concentrations (10, 20, 30, & 60%) for antifungalactivity in vitro. The study found that aqueous extract at 60% concentration was most effective in vitro when mycelial growth was < 3.8 mm, growth inhibition was $> 52\%$, and growth rate was < 1.05 mm/h. In vivo, combined treatments of tomato seeds reduced gray mold damage by 8.67%. The most favorable growth parameters of seedlings were chlorophyll a > 1.50 mg/g.f.Wt., chlorophyll b > 1.76 mg/g.f. Wt., total chlorophyll content > 3.26 mg/g.f.Wt., seedling fresh weight > 0.43 g, and seedling length > 12.43 cm, respectively. The aqueous extract of Capsicum annuum seeds coupled with salicylic acid suppressed B. cinerea, indicating that it might be a viable and environmentally friendly alternative to chemical fungicides for long-term agricultural sustainability in the face of climate change.

Syeda Noureen Fatima et al. (2023) compared the antifungal activity of plant extracts with standard fungicides against capsicum capsici. Morphologically identifiable strains of capsicum capsici were examined for infectiousness, with strain CC-2 demonstrating a highly virulent response. In-vitro experiments found that Ginger (15% concentration) inhibited fungal mycelial development and spore germination at levels comparable to Nativo and Antracol at

1000 ppm. In the protective and curative experiments, ginger extract at 15% showed the highest crop protection activity (84%) and medicinal value (70%). As a consequence, among fungicides, Antracol at 1000 ppm had the highest crop protection activity (92%) and curative effectiveness (96%). Pot experiments found that Ginger significantly decreased capsicum capsici and improved plant growth, while Antracol outperformed Nativo as a fungicide. PCA looked explored the association between growth indices in chili plants injected with plant extracts and fungicides.

S.K. Sudirga et al. (2023) investigated plant extracts' ability to inhibit the growth of the pathogenic fungus Colletotrichum acutatum, which causes anthracnose disease in chili. This study identified twenty potential plant species for future investigation. The leaf was extracted using the maceration method in methanol and n-hexane. The chemical element composition was constant throughout the GC-MS examination. All of the leaf extracts tested for bioactivity did well in colony and diffusion assays. Six of the 20 plant species studied were shown to be capable of inhibiting Capsicum acutatum fungus growth: Piper nigrum, Piper ornatum, Piper retrofractum, Ficus septica, Samanea saman, and Tithonia diversifolia.

Cheng et al. (2022) found that ethanolicextract of pomelo fruit inhibited the development of Colletotrichum gloeosporioides. The IC50 for pomelo fruit extract was reported to be 3.2 ml/l.

Sousa et al. (2022) investigated antifungal activity of ethanolicextracts of Dipteryx punctata leaves, stems, and fruits at concentrationsof 10 percent, 20 percent, 30 percent, 40 percent, and 50% (w/v). At 40% and 50% concentrations, D. punctate stem and fruit extracts reduced the diameter of Colletotrichum musae spots on banana fruit.

Kumaret al. (2021) investigated in vitro & in vivo actions of neem (Azadirachta indica), kusum (Schleichera oleosa), karanj (Pongamia pinnata), and jatropha (Jatropha curcas) essential oils against Colletotrichum musae. Schleichera oleosa oil outperformed the others in terms of in-vitro & in-vivo activity percent against capsicum Musae, which causes banana anthracnose disease.

Jadesha and Velappagounder (2021) evaluated fungicidal effect of 25 medicinal plants; Ageratum conizoide (Floss flower),Ocimum sanctum (Tulasi), Azadirachta indica (Neem),

Allium sativum (Garlic), A. cepa (Onion), Ocimum basilicum (Sweet basil), Plectranthus barbatus (Marunthukoorkan) , Adenocalymma alliaceum (Garlic creeper),Catharanthus roseus (Red periwinkle), Datura metel (Oumathum), Eclipta alba (Karisalankani), Eucalyptus lobules (Eucalyptus), Jatropha curcas (Jatropha), Lantana camara (Lantana), Nerium odorum (Arali), Psoralea corylifolia (Karpogaarsi), Bougainvillea spectabilis (Bougainvillea), Ricinus communis (Castor), Solanum torvum (Turkeyberry), Prosopis juliflora (Mesquite), Vitex negundo (Notchi), Andrographis paniculate (Nilavembu), Solanum trilobatum (Purple-fruited pea Eggplant),Tridox procumbens (Tridax daisy) and Aegle marmelos (Bael), belonging to 17 different families against banana anthracnose disease caused by capsicum musae. Maximum antifungal activity was shown by Solanum trilobatum, among all medicinal plants.

Dias et al. (2020) studied aromatic extracts from noni fruits (Morindacitri folia L.) & leaves of the lemongrass (Cymbopogon citratus DCStapf), Mastruz (Chenopodium is ambrosioides L.), Citronella(Cymbopogon nardus L. Rendle), & Rosemary pepper (Lippiasidoides Cham) against the conidial development and mycelialgrowth of Colletotrichum gloeosporioides, & observed that loss of fruits fresh mass was 7% reduced contrasted to the untreated papa.

Santos et al. (2019) investigated antimicrobial activity of Cymbopogon citratus leaf extracts (aqueous, ethanolic, & methanolic) against Colletotrichum gloeosporioides during guava postharvest. Capsicum citratus extract suppressed Capsicum gloeosporioides growth in vitro, but was ineffective in vivo.

Costa et al. (2019) investigated the metabolite interaction of Penicillium digitatum and Penicillium citrinum using mass spectrometry. The former isa postharvest disease ofcitrus fruits that causes significant losses. During the interaction, two tetrapeptides (deoxycitrinadin A, citrinadin A, chrysogenamide A, & tryptoquialanines) were discovered and shown antifungal efficacy against P. digitatum and P. citrinum.

Savi et al. (2019) identified the new metabolite dioxolanone phenguignardic acid butyl ester from citrus phytopathogen Phyllostictacitricarpa LGMF06. The isolated metabolite demonstrated antifungal and antibacterial actionagainst methicillin-sensitive & resistant Staphylococcus aureus.

Zhao et al. (2018) identified 31 fungal isolates from maritime plants and investigated their antibacterial and antifungal properties against phytopathogens. The most common fungus among the 31 detected strains were Alternaria sp. and Fusarium equiseti. The extracts of Fusarium equiseti (isolate No. P18) and Alternaria sp. (isolate No. P8) included two anthraquinone derivatives (compounds 1 and 2) and two perylenequinones (compounds 3 and 4). These extracts were chosen because they demonstrated strong antifungal activity against two phytopathogenic fungus (Pestallozzi atheae and Alternaria brassicicola) and a phytopathogenic bacteria (Clavibacter michiganensis).

Birari et al. (2018) investigate the effect of Bavchi seeds (Psoraleacorylifolia), datura leaves (Datura sp.), & ghaneri leaves (Lantanacamera) on Colletotrichum capsici. The poisoned food approach was used to evaluate doses ranging from 250 to 1000 µl. At 1000µl concentration, a methanolic extract of Psoralea corylifolia inhibited Capsicum capsici the most effectively.

Choudhury et al. (2017) used the poisoned food approach to test the effects of a chloroformextract of ginger (Zingiber officinaleRoscoe.) rhizome and a methanolic extract of mature leaves ofClerodendrum (Clerodendruminfortunatum L.) and Polyalthia (Polyalthialongifolia) on Capsicum capsici. The study found that extract doses of 20, 100, 200, and 400 µg/ml decreased Capsicum capsici radial development, spore germination, and biomass production.

Boonrung et al. (2017) investigated antifungal characteristics of 2 volatile chemicals, thymol and R-(-)-carvono, at 12 and 25 °C. At 12 °C, 20% Thymol alone inhibited fungal growth, whereas a combination of 15% carvono and 20% Thymol suppressed Colletotrichum gloeosporioides more effectively.

Sarkhosh et al. (2017) found antifungal activity in oil extracts derived from mint, thyme, savory, cinnamon, and lavender against Colletotrichum gloeosporioides. The oil extract of savory and thyme plants included significant levels of carvacrol, thymol, and cis cinnamaldehyde. Applying 2000 µl of this extract reduced lesion surrounding the inoculation site on fruit.

Aqueveque et al. (2017) investigated antifungal activity ofethyl acetate (EtOAc-extract) and methoanolic (MeOH-extract) extracts of Chileanfungus Stereum hirsutum (Sh134-11) grown in liquid-state submerged fermentation against Botrytis cinerea, a fungus that causes grey mould on plants. EtOAc extract was shown to be more efficient than MeOH extract. Four compounds were identified from the extract: MS-3, vibralactone, vibralactone B, & sterenin D, the latter of which exhibited strong antifungal action against B. cinerea. The MIC of sterenin D was 20μg/ml, and at 500μg/ml, it prevented 96% spore percent germination of B. cinerea.

Akremi et al. (2017) tested effects of extracts from a Mediterranean brown alga, Dictyopteris membranacea, on yeast and eight bacterial pathogens. Theethanol & acetone fractions had strong bactericidal activity. The ethanol fractionwas high in flavonoids, but the acetone fraction was high in phenolics and tannins, which accounted for their antibacterial properties.

Bhuyan (2017) conducted phytochemical analysis and antifungal assays on an aqueous extract of Eucalyptus microcory. The extract included phenolics, flavonoids, proanthocyanidins, and saponin, and it demonstrated strong antifungal activity against two phytopathogenic fungus, Aspergillus brasiliensis and The plant Geotrichum candidum.

Morais et al. (2017) showed that herbal extracts can enhance the antifungal efficacy of chemical fungicides. In their investigation, the ethanolic extract of Guazuma ulmifolia enhanced fluconazole's antifungal effectiveness against Candida tropicalis. Furthermore, the extract demonstrated antioxidant and anticholinesterase action due to presence of phenolic percent components (calechin, chlorogenic and caffeic acid) and flavonoids (rutin, quercitrin, quercetin, and lutealin).

Sharma et al. (2017) investigated antifungal properties of the essential percent oils of Syzygium aromaticum, Cymbopogon percent citratus, Eucalyptus globulus, and Mentha piperita against Fusarium oxysporum f. sp. lycopersici 1322, cause of wilt disease. S. aromaticum has the strongest antifungal potential when compared to other essential oils. The oil of S. aromaticum included antimicrobial metabolites such as eugenol (75.41%), ecaryophyllene (15.11%), α-humlene (3.78%), and caryophyllene.

Yadav A. L. (2017) observed Colletotrichum capsici infection on five kharif crops, including seasamum, the groundnut, cowpea, soyabean, and urdbean, as well as two weeds, motha and jangli chaulai.

Adeogun et al. (2016) tested acetone, aqueous, ethanol, and hexane extracts of Thaumatococcus daniellii leaves against 11 food spoiling fungi (Aspergillus aculeatus, A. niger, A. flavus, Rhizopusstolonifer, Issatchenkiaorientalis, Meyerozyma guilliermondii,Fusarium oxysporum, Paecilomycesvariotii, Penicillium is crustosum, Tricoderma harzianum). Acetone & ethanol leaf extracts had antifungal action against all examined fungi, and the extracts included alkaloids, saponins, tannins and flavonoid.

Balashanmugam et al. (2016) shown that plant extracts may also be used to synthesize nanoparticles. In their investigation, silver nanoparticles were produced using anaqueous leaf extract of Cassiaroxburghii. Plant-assisted nanoparticles were evaluated against three plant fungal diseases (Rhizoctoniasolani, Fusarium oxysporum, & Curvullaria sp.), and the nanoparticles had more antifungal activity than the conventional antifungal medication amphotericin B.

Kacem et al. (2016). Genista quadrifloro oil contains sesquiterpenes such as murolan-4, 7-α-cadinol, caryophyllene oxide, and germacra-4 (15) 5, 10 (14) - triene-1-α-01. The crude essential oil shown antifungal efficacy against Fusarium oxysporum.

Li et al. (2016) investigated activity ofan ethanolic extract of Chloroanthus japonicas against Botrytis cinerea and Sclerotinia sclerotirum. The extract included sesquiterpenoid and sesquiterpenoid lactones, which accounted for its antifungal action. Sesquiterpenoid lactones inhibited S. sclerotirum growth by 82.61% at a concentrationof 50 μg/mL.

Singh et al. (2016) isolated and discovered a phytosterol from Duranta repens known as durantol. Durantol was shown to be particularly efficient against sorghum mildew-like at a plant oil concentration of 5%.

Falade (2016) investigated the effects of extracts of Jatropha gossypifolia, Tridax procumbens, Sidaacuta, Blighiasapida, Datura stramonium, and Ricinus communis on

Colletotrichum lindemuthainum. D. scandens had a greater fungal inhibitory effect than R. communis & J. gossypifolia, while B. sapida exhibited least growth inhibition.

Mongkol et al. (2016) analyzed the dichloromethane extract of Mansoniagagei drum and tested it against Alternariaporri, Colletotrichumgloeosporioides, Fusarium oxysporum, & The fungus parasitica. Theextract contained mansorins A, B, & C, as well as mansonones C, E, G, & H, and the presence of mansonone made the plant extract more effective against Colloetotrichum gloeosporioides.

Bhuyan et al. (2015) investigated the resistance of six plant species, Cinnamomumimpressinervium, Cinnamomumtamala, Cymbopogoncitratus, Cymbopogon jwarancusa, Catharanthusroseus, and Tithonia diversi, against Alternaria Colletotrichum gloeosporioides and Fusarium monilforme. Cinnamomum impressinervium has the greatest antifungal action against capsicum gloeosporiodes and A. alternative when compared to Cinnamomum tamala, Cymbopogon jwarancus, and Cymbopogon citratus.

Fardin and Young (2015) tested an extract of Avicennia schaueriana against the postharvest disease anthracnose produced by Capsicum gloeosporioides. The extract exhibited the strongest antifungal efficacy against Capsicum gloeosporioides. Lupeol and naphthoquinones were identified as active compounds that suppress fungus growth.

Prasad (2015) examined the extracts and aqueous extracts of Lanatana camara, Mikaania micrantha, Sphagneticola trilobata, Cyperus rdundus, Mangifera indica, Carica papaya, Citrus limon L., and Perseaamericana mill against Colletotrichum gloeosporiodes. Lantana camara has more antifungal action against Capsicum gloeosporides than Citrus limon or Persea Americana. Mikania micranthaKunth, Sphagneticola trilobata (L.) Pruski, 7 Cyperus rotundus L had no inhibitory impact on fungi.

Silva et al. (2015) identified the Ricinus communis trypsin inhibitor (RcTI) and discovered that it was an active inhibitor of Colletotrichum gloeosporioides growth.

Qing-Hu et al. (2015) extracted the flavonoids sacriflavone A and sacriflavone B from a chloroform extract of Artemisia sacrorum Ledeb. Both flavonoids were shown to be efficient at inhibiting the development of Fusarium oxysporum.

Hussain et al. (2015) identified scandenin, scandenin A, betulinic acid, lupeol, and β-sitosterol glucopyranoside in the root and stem extract of Derris scandes. It was discovered that the presence of these chemicals caused the extracts to exhibit antibacterial (against Escherichia coli and Bacillus megaterium) & antifungal action (against Microbotryumviolaceum).

According to Juarez et al. (2015), antifungal activityof the oils obtained from Agastache mexicana ssp. Xolocotziana & Porophyllum percent linaria can be attributed to the presence of estragole and methyl eugenol in A. mexicana and linoleic acid and phytol in P. linaria.

Kalidindi et al. (2015) tested aqueous, chloroform, & methanolextracts of Annona squamosal Linn. leaves against five fungal phytopathogens: Alternaria alterna, Candida albicans, Fumarium solani, Microsporum canis, and Aspergillus The extracts contained glycosides, saponins, tannins, flavonoids, which phenols, and other chemicals, which resulted in high antifungal and antioxidant activities. The tannin in the chloroform extract had the strongest antifungal efficacy against Fusarium solani.

Khanam et al. (2015) obtained methanol, acetone, ethyl acetate, chloroform, and petroleum-based ether from Eurycomalogi folia. Terpenoid was found in all stem and root gathers, with the ethyl acetate stem extract inhibiting Aspergillus niger growth significantly.

Xie et al. (2015) investigated antifungal activityof clove oil against three fungi: Trametes hirsute, Schizophyllum commune, and Pycnoporus sanguineus. Because of presence of eugenol,clove oil has antifungal properties; additional compounds such as eugeno, methyleugenol, and acetyl-isoeugenol have also been found from clove oil.

Sharma and Kulshreshta (2015) found that anthracnose disease of chilli cousing Colletotrichum capsici displayed numerous morphological and physiological changes, as reported by Arx von (1957), who characterized the fungus's morphology and spores as irregular & appearing as brown toblack spots.

Saket et al. (2015) investigated cultural & morphological characteristicof Colletotrichum capsici isolates grown in PDA medium. They discovered that colony growth

begins in 1 to 2 days at 27 + 20C under darkness on PDA, and the colony color appears to be grey in the dark and white in Oat meal agar.

Alvarez et al. (2014) investigated the antifungal impact of flavonoid-containing asparagus extract. The aqueous extract was shown to suppress Fusarium oxysporum, F. oxysporumf.sp. dianthi, F. oxysporumf.sp. asparagi, and F. oxysporum.

Omezzine et al. (2014) investigated influence ofdifferent developmental stages and ploidy levels on the antifungal capability of Trigonella foenum-graecum L. Aqueous extracts of T. foenum-graecum were produced throughout vegetative, blooming, and fruiting phases using diploid and mixploid plants. The extract from diploid plants during vegetative growth showed the greatest suppression of Fusarium oxysporumf sp. Lycopersici when compared to extracts from other stages.

Rashed et al. (2014) found that methanolicextract of Diospyros virginiana fruits has antifungal activity against six fungi: Aspergillusfumigatus, Aspergillus versicolor, Aspergillus ochraces, and Aspergillusniger. Trichodermaviride, Penicillum cyclopium. The inclusion of flavonoids, as well as phenolics, contributed to the extract's high antifungal activity.

Ademe et al. (2014) investigated 18 plant extracts againstpapaya anthracnose (Colletotrichumgloeosporioides). Nine plants out of 18, including Artemisia afra, Echinops sp., Lantana viburnoides, Ocimum lamifolium, Ocimum sp., Rutacha lepensis, Thymus serrulatus, Vernonia amygdalina, and Zingiber officinale,were found tobe more effective against Capsicum gloeosporioides. The 50 mg/ml methanol extracts ofEchinops sp., Thymus serrulatus, & Ocimum laifolium successfully reduced Capsicum spore germination. gloeosporioides.

Baize et al. (2014) tested aqueous and methanol leaf extracts from 21 plants against Colletotrichum musae. Compared to Prosopis julifloria, 2% leaf extract of Acacial albida inhibited fungal conidial germination more effectively. The aqueous extract was shown to be heat stable in antifungal activity, followed by P. julifloira.

Dooh et al. (2014) tested aqueous, ethylacetate, and methanol extracts from Thevetia peruviana seeds against Colletotrichum gloeosporides. Only acetone and methanol exhibited

antifungal action against Capsicum gloeosporioides. Only the acetone seed extract completely inhibited spore germination at all concentrations tested.

Handiso & Alemu (2014) found antifungal activity in five distinct noxious plants: Sennaoccidentalis, Melia azendrachta, Partheniumhysterophorus, Calotropis procera, & Argemonemexicana, against Colletotrichum kahawae. These plants' leaf and seed extracts, both alcoholic and aqueous, were tested. The aqueous extract of two examined sections of Melia azendrachta and Senna accidentalis had the largest zone of inhibition, whereas the alcoholic extract of Calotropis procera and Senna occidentalis leaf and seeds showed potential antifungal activity. The aqueous seed extracts of Capsicum procera and S. occidentalis had more inhibitory efficacy than the leaf extracts.

Harsha et al.(2014) investigated antifungal efficacy of methanol leaf extracts from three citrus species againstColletotrichum capsici. Citrus reticulate was shown to be more effective in suppressing fungal mycelium development.

Prashitk-Kekuda et al. (2014) investigated impact of cow urine plant extract on Colletotrichum capsici. Extract from Anacardiumoccidentale L., Pimenta dioica (linn) Merill., and Alpinia galangal Wild & Anisomeles indicaLinn demonstrated growth inhibition of fungal mycelium.

Shinde and Gawai (2014). Extracts from eleven plants, including Azadirachta indica, Ocimum sanctum, Tridex procubens, Clerodendron innermis, Cathranthus roseus, and Ricinus communis, were evaluated against Colletotrichum capsici. A 15% alcoholic extract of A. indica and O. sanctum demonstrated significant antifungal activity.

Kumar et al. (2014) discovered that Colletotrichum capsici infection affects three kharif crops (mungbean, bottle gourd, and soyabean) as well as three weeds (chilmil, kondhra, and santhi).

Gautam (2014) observed that in India, many plant diseases are caused by Colletotrichum species. the fungus Coll capsici, Colletotrichum gloeosporioides, the fungus Coll truncatum, the fungus Coll falcatum, the fungus Coll acutatum, Colletotrichum sansevieriae, and Colletotrichum coccodes all caused around 25 plant diseases. According to

the study, even a single species of Colletotrichum can impact many hosts. Colletotrichum are among world's most importantplant infections, causingeconomically significant plant diseases.

Ademe et al. (2013) investigated their antifungal activity. Lantanacamara, Lantana viburnoides,Echinops sp, & Rutachalepensis have high antifungal properties, while Lanatana camara ethyl acetate extract inhibited fungal growth.

Chen et al. (2013) tested an extract of Jerusalem artichok (Helianthus tuberosus L.) against nine fungi, including Botrytis cinerea, Colletotrichum gloeosporioides, Phytophthora capsules Leonian, the fungus Rh cerealis, Exserohilum turcicum, Gaeumannomyce sgramin, Gibberella zeae, Pyricularia, and Sclerotinia sclerotiorum. The leaf extracts from these plants included six phenolic compounds. Only three compounds, caffeic acid, 3,4-dicaffeoylquinic acid, and 1,5-dicaffeoylquinic acid, demonstrated significant antifungal activity against B. cinerea, capsicum gloeosporioides, Phytophthora capsici Leoniam, and R. cerealis.

Kumaran et al. (2013) examined an extract of Rauvolfia tetraphyla and the chemical fungicide dithane-M45 against Colletotrichum, generating polymethyl galacturonase, pectin transelimnase, and carboxymethyl cellulase enzymes. Rauvolfia tetraphyla extract inhibited the enzyme generated by Colletotrichum more effectively than the chemical fungicide dithane-M45.

Sangeethaet al. (2013) investigated effectiveness of plant leaf extracts from Zimmu (an interspecific hybrid ofAllium cepa L. × Alliumsativum L.) against Lasiodiplodia thebromae & Colletotrichum musae. The aqueous tuber extract of Zehneria scabra had the best antifungal effectiveness against fungal mycelium development and spore germination at a concentration of 25%. The treated banana fruit demonstrated enhanced shelf life and 100% rot disease inhibition at 14°Cfor 35 days & 85% at 28°C for 12 days. Fruit shelf life was extended by significantly increasing phenylalanineammonia-lyase (PAL), chitinase, & β-1,3 glucanase levels, whichare fungi hazardous to pathogens.

Cruz et al. (2013) found that Azadirachta indica and citric plant extracts have antifungal action against Colletotrichum musae in immature and mature banana fruit during post-harvest at concentrations of 2% and 4%. At a concentration of 2%, A. indica extract demonstrated 75.13% illness severity and 20.85% disease control. The most successful therapy was a 4%

concentration of citric plant extract, with illness incidence, severity, and control rates of 19.44%, 9.34%, and 90.16%, respectively.

In astudy by Ammar et al. (2013), the methanolicextract of Tephrosia apollinea L. was tested against fourphytopathogenicfungi: Alternaria alternata, Helminthosporium sp.,Colletotrichum acutatum, & Pestalotiopsis sp. The extract inhibited the fungi by 32.8-58.3%. The phytochemicalanalysis revealed presence of four prenylated flavonoids: isoglabratephrin, (+)-glabratephrin, tephroapollin-F, and lance.

Mostafa et al. (2013) extracted spirostane-type glycoside aginoside from Allium nigrum leaf, root, and bulb extracts using hexane and methanol. The isolated metabolite was evaluated for antifungal activityagainst Colletotrichum gloeosporioides, Botrytis sqamosa, & four subspecies of Fusariumoxysporum. At 400 ppm concentration, anginoside was shown tobe efficient in inhibiting growth of Capsicum gloeosporioides, F. verticillioides, & Botrytis sqamosa, followed by F. oxysporumf. sp. cepae and F. oxysporumf. sp. radices-lycopersici in decreasing order.

Vogt et al. (2013) studied the antifungal activity of hexane, chloroform, and methanol-based extracts of Larrea divaricate against Fusarium graminearum. The extract of chloroform included apigenine-7-methylether, nor-dihydroguaiaretic acid, and 3,4-dihydroxy-3,4-dimethoxy-6,7'-cycloignan, all of which have antifungal properties.

Parey (2013) investigated the pathogenicity of Colletotrichum capsici isolates from detached chilli fruits using various inoculation techniques. The chilli fruit variety pussa jawala was the most virulent,with an average lesionsize of 10.95mm. The evaluationof chilli cultivars against Colletotrichum capsici indicated thatnone were resistant. However, LCA-235, LCA-301, LCA-333, Ankalohit, and DC-4 demonstrated considerable resistance under field pot growing conditions and minimal lesion size using the detached approach.

Kartar (2013) discovered that Colletotrichum capsici infects four kharif crops, including soyabean, urdbean, cowpea, and sesamum, as well as two weeds, bill goat and begger's weed.

Kartar (2013) conducted an in vitro test against Colletotrichum capsici using four plant extracts. Siras (50%) was determined to be the most significant, inhibiting 100 percent of spore germination and lowering mycelial development by 84.25 percent.

Bussaman et al. (2012) extracted Piper sarmentosum using 80% ethanol, methanol, and chloroform and discovered that it has extremely significant antifungal activity against Colletotrichum gloeosporioides. Methanolic extracts completely inhibited fungal mycelium development, followedby chloroformextract (81.85%) & ethanol extract (45.50%).

Masangwa et al. (2012) conducted assays against Colletotrichum lindemuthianum and Colletotrichum dematium using acetone and aqueous extractsof Ipomoea batatas,Carica papaya, Allium sativum,Syzygium cordatum,Chlorophytum comosum, & Agapanthuscaulescens at concentrations of 0.78, 1.56, 3.13, 6.25, and 12.5 mg/l.

According to Prakash et al. (2012), Colletotrichum copsici infects three kharif crops (black gram, cotton, and raddish) as well as four weeds (kagaroti, kharjal, sawank, and mirch booti).

Dean et al. (2012) observed that Colletotrichum spp., one of primary plant pathogenicgenera, produces anthracnose disease on a wide range ofhosts, including grasses & trees.

Ismet Ara et al. (2012) investigated the antagonistic impact of actinomycetes on the Colletotrichum musae pathogen duringpost-harvest anthracnose ofbanana. Actinomycents isolates were cultivated with Colletotrichum musae in vitro using the dual culture technique to test their antagonistic ability.

Mukherjee et al. (2011) found that 30%, 40%, 50%, 60%, and 70% aqueous leaf extracts of tobacco and seeds of keora, mahogoni, garlic, and ginger have antibacterial activity against Colletotrichum gloeosporioides. At a 50% concentration, garlic extract was shown to be effective against Capsicum gloeosporioides, followed by keora seed, ginger, mahogany, and tobacco.

Shinde and Gawai (2011) investigated the effectiveness of Azadirachtaindica, Ocimum sanctum,Tridex procubens, Clerodendron inermis, Cathranthusroseus, Ricinus communis, and Citrus limonagainst Colletotrichum gloeosporioides. The whole 15% aqueous and alcoholic extract of Ocimum santum and Clerodendron inermis inhibited fungal growth the most effectively.

Song et al. (2011) investigated the efficacy of Astilbe myriantha Diels against Rhizoctonia solani, Fusarium oxysporum f sp. Colletotrichum lagenarium, and Penicillium digitatum. The portion of ethanolic root extract included seven terpenoids. 3β 6β 2-4 trihydroxyurs -12-27-oic acid (7) effectively suppressed the development of Colletotrichum gloeosporioides.

According to Phoulivong et al. (2011), Colletotrichum can cause damage to any portions of chilli plant at any stage ofgrowth.However, fruitlesions on fruits are most commercially significant part of anthrax disease.

Madhumith and Saral (2011) investigated antifungal activityof ethylacetate, methanol, & petroleum etherextracts of Crossandra infundibulformis against Aspergillus niger, A. flavus, A. fumigatus, & Penicillium chrysogenum. The petroleum ether extract included phenolics, tannins, flavanoides, and terpenoids, which likely contributed to its superior antifungal activity against the tested fungus comparedto the otherextracts.

Sangdee et al. (2011) investigated conidia morphology of the various groups and discovered that it was fusiform with pointy ends.

Thangamani et al. (2011) demonstrated the pathogenicity of Colletotrichum musae by inoculating banana fruits using the pin prick technique. Following inoculation, the fruit surfaces were covered with damp cotton and maintained inside the moist chamber. Infection was detected after seven days.

Pandey (2011) discovered that mango anthrancnose infection requires moisture and warmth. It commonly emerges after a rainstorm or an extended period of dampness. Weir et al. (2012) found that the majority of crops farmed throughout the world are vulnerable to one

or more Colletotrichum species. According to Saket et al. (2015), Colletotrichum capsici infects chillies under high humidity during mature and immature fruit conditions.

Kumar et al. (2011) investigated antifungal efficacy of aqueous leaf extracts from three Plumbago speciesagainst Colletotrichum gloeosporioides, fungus responsible for anthracnose disease. Plumbago indica leaf extract significantly inhibited radial mycalial growth (98.75%) and conidial germination (98.9%) of the pathogen.

According toJohnny et al. (2011), methanol crude extracts of Piper betle at concentrations of 12.5, 15, 17.5, & 20 μg/ml had the highest antifungal activity, inhibiting Colletotrichum capsici by 72.30 to 85.18%, followed by A. galangal at 68.77 to 74.60% and Centella asiatica at 57.60 to 71.87%.

Machowicz-stefaniaki and Zalewska (2011) discovered that the fungicides dithane neo tec and helm-cymi 72.5 were most effective against Colletotrichum dematium at 100 ppm concentration in terms of growth and development.

Jamadar and Lingaraju (2011) evaluated systemic fungicides in vitro against Elsinoe ampelina, a fungus that causes grapevine anthracnose. They observed that hexaconazole 5E was the most effective fungicide, inhibiting fungus completely, followed by carbendazim (97.5%) and thiophanate methyl (89.3%) at various dosages.

Akinbode O.A. et al. (2011) investigated antagonistic impact of two Trichodermaspecies, Trichoderma pseudokoningii and Trichoderma harzianum, on the pathogen Colletotrichum destructivum, which causes anthrocnose of cowpea. It was discovered that Trichoderma pseudokoningii and Trichoderma harzianum were the most effective against the infection.

Al- Reza et al.(2010) investigated hexane & methanol extractsof Cestrum nocturnum & its essential oil against plant pathogenic fungus suchas Botrytis cinerea, Colletotrichumcapsici, Fusarium oxysporum,Fusariumsolani, and Scleotinia scleotiorum. The chloroform extract of plant had stronger antifungal activity against Colletotrichum capsici than ethanol and methanol extracts, and the essential oil of the extract did not impede capsicum capsici conidia germination.

Veloz-Garcia et al. (2010) investigated the Caesalpinia calaco plant and determined that it contains phenolic compounds such as gallic and tannic acid. These phenolic compounds (gallic and tannic acids) showed potential action against Colletotrichum lindemuthianum. The highest mycelium growth inhibition was discovered to rise with increasing concentration of phenolic component.

Kanchalika et al. (2010) obtained 34 Colletotrichum species isolates from bell pepper anthracnose disease, including two species, Colletotrichum capsici and Colletotrichum gloeosporioides. Pathogenicity assays classified pathogenic potential into low, medium, & high virulence groups among the three hosts, and Colletotrichum capsici was obviously the most virulent isolate.

Ratanacherdchai et al. (2010) identified thirty-four Colletotrichum spp., including two species, Colletotrichumgloeosporioides & Colletotrichum capsici, from anthracnose on bell pepper, long cayenne pepper, & bird's eye chili & demonstrated their pathogenicity by fruit inoculation.

Deyol (2010) revealed that garlic oil (100%) was effective when red chilli fruit were initially sprayed with oil, followed by pathogen inoculation 48 hours later.

According to Ngullie et al. (2010), plant extracts of Allium sativum and Azadirachta indica inhibited Colletotrichum gloeosporioides mycelia growth to the greatest extent at a 10% concentration.

Munoz et al. (2009) investigated antifungal activity on the tomato and grape plants. Colletotrichum sp. was isolated from diseased tomato and grape fruit. Isolated Colletotrichum was treated with chitosan at concentrations of 1, 1.5, 2, and 2.5%, and an aqueous chitosan solution was shown to be more efficient in reducing lesion width on tomato and grape fruits.

Damm et al. (2009) stated that Colletotrichum dematium was first recovered from an Eryngium stem in France, as well as from Solanaceous hosts.

According to Robert et al. (2009), Colletotrichum spp. causes illness in all regions of pepper plant at any stage of growth. Lesions on fruit that are initially water-soaked grow soft and somewhat sunken with time. Multiple lesions appeared on the fruit, and the surface of the lesions was coated in moist, gelatinousspores from salmon-colored fruiting bodies known as acervuli, as well as many black spines.

According to Hyde et al. (2009), anthracnose disease growth under favorable conditions can cause fruit damage of up to 50%. Fruit rot occurs at 280 degrees Celsius and 95.7% relative humidity.

According to Roberts et al. (2009), Colletotrichum spp. may be found on different solanaceous crop hosts such as potatoes, egg plants, and tomatoes.

Vinaya et al.(2009) gathered chilli seed samples from several chilli growing regions in Northern Karnataka and discovered Colletotrichum capsici to be the most prevalent fungus. Conidia are somewhat pink or hyaline, with rounded ends. Conidia germinates and develops one to four germtubes, which give birth tomycelium. The mycelium is septate and highly branched, starting out hyaline and darkening as it matures.

Yun et al. (2009) discovered that Colletotrichum capsici generates a clean circular border in the colony on the second day, as well as grey white mycelium in isolation cultures. Colletotrichum capsici measured 13.21 x 14.21 μm length and 1.79 to 3.28 μm wide.

According to Roberts et al. (2009), anthracnose disease is more likely to occur on both ripe and immature fruits. During wet conditions, spores are washed or splattered, causing significant losses.

In vitro investigations by Anand and Bhaskaran (2009) revealed that leaf extracts ofAbrus precatorius (gundumuthu) & Aegle marmelos(vilvum) inhibited mycelial development & spore germination of Colletotrichum capsici most.

Nduagu et al., (2008) studied the effect of the aqueous extracts of leaf, stem bark and root of twelve plants; Aonnona senegalensis, indica Azadirachta Chromolaena odorata, Citrus limon, Cochlospermum planchonii, Hymenocardia acida, Ocimum gratissimum, Psidium

guajava, Ricinus communis, Tephrosia vogelii and Vernonia amygdalina on the mycelium growth and spore germination of Colletotrichum capsici. They observed that only the stem and root bark extracts of A. indica, V. amygdalina, and Capsicum planchonii could control Capsicum capcisi, whereas leaf extracts from other plants had no influence on it.

Than et al.(2008b) stated that Colletotrichum dematium has recently been isolated from a variety of hosts, including a chilli pathogen.

According to Ramachandran et al. (2008), chilli anthracnose disease has become a significant constraint in all chilli growing locations across the world, regardless of crop type. Nduagu et al., (2008) studied the effect of the aqueous extracts of leaf, stem bark and root of twelve plants; Aonnona senegalensis, indica Azadirachta Chromolaena odorata, Citrus limon, a Cochlospermum planchonii, Hymenocardia acida, Ocimum gratissimum, Psidium guajava, Ricinus communis, Tephrosia vogelii and Vernonia amygdalina on the mycelium growth and spore germination of Colletotrichum capsici. They observed that only the stem and root bark extracts of A. indica, V. amygdalina, and Capsicum planchonii could control Capsicum capcisi, whereas leaf extracts from other plants had no influence on it.

Mistry et al.(2008) revealed that, disease has been seen in 2 phases.They are a) leaf spot & Dieback & b) anthracnose orfruit rot. Capsicum fruitrot affects fruit dry weight & amounts of oleoresin & capsacin.

Taylor et al. (2008) found that isolates from chilli fruits exhibiting anthracnose the fungus Coll symptoms were Colletotrichum capsici, the bacterium Coll gloeosporioides, and the fungus Coll acutatum.

Kumar (2008) discovered that an in vitro extract of the datura plant inhibited Colletotrichum capsici spore germination completely.

Tiwari et al.(2008) discovered thatplant extracts of onion, garlicbulb, and Datura leaf had an antifungal impact on Colletotrichum capsici, fully inhibiting mycelia development and sporulation.

Poonpolgul and Kumphai (2007) showed that anthracnose disease affected commercial chilli yields in Thailand by 10 to 80 percent.

Hingole and Kurundkar (2007) found chilli output losses of up to 50-55% in the Marathwada area.

According to Baliyan and Vishunavat (2007), infected soybean seeds have an irregular brown to uneven gray discoloration on their seed surfaces. Infected soybean seeds are withered and smaller in size.

Kumar and Yadav (2007) found symptoms on betelvine (Piper betel) leaves, creating leafspot or marginal blight, as well as anthracnose signs on the stem, which surrounded the internodes with brown to black lesions and caused significant harm to the plant.

Webster and Weber (2007) described a saucer-shaped acervulus encircled by stiff, unbranched black hairs known as setae. Slimy droplets generate curved elongated conidia that are kept inplace by stiff black setae that surround acervulus. The conidia are fusoid, aseptate, and somewhat curved or sickled formed.

Rajapakse et al.(2007) discovered that after 10 days, all Colletotrichum capsici isolates produced spores on PDA medium. After 10 days, the amount of spores grows.

Gopalkrishna and Prakasam (2007) obtained five Colletotrichum isolates from naturally infected chilli fruits, French bean green pods, lab lab, sugarcane, and turmeric leaves, representing three species: Colletotrichum capsici, Colletotrichum lindemuthianum, and Colletotrichum falcatum. They discovered that in cross-inoculation tests, chilli and sugarcane isolates did not infect other fruit hosts, however turmeric isolates did. French bean and lablab isolates were shown to cause identical symptoms in both hosts.

Sunil Kumar et al. (2007) compared phytoextracts of Allium sativum, Azadirachata indica, and Datura stramonium to Colletotrichum gloeosporioides and Colletotrichum capsici. They discovered that among the three phytoextracts, Allium sativum was the most efficient in inhibiting the conidial germination of Colletorichum gloeosporioides and Colletotrichum capsici.

Venkataravonappa and Nargund (2007) discovered that at a three percent concentration of Ocimum sanctum, Azadirachta indica, and Prosopis juliflora inhibited Colletotrichum gloeosporioides spore germination the most effectively.

Suthin et al. (2006) recovered the Colletotrichum capsici pathogen from chili fruit samples collected in Chidambasam. Chilli plants that were 105 days old were inoculated and housed in a glass house. They were sprayed with sterile water before being treated with Colletotrichum capsici. Conidial suspension with an atomizer in lateevening. The controlplants were treated with sterile distilledwater. Fruit rot was found on a periodic basis.

Farr et al. (2006) discovered that the pathogen's distribution is global, since the main inoculumis extensively disseminated bywind or rain. Thepathogen loves humid and warm weather conditions to transmit anthracnose to a variety of plant hosts, including vegetables, crops, grasses, ornamental and fruit plants, angiosperms, and gymnosperms.

Raj et al. (2006) investigated the efficiency of selected plant products Allium sativum (20%), Eucalyptus globules (60%), Datura metel (60%), and Prosopis juliflora (60%), against Colletotrichum capsici. Among the evaluated plant items, Allium sativum had the lowest illness incidence, followed by Eucalyptus globules.

Gorawar et al. (2006) evaluated efficiency of several plant extractsagainst Colletotrichum capsici, which causes turmeric leaf spot. They discovered that at 10%, Datura leaf extracts reduced mycelial growth the greatest (84.42%), followed by Parthenium (74.08%), ginger (55.18%), Asatoetida (42.97%), and Honge (33.16%).

Imtiaj et al. (2005) evaluated fungicidal efficacy of aqueousextracts of Tagetes erecta (leaf), Curcuma longa (rhizome and leaf), and Zingiber officinales (rhizome) to five fungicides: cupravit, bavitin, dithane M-45, thiovit, and redomil against Colletotrichum gloeosporioides. Their findings showed that the plant extract was more efficient than synthetic fungicides against Capsicum gloeosporioides.

Srinivas (2005) revealed that Colletotrichum capsici were the most significant infection found in seed samples collected in Mulbagal village, Kolar district, Karnataka. Seed-borne diseases appear tobe widespread in harvested seedsamples from impacted farms.

Pakdeevaraporn et al. (2005) found that chilli output losses in Thailand severely infected with capsicum capsici can be as high as 50%.

According to Lakshmeshsa et al. (2005), disease symptoms appeared only on fully matured chilli pods or damaged chilli fruits. On the third day, water-soaked lesionsappeared on surface of chilli fruit, as did browinish discolouration lesions and visible mycelia. In later stages, lesions combine to produce a huge anthracnose-infected region, followed by production ofacervuli with concentric rings on the fruit surface.

Srinivas et al. (2005) discovered that infected chilli fruits lose their natural red color and turn yellow or, in some cases, light white. The infection also causes delicate twig necrosis, which progresses to the entire branch.

According to Agrios (2005), the acervuli pathogen generates conidia that measure 17.18 x 3.4 μm, are one-celled,cylindrical, colorless, & can be dumbbell shaped or curved.

Kaur et al. (2005) utilized inoculums with a density of 5×105 spores per milliliter as standard. Freshly picked matured fruits from healthy plants cultivated in glasshouses are pin pricked using a consistent drop ofspore solution. Theinoculated fruits were put in ahumidity room, & symptoms appeared seven days later.

Lakshmesha et al. (2005) found that the average length & breadth of conidia ranged from 23.5 to 35.0 μm & 2.5 to 3.75 μm.

Rao and Narayana (2005) discovered in vitro that six plant extracts have antifungal efficacy against Colletotrichum dematium, which causes chickpea blight. They found that a 10% leaf extract of Polyathia longifolia considerably suppressed the mycelial development of test pathogen by up to 30.55%.

Jalali et al. (2005) found that extracts of bougenvillea flower suppressed Colletotrichum falcatum's radial development.

Voorrips et al. (2004) discovered that typical signs of anthracnose disease on chili fruits include water-soaked lesions. In some cases, lesions develop dark to black and can grow to 2 - 3 cm indiameter on big fruits, sunken necrotictissues, and concentric rings ofacervuli. Anthracnose disease begins at the flower bud's growth point, and damaged upper branches wither and turn brown. Infected plants yield few low-quality fruits.

Kim et al. (2004) discovered that anthracnose disease occurs on fruits and foliage as tiny, circular spots that merge to become huge elliptical patches.In extreme circumstances, afflicted plants lose their leaves.

Ray (2004) found that mature chilli fruits had tiny, sunken circulardepressions up to 30 mm indiameter. The center of lesions turns brown, while underneath the tissue, the lesions grow lighter in color and are dotted with many black fruiting bodies with concentric rings. Green fruits may alsobe infected with fungus, although symptoms may not manifest until fruit ripens at harvest time. A latent infection is one that is not fully active.

Melanie et al. (2004) found that on immature fruits, round or angular sunken lesions occur, and when illness is severe, lesions may combine. Acervuli are black structures that occur within a lesion. On the surface of lesions, pink to orange masses of fungal spores grow in concentric rings. The disease can spread quickly across a pepper crop, resulting in a 0% yield loss.

Bagri et al. (2004) researched chilli fruit rot and found that mature chilli fruits lost 10-15% of their yield.

Rathore (2004) found severe losses of up to 50-55 percent in fruit production of red fruits caused by Colletotrichum capsici.

According to Kim et al. (2004), different species of Colletotrichum cause disease on various parts of chilli plants. Colletotrichum and Colletotrichum acutatum infect chilli fruits at

all developmental stages but rarely infect leaves orstems, which are mostly damaged by Colletotrichum dematium and Colletotrichum coccodes.

Oanh et al. (2004) demonstrated pathogenicity on chilli seedlings grown in a plastic tray, and inoculums were sprayed into chilli plant leaves using an atomizer spray.

Saikia et al. (2004) identified and purified Colletotrichum capsici from sick chilli fruits, demonstrated its pathogenicity, and cultured it on PDA medium.

Ray (2004) discovered that Colletotrichum capsici may survive on alternative hosts such as potato, tomato, cucumber, egg plant, & many other cultivatedcrops. Infection does not need fruit wounds, although moist conditions are required for spore germination and infection. Water splashes or wind-driven rain are necessary to disperse microsclerotia or fungal spores.

Dubey and Ekka (2004) found that Colletotrichum capsici isolates from the chilli tree infect mungbean, bittergourd, or urd bean.

Melanie et al. (2004) discovered that Colletortichum acutatum causes pepper anthracnose by feeding on plant debris from infected crops & other sensitive plant species.

Pratibha et al. (2004) stated that during wet weather, substantial lossesoccur because sporesare splashed or washed onto otherfruits, resulting in additional illnesses. Also noticed was that the highest severity of fruit rot occurred in the morning when the leaf surface was moist with dew deposition, with amaximum temperatureof 32.60C and a minimum temperature of 190C.

According to Ray (2004), chilli fruits on soil surface are most susceptible to infection via rain splashes or direct soilcontact. The optimal temperature for fruit infection is 280C to 320C with fruitsurface wetness, however infection can occur at temperatures ranging from ten to 300C. The longer the fruit is moist, the more severe the anthracnose.

Meera et al. (2004) found that extract of Allium sativum totally prevented conidial germination & mycelial development of Colletotrichum capsici, & that a 20% spray on chilli crop provided maximal control of anthracnose disease with increased yields.

Bagri et al. (2004) investigated antifungal effects of Datura (Datura stramonium), bitter temru (Diospyros cordifolia), babool (Acacia nilotica), amaltas (Cassia fistula), brhati (Solanum indicum), mehandi (Lawsonia inermis), & sandal (Santalum album) extracts for the treatment of chillifruit rot. They discovered that using poison food approach method resulted in greatest mycelia growth and spore germination suppression in bittertemru fruit extracts, followed bydatura leaves.

Yadav (2004) discovered that at 4% concentrations, Allium sativum and Azadirachta indica totally suppressed mycelial development of the fungus Coll gloeosporioides, followed by Datura stramonium.

Singh et al. (2003) investigated anthracnose disease of hybrid strawberries caused by Colletotrichum dematium and discovered tiny, dark brown patches on the leaves.

According to Bosland & Votava (2003), anthracnose disease exhibits quiescence, which means that symptoms do not appear until fruit ripens. The problem generates significant losses in both before and post-harvest fruit degradation.

Rangarajulu et al. (2003) isolated Colletotrichum capsici from infected chilies and tested it for pathogenicity using the pin prick technique.

According to Sanders and Korsten (2003), Colletotrichum capsici is cosmopolitan, meaning it might be a single species that lives on numerous hosts or multiple species that live on the same host.

Pernezny et al. (2003) discovered that Colletotrichum species may live in and on seeds as microsclerotia and acervuli.

Kumaran et al.(2003) investigated the fungitoxic impact of root ethanolic extracts from 18 different plant species on Colletotrichum capsici, which causes chilli anthracnose. They discovered that ethanolic root extracts of Rauvolfia tetraphylla and Abrus precatorius inhibited radial growth & conidial germination of Colletotrichum capsici.

According to Chandrasekaran and Rajappan (2002), anthracnose and pod blight induced by Colletotrichum truncatum resulted in 30 to 35 percent losses in soybean. Gaikwad et al. (2002) observed that fruit rot of custard apples induced by Colletotrichum gloeosporioides resulted in yield losses of 60-70% or higher in Maharashtra.

Narasimhudu and Balasubramanian (2002) investigated turmeric yield leaf spot caused by Colletotrichum capsici and found yield losses ranging from 15 to 60 percent in Andhra Pradesh's Cuddapah area.

Narasimhudu and Balasubramanian (2002) discovered that signs of leaf spot caused by Colletotrichum capsici occurred two months after planting in a favorable climatic setting.

Gaikwad et al. (2002) discovered that Colletotrichum gloeosporioides causes anthracnose in custard apples, with symptoms including blackish brown, round, and sunken patches on the fruits. Which, under favorable conditions, spreads throughout the fruits, producing shriveling, premature fruit drops, and rotting of mature fruits.

Rajapakse et al. (2002) devised a screening approach for chilli anthracnose in the field and discovered that all isolates tested using the pinprick method (wound inoculation with conidial suspension) had anthracnose symptoms on the fruits.

Meenu and Gerg (2002) investigated effects of rainfall, temperature, & humidity on incidence & severity of fruit rot inchilli caused by the fungus Coll capsici. They found that the cumulative effect of rainfall was most significantly and positively correlated with the incidence of fruit rot disease and was not significantly correlated with other weather-related variables studied.

Madhusudhan (2002) examined 15 plant extracts in vitro against Colletotrichum truncatum. He discovered that Parthenium leaf extract inhibited fungal spore germination most effectively at 5 and 10% doses.

Hedge et al. (2002a) found that neem kernel extract (nimbicidin) at 0.3 percent suppressed Colletotrichum capsici growth much more than the control under the conditions of the greenhouse.

Roberts et al. (2001) found that illness spreads from plant to plant, and sick fruits serve as asource of inoculum in field. During rainy and warm seasons, rain or irrigation water sprayed conidia from acervuli micro-sclerotia from infected to healthy fruit and foliage. Other field crops that can serve as alternate hosts include potatoes, tomatoes, and egg plants.

According to Roberts et al. (2001), optimal conditions foranthracnose disease development include rainy weather, temperatures of 270°C, and relative humidity levels around 80%.

Chidanandaswamy (2001) investigated the antifungal activities of some plant extracts on Colletotrichum capsici, fungus that causes turmeric leaf spot in vitro, and discovered that Parthenium leaf extract was most effective in inhibiting Colletotrichum capsici growth, followed by garlic bulb extract.

Vibha Varshney (2001) investigated antifungal activity of plant extracts against Drechslera graminca, which causes stripe disease in barley. They combined leaf extracts of Lantana camara, Azadirachta indica, Tagetes erecta, and Pinus roxburghii with a water-soluble fraction of mustard oil cake. He discovered that Azadirachta indica and Tagets erecta extracts were the most inhibitory against Drechslera graminca.

Chitra and Kannabiran (2001) tested the antifungal efficacy of floral and fruit juices of Datura innoxia L. against Colletotrichum capsici in vitro. They discovered that both floral and fruit extracts reduced the fresh and dried weights of the fungus Coll capsici when compared to the control group without treatment.

Prusky et al. (2000) investigated the process of initial infection of Colletotrichum spp., which includes conidia attachment to plant surfaces, conidia germination, adhesive appressortia production and epidermis entry, plant tissue colonization, acervuli production, and sporulation.

Fernandez et al.(2000) discovered that infecting Colletotrichum species with a sensitive cultivar of Phaseolus vulgaris in suitable conditions results in 100% yield losses.

Bhale et al.(2000) discovered that the fungus Colletotrichun dematium generated many sickled-shaped conidia that could be viewed using a combination microscope.

Gomathi and Kannabiran (2000) gathered fruit samples from chilli producing areas near Pondicherry, which had classic fruit rot signs. Gloeosporium piperatum Ell and EV, as well as Colletotrichum capsici, were discovered and evaluated for pathogenicity.

According to Freeman et al. (2000), fungus-host connections are vast, ambiguous, and frequently overlap.

Gomathi & Kannabiran (2000) examined aqueous leafextracts from 23 wild plants against the anthracnose fungus Gloeosporium piperatum and Colletotrichum capsici, which infect Capsicum annuum. They discovered that leaf extracts of Datura metal, Solanum forvum, & Prosopis juliflorawere more efficient ininhibiting mycelia development and conidialgermination of these pathogens.

Bairwa et al. (2000) investigated integrated management against Colletotrichum capsici, which causes chilli anthracnose disease, utilizing bio-agents, plant extracts, and fungicides that Extracts of Ipomea SP., Datura stramonium, and The sativum inhibited Colletotrichum capsici growth by up to 59.4%.

Varaprasad (2000) discovered that a 10% extract of Polylathia longifolia suppressed the mycelial development of Colletotrichum dematium.

Chapter 3

Materials and Methods

3.1 Chemicals Used

Sodium hypochlorite	HCl	H2SO4
TLC silica gel 60 F254 plate	Chloroform	Ethyl acetate
Formicacid	Poly ethylene glycol	Phenolphthalein
NaOH	Sodium phosphatedibasic heptahydrate	
Sodium phosphate monobasic monohydrate		Boric acid
NaCl	Sodium tetraborate	Catechol
Guaiacol	Hydrogen peroxide	Sodium Acetate
Acetic Acid	L-Methionine	Sodium carbonate
Gallic acid etc.		

3.2 Glassware and Equipments

- Petri plates
- Measuring cylinder (100 and 20 ml)
- Micropipette (10, 100, 1000 µl)
- Pipette (10 ml)
- Test tubes
- Culture tubes
- Slides
- Beakers (20, 100, 250, 500 ml)
- Conical flasks (100, 500 ml)
- Concentration bottles
- Eppendorf tube
- Cover slips
- Funnel
- Filter paper
- Inoculum loop
- Glass rod
- L- shaped glass rod

- Tissue paper
- Microtip box
- Washing tray
- Washing bottle
- Compound microscope
- Systronics controller based spectrophotometer
- Autoclave
- Refrigerator
- Centrifuge
- Cooling centrifuge
- Hot air oven
- Incubator
- Forceps
- Heating mantle
- Water bath
- Scissors
- Lyophilizer
- Needle
- Spatula
- Brush
- Sprit lamp
- Cork borer etc.

3.3 Collection of Plants and their Identification

Different parts of medicinal plants were collected those are-

Acalypha indica**,**

Adhatoda vasica,

Alternanthera sessilis,

Cocculus hirsutus,

Mitragyna parvifolia,

Peristrophe paniculata

Terminalia bellirica

3.3.1 Isolation and Identification of Colletotrichum Capsici

It was simple to gather contaminated chilli fruits for study purposes from the market. The infected parts of the fruits were surface sterilized with 4% of an aqueous solution of sodium hypochlorite (NaOCl), & fruits werewashed again with sterilized distilledwater. Following cleaning, diseased area was aseptically transferredto a petriplate containing potatodextrose agar(PDA) media (Hi-media). Inoculated plates were sealed withparafilm & incubated at 25 ± 2 °C for seven days in darkness. After three days, incubated plates were examined, and mycelial growth was detected surrounding the inoculum. Mycelium was aseptically transferred/subcultured onto a new autoclaved pour plate with malt extractagar (MEA) & potato carrot agar (PCA). Cultural parameters such as colony color, growth pattern, exudate, texture, reverse color, conidia size & shape, presence of setae & appressoria, and so on were recorded for morphological identification. The identify of the fungus was also validated by the National Fungal Culture Collection of India, ARI, Pune, Maharashtra, using the Methuen Handbook of Colour.

3.3.2 Preparation of Extract

After collection, plant leaves and stems were weighed and washed4-5 times with tapwater before being rinsed again with distilledwater. The washedleaves and stems wereair-dried for three weeks in shade at roomtemperature. Furthermore, air-driedsamples were weighed again to assess the moisture content after being ground into powder in a grinder.

Thirty grams of powder were extracted using the soxhlet apparatus with 70% methanol, 70% ethanol, hexane, and distilled water, respectively. Using the normal extraction methodology, 30 g of plant martial powderwas put in a soxhlet thimble & extracted with 250 ml of solvent during 8 soxhlation cycles. The extract was concentrated using rotavapor and dried in a lyophilizer at -55 °C and 1.0 torr pressure for 2 hours (BioEra -55 °C model clout). To calculate the yield % of the crude extract, it was weighed after drying and kept at 4 °C in sterile vials.

The following formula was used to calculate Crude extract yield (CEY):

$$\text{Yield } (\%) = (\text{w1} \times 100)/\text{w2}$$

Where, W1 = weight of dry crude extract, & W2 = initial weight of dry plant material packed in the Soxhlet.

3.4 Antifungalactivity of Crude Extracts

The antifungal efficacy of medicinal plant leaf and stem extracts will be evaluated using food poison technique. The plantextracts will be dissolved in 0.5% DMSO (SRL) at five concentrations (1, 2, 3, 4, and 5 mg/ml) in a 500 µl container. After combining the extract, put 500 µl to a 90 mm petri dish, followedby 9.5 ml of potato carrot agar. The plate was kept at room temperature to allow the extract to permeate into media. Carmel antifungal (carbendazim 12% + mancozeb 63%) will be used as a positive control, with DMSO as the negative control. A 4 mm diameter mycelial disc will be put in the center of each petri dish using a cork borer and stored in an incubator at 25°C±2. Radial growth from the center will be monitored after the first, third, and fifth days of incubation. The percentage ofgrowth inhibition will be determined using followingformula.

Growth Inhibition % = $\frac{(C-T)}{C} \times 100$

Where, C = diameter of a fungalcolony in control.

T = diameter of a fungalcolony in treatment.

The food poison technique will also be used to determine minimal inhibitory concentration (MIC) & inhibitory concentration (IC50), which are defined as more than 50% fungal growth.

The growth inhibition of conidia from each extract and control will be estimated using the following formula: -

Conidia Germination % = $\frac{(GC-GT)}{GC} \times 100$

Where, GC = germination in control; GT = germination in the treatment.

The disease incidence wasdetermined using following equation(Bill et al., 2014):

Disease incidence = $\frac{Number\ of\ infected\ wounds}{Total\ number\ of\ inoculated\ fruit} \times 100$

The fresh weightloss was calculated by following formula.

$$\text{FWL \%} = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

Plant defense enzyme assays for phenylalanine ammonia-lyase (PAL), peroxidase (POD), polyphenol oxidase (PPO),superoxide dismutase (SOD), & catalase (CAT) weredetermined from treated extract, fungicide and untreated control by the modified method of Yeoh and Ali (2016). 3.0 g of sample tissuewas mixed, and homogenizedwith 15ml of ice-cold 100 mM L−1sodium phosphatebuffer (pH 7.8) for PPO, POD, & CAT enzyme analysis & centrifuged at 10,000 × g for 25 min at 4 °C. Then, supernatant will be taken from the homogenate sample to determine the PPO, POD, and CAT activity.

The heat stability test was performed by heating extracts to 50 and 100 °C for 5 minutes. The foodpoisoning approach was employed to assess antifungal activity following therapy. Rizzello et al. (2011) showed how to determine proteolysis by treating extracts with trypsin. The trypsin was dissolved in 1%, w/v of 0.25 M Tris– HCl (pH 5.8). 500 µl of plant extract dissolved in an appropriate solvent and the 100µl buffered enzyme solution was mixed. Mix solution incubated for 5 hat 25 ±2 °C, and reaction was stopped after boiling mixture for three min. The pH of the solution was then changed to 6.0, and antifungal activity was assessed using the food poisoning approach.

The time-kill experiment was performed using method described by Ribas et al. (2015) to determine effect of extract on fungal colony development over time. After 7 days, the fungal inoculum was scraped and distributed in tubes with extract concentrations ranging from 1 × MIC to 4 × MIC. The time duration was 1, 6, 12, 24 and 48 hours, serial dilution of conidial suspension from each MIC concentration was taken out to make conidial dilution 10^{-1} and 10^{-3}. Then, 100 µl diluted conidial suspensionwas spread on a PCA plate & incubated at 27 ±1°C for 48 hours and observed CFU ml-1.The time-kill curve was depicted using log10 CFU ml^{-1} against time interval.

The modified Yeoh and Ali (2016) approach was used to assess plant defense enzyme tests for phenylalanineammonia-lyase (PAL),peroxidase (POD), polyphenol oxidase(PPO), superoxide dismutase (SOD), and catalase (CAT) in treated extract, fungicide, & untreated control. To analyze PPO, POD, and CAT enzymes, 3.0 g of sample tissue washomogenized in 15ml of ice-cold 100 mM L−1 sodiumphosphate buffer (pH 7.8) & centrifuged at 10,000 × g

for25 minutes at 4°C. Thsupernatant was then extracted from the homogenate sample and tested for PPO, POD, and CAT activities.

3.5 Column Chromatography (Silica Gel) Analysis

The solvent-free crude powder from the sample was dried and subjected to silica gel column chromatography (size 60-120 mesh). For the admixture, 10 g of the dried solvent-free crude extract was combined with 30 g of pure silica gel and carefully mixed. Then, the produced admixture was put into a silica gel column. Elution was performed using the solvent system (v/v): hexane:chloroform; chloroform:ethyl acetate; ethyl acetate:methanol; methanol: ethanol in various ratios: 100:0; 70:30, 50:50; 30:70, 50:05; 30:70; 100:0.The obtained eluted plant extract samples were spotted on a dry TLC plate.

3.6 Thin Layer Chromatography (TLC) Analysis

TLC plates (10 × 10 cm) coated with 0.25mm layers of silica gel60 F254 (Merck, #5554). Extract solutions (20µl) were loaded with a micropipette in aline 1 cmwide. The prepared plates weredeveloped using different mobilesystems of varying polarity: ethyl acetate: acetic acid:formic acid: water (100:11:11:26, v/v),toluene: diethyl ether:acetic acid (60:40:10, v/v) and chloroform: ethylacetate: acetone: formic acid (40:30:20:10, v/v). The leftover solvent was removed from the chromatograms by drying them at room temperatureunder a stream of airovernight. The characteristic-colored specks were seen in UV after spraying a solution of polyethylene glycol (PEG) (Valli and Gowrie, 2021).

3.7 Identification Andanalysis of Phytochemicals Byhigh Resolution-Liquid Chromatography-Massspectroscopy (HR-LC-MS)

The phytochemistry of A. sessilis and M. parvifolia crude extracts was analyzed using an agilent system (6550A Funnel Q-TOF) using HR-LCMS. The liquid chromatography system included a HiP sampler, a binary gradient solvent pump, a column compartment, & a quadrupole time of flight mass spectrometer (MS Q-TOF) with a dual agilent jet stream electrospray (AJS ES) ionsource. A 5 µl ethanolic sample wasinjected with a needle and separated using a G1316C column. The material was eluted with 0.1 percent formic acid in water (solvent A) & 90% acetonitrile, 10% H2O, and 0.1% formic acid (solvent B). The

flow rate was 0.300 ml/min for up to 30 minutes, and MS detection was accomplished using a Q-TOF massspectrometer. Compounds were identified based on mass spectra & distinct mass fragmentation patterns. The phytochemical components were discovered utilizing software such as Compound Discoverer 2.1, Chemspider, and Pubchem. Ionization for the MS experiment was performed using a Dual AJS ESI system with the capillary voltage set to 3500 V, the nozzle voltage set to 1000 V, the gas temperatures set to 250 °C, the nebulizer pressure set to 35 psi, and the drying gas flow rate set to 13 l/min. The Mass Hunter program was used to collect Q-TOF data and analyze mass spectrometry results. The LC-MS experiment was outsourced to SAIF-IIT in Mumbai, Maharashtra.

3.8. Nuclear Magneticresonance Measurement (NMR)

NMR experiments were carried out on aBruker 400 AV III HD-300 (FT NMR) spectrometer equipped with a 5 mm broadbandInverse Probe (Z-Grd) withVT unit. The spectrometer was also equippedwith an HR-MAS dual 13C/1H (Z-Grd) 4mm probe(Bruker Biospin, Rheinstetten, Germany) and a cryogenically cooled probe. Samples were dissolved in 0.5 mL ofchloroform-d and put into 5-mmNMR tubes. The samples were vortexed for 1 minute, ultrasonicated for 10 minutes without heating, and then centrifuged for10 minutes at 10,000 rpm. About 0.6 ml of supernatant was transferred to anNMR tube for 1H-NMR analysis. TheNMR spectrometer was operated by the Top Spin 3.2 software. In one-dimensional 1H NMR spectra, acquisition and relaxation delays were measured at 10.3 s and 20.60 s, respectively. The spectral breadth and pulse length were 12 and 140 ppm, respectively. The 1H-13C HSQC was performed with normal Bruker software, whereas the HMBC tests and parameters were optimized for coupling values of 145.0 Hz and 10.0 to 2.5 Hz, respectively. One-dimensional TOCSY investigations were carried out at 298 K using a Bruker AV III HD 800 spectroscopy with the selmlgp pulse scheme, 32 to 256 scans, and an excitation delay of 5 s. The NMR experiment was carried out through outsourcing at SAIF CDRI in Lucknow, Uttar Pradesh.

3.9. STATISTICAL ANALYSIS

The experiment's findings were analysed using ANOVA and Duncan's multiple range test (DMRT). SPSS Statistics for Windows, version 29.00, was used to determine standard errors for all mean values and detect significant deviations ($p < 0.05$).

CHAPTER 4

Plants Extract used in Study

4.1. Introduction

The currentstudy sought to understand use of plant extractsfrom seven various species of plants, including Terminalia bellirica, Adhatoda vasica, Peristrophe paniculata, Alternanthera sessilis, Acalypha indica, Cocculus hirsutus, and Mitragyna parvifolia, and to determine their phytochemical features in preventing the spread of fungal infection of the bacterium Coll capsici without affecting the nutritional value of chilli.

4.2. Terminalia Bellirica (Gaertn.) Roxb

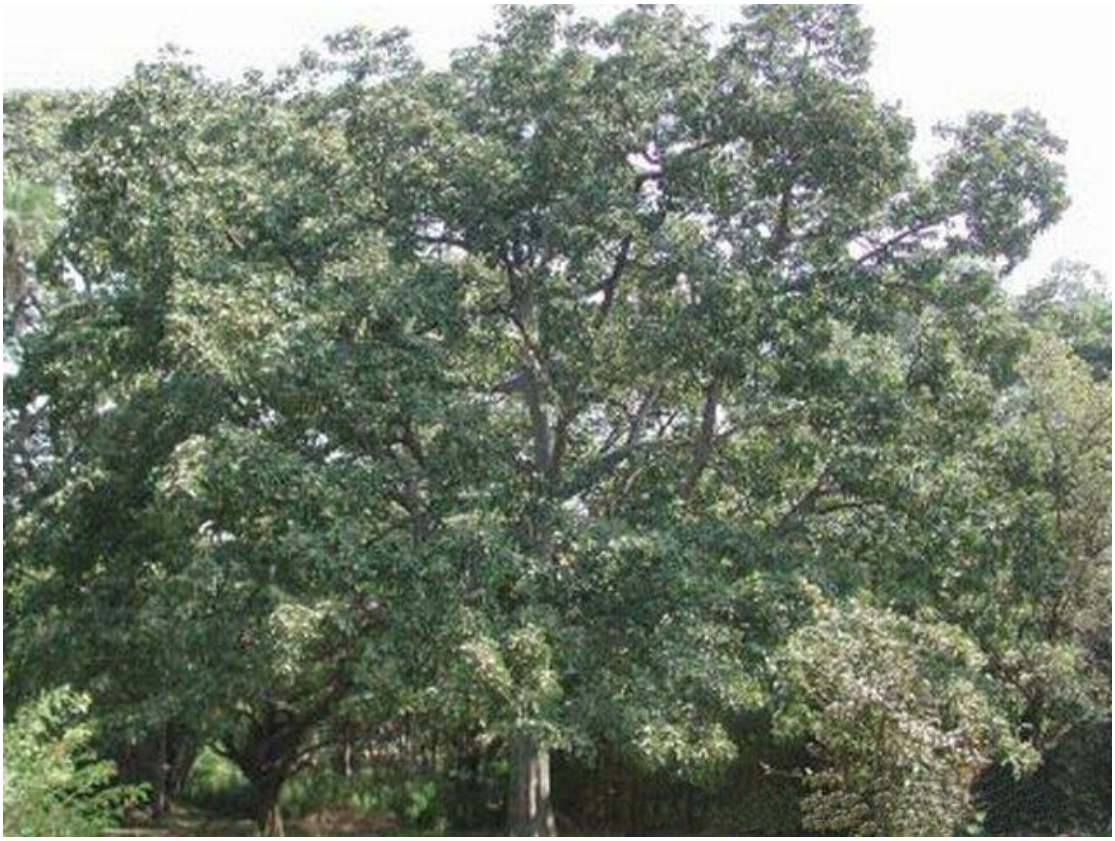


FIG. 4.1 TERMINALIA BELLIRICA (GAERTN.) ROXB

Theterm 'Terminalia' comes from Latinword 'terminus' or 'terminalis', which means that the leaves are clustered around the point of the shoot apex. Terminalia bellirica, commonly called asbaheda, bahera, behada, beleric, or bastard myrobalan (Arabic: beliledj بليلج, adopted from Middle Persian Balilag), Persian بليله (Balileh), Sanskrit: Vibhītaka, Aksha) is a huge

deciduous tree in the Combretaceae family. It grows in the plains and lower slopes of South and Southeast Asia, where it tends to be planted as an avenue tree. The basionym is Myrobalanus bellirica Gaertn. M. bellirica was relocated to Terminalia by William Roxburgh under the name "T. bellerica (Gaertn.) Roxb." This spelling error is now often used, causing confusion. The correct name is Terminalia bellirica (Gaertn.) Roxb.

It is a huge deciduous treethat thrives in Southeast Asia's plains & lower hills. The tree is approximately 50 meters tall, 3 meters in circumference, and has a circular crown. The base's branches are around 20 meters in length. The tree's bark is bluish or ashy-grey in hue and covered withnumerous small longitudinal fractures, while the inner bark is yellowish. The leaves are around 24 cm and 11 cm long, glabrous, alternating, broadly elliptic to obovate-elliptical. Secondary and tertiary veins are seen on both the adaxial and abaxial surfaces, clustering toward the apex of branchlets. The leaf petiole is around 9 cm in length.

Young leaves turn parrot green, then copper-red, and eventually dark green. T. bellirica leavesare around 15 cm long & densely packed toward ends of branches. The blooms are solitary and tiny, measuring around 15 cm inlength. Flowers are greenish-white and straightforward. Fruits are sub-globular to ellipsoid, measuring around 4 × 2.2 cm, light-yellow with five angles, and brownish.

FIG. 4.2 BAHERA (TERMINALIA BELLIRICA) FRUITS

The leaves are around 15 cm long and thickly packed at the branches' ends. It is regarded as good cattle feed. Terminalia bellirica seeds contain 40% oil, and the fatty acid methyl ester meets all major biodiesel requirements in the US (ASTM D 6751-02, ASTM PS 121-99), Germany (DIN V 51606), and Europe (EN 14214). The seeds are called bedda nuts.

The kernelsare consumed by Lodha people of Indian subcontinentfor their mind-altering effects. The tree's nutsare spherical, but have five flat edges. It refers to the usage of dice in the epic poem Mahabharata and the Rigveda book 10, song 34. A handful of nuts would be placed on a gaming board, and the participants would have to determine whether an odd or even number of nuts were tossed. In the Nala, King Rituparna exhibits his ability to quickly compute huge numbers by counting the quantity of nuts on a single limb of tree.

4.2.1. Classification

KINGDOM:	PLANTAE
PHYLUM:	TRACHEOPHYTA
CLASS:	MAGNOLIOPSIDA
ORDER:	MYRTALES
FAMILY:	COMBRETACEAE
GENUS:	TERMINALIA
SPECIES:	BELLIRICA (GAERTN.) ROXB
LOCAL NAMES/COMMON NAMES	
HINDI:	BAHERA
ENGLISH:	BELERIC OR BASTARD MYROBALAN
SANSKRIT:	BIBHITAKI

4.2.2 Traditional Use of Terminalia Bellirica

T. bellirica has long been utilized in Ayurveda, Siddha, Unani, and Chinese medicine to treat a variety of ailments. In India, a town in Madhya Pradesh's Malwa area is a significant trading hub for de-skinned and entire T. bellirica fruits. The Lodha tribe of India consume these kernels because of their mind-altering properties.

Its fruit is used in popular Indian herbal rasayana "Triphala". T. bellirica comes in two types in India: one with roughly spherical fruit that is 1/2 to 3/4 inch in diameter, and other with ovate & bigger fruits. The pulp of fruit belericmyrobalan is combined with salt and long pepper to cure chest and throat infections. It is used to treat a variety of ailments since it is a component of triphala (three fruits), which includes emblic, beleric, & chebulic myrobalans. The fruits of this plant are used as laxatives, astringents, and anthelmintics, and a decoction of green fruit is used to cure coughs. The pulp of the fruit is commonly used to treat ailments such as diarrhea, dropsy, piles, and leprosy. The kernels are utilized as narcotics. Seed oil is used to treat rheumatism. The seed contains oil that is used to treat skin problems, premature graying of hair, and can be applied to uncomfortable, swollen areas. The plant's principal constituents include glucosides, tannins, gallic acid, ethyl gallate, & chebulinic acid, which act as an antioxidant, antimicrobial, antidiarrheal, anticancer, and antipyretic agent (Deb et al., 2016).

4.2.3 Pharmacological Activity of T. Bellirica

Fahmy et al. (2015) found that acetone fruit extract of T. bellirica had free radical scavenging activity & strong antioxidants in in vitro experiments. Devi et al. (2014) observed that aqueous fruit extract of T. bellirica has antibacterial and antifungal action against a variety of pathogenic bacteria, including Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Shigella flexneri, & Salmonella typhi. Saraphanchotiwitthaya et al. (2008) tested stem and leaf extracts against both Gram-positive & Gram-negative microorganisms. Valli et al. (2013) found that an ethanolic fruit extract of T. bellirica was employed against clinical and environmental isolates of Cryptococcus neoformans using the disc diffusion technique.

4.2.4. Medicinal Use

In traditional Indian Ayurvedic medicine, Beleric isknown as "Bibhitaki" (Marathi: "Behada orBhenda"). The fruit is utilized in triphala, a traditional Indian herbal rasayana therapy. In Sanskrit, it's called bibhītaka. In India, Neemuch, a town in Madhya Pradesh's Malwa Region, is a significant trade center for skinless baheda and complete T. bellirica fruits. In Madhya Pradesh's Malwa area, the fruits are commonly gathered wild. According to Dymock, Warden, and Hooper's Pharmacographia Indica (1890), "This tree, in Hindu Bibhita and Bibhitaka (fearless), is avoided by the Hindus of Northern India, whose will not sit in its

shade, because it is believed to be inhabited by demons." In India, there are two types of T. belerica: one with virtually spherical fruit that is 1/2 to 3/4 inch in diameter, and another with ovate and larger fruit.

Ayurvedic physicians regard pulp of fruit (Belericmyrobalan) tobe astringent and laxative, & it is used with salt & long pepper to treat throat and chest infections. It is utilized in a variety of ailments as a component of triphala (three fruits), which include emblic, beleric, & chebulicmyrobalans, and the kernel is occasionally applied externally to inflamed areas. Because of its therapeutic powers, the tree is known as Anila-ghnaka, or "wind-killing." According to Nighantus, the kernels are narcotic.

The CharakaSamhita, an ancient Ayurvedic treatise, mentions Bibhitakifruits as havingdisease-relieving properties as well as bestowing longevity, intellectual prowess, and power. The Charaka Samhita describes numerous "rasaayan" that make use of Bibhitaki.

Descriptionof the Fourth Amalaka Rasaayan,which contains Bibhitaki among its fruits: By this cure, sages recovered their youth & lived disease-free for several hundred years, & equipped with vigor of physically, brain, & senses, they did penance with extreme dedication.

4.3. Adhatoda Vasica (L.) NEES

FIG. 4.3: ADHATODA VASICA (L.) NEES

Justiciaadhatoda, also known in English as Malabar nut, adulsa, adhatoda, vasa, or vasaka, is endemic toAsia. In Tamil, Adathoda means 'untouched bygoats'. The term is derived from fact that animals such as goats avoid eating this plant owing to its intense bitterness. The plant is endemic to Afghanistan, Indian subcontinent (Bangladesh, India, Pakistan, Nepal, & Sri Lanka), Laos, Myanmar, and Vietnam. It has been introduced elsewhere. Adhatoda vasica (L.) Nees (Accepted name: Justicia adhatoda L.) lives in the lower Himalayas, India, Sri Lanka, Burma, Malaysia, & other regions of Asia. It is a medicinal herb found in Ayurveda and Unani. It is a perennial shrub that grows 1 - 2.5 m tall on open plains and has opposed climbing branches. The leaves are simple, ovate-lanceolate, opposite in shape, hairy, leathery, light green top, dark green below, 7-19 cm long, & 4-7 cm broad. The blooms are white with red- or yellow-barred throats, thick, big bracts, and appealing white petals.

FIG. 4.4 ADHATODA VASICA NEES FRUITS

Four spherical seeds form the fruits, which are tiny, clavate capsules with longitudinal channels. A. vasica leaves contain the alkaloid vasicine. The flowers, leaves, bark, & roots have all been employed in various therapeutic compositions. A. vasica has been used to treat a variety of conditions, including leprosy, blood diseases, heart problems, thirst, fever, vomiting, memory loss, and speeding up labor. A. vasica is used to treat respiratory diseases such as colds, coughs, asthma, whooping cough, chronic bronchitis, and TB.

Justicia adhatoda is a shrub with 10-20 lance-shaped leaves about 8-9 centimeters in length and four broad. They are organized in opposing directions, have smooth edges, and are

supported by slender petioles.[Citation required] When dried, they have a drab brownish-green hue. They taste bitter. When a leaf is cleansed with chloral hydrate and examined under a microscope, the oval stomata may be seen. They are surrounded by two crescent-shaped cells that make a right angle to the ostiole. The epidermis contains basic warty hairs with one to three cells and small glandular hairs. Cystoliths grow beneath the epidermis on the underside of the blade. The stem contains numerous long, opposing ascending branches with yellowish bark. Flowers are usually white, and their arrangement includes massive, thick axillary spikes. Fruits are overgrown and have club-shaped capsules.

4.3.1. Classification

KINGDOM:	PLANTAE
PHYLUM:	TRACHEOPHYTA
CLASS:	MAGNOLIOPSIDA
ORDER:	LAMIALES
FAMILY:	ACANTHACEAE
GENUS:	ADHATODA
SPECIES:	VASICA (L.) NEES
LOCAL NAMES/COMMON NAMES	
HINDI:	ADOSA
SANSKRIT:	VASAKA
BANGLA:	BASAK
ENGLISH:	MALABAR NUT

4.3.2. Traditional use of Adhatoda Vasica:

A. Vasica leaf parts are used to treat fever in Bastar, Madhya Pradesh (Shah and Joshi, 1971); asthma, cough, colds, dysentery, & diarrhea in Northeast Haryana, India; jaundice in Anantapur, Andhra Pradesh; urinary problems in Sitapur, Uttar Pradesh; and'stimulating & healing' before & after delivery in Neterhat, Bihar. The root has been used to treat gonorrhoea in Hardoi, Uttar Pradesh, as well as fever, malarial fever, rheumatism, leucorrhoea, bilious vomiting, and diuretics. The bark of A. vasica has been used to treat chest illnesses, asthma, expectorants, antispasmodics, and phthisis (Hossain et al. 2016).

4.3.3. Pharmacological Activity of A. Vasica:

Sarker et al. (2009) revealed that A. vasica leafextract has antibacterial efficacy against Bacillus subtilis and Vibrio cholera. Dorsch and Wagner (1991) discovered that the major alkaloids of A. vasica are vasicine & vasicinone, which are employed as therapeutic respiratoryagents. Shrivastava et al. (2006) foundthat leaf powder of A. vasica has significant anti-ulcer action in rats. Srmivasarao et al. (2006) found that the vasicine of A. vasica has anti-inflammatory properties. According to Narimaian et al. (2005), vasicine from A. vasica has a significant supplementary function in treatment of tuberculoses. Other activities include insecticidal activity (Srivastava et al., 1965), anticholinesterase activity, sucrose inhibitory action, antimutagenic activity (Jahangir et al., 2006), cardioprotective activity, radioprotective effects, and abortifacient activity (Ahmad 2009).

4.3.4 Chemical Composition

Justicia adhatoda's leaves include chemicals such alkaloids, tannins, saponins, phenol compounds, and flavonoids.[Citation Required] The most prominent is vasicine, which is a quinazoline alkaloid. The herbage's vasicine yield is expected to range between 0.541 and 1.1% dry weight. Bromhexine, a serine protease inhibitor with mucolytic properties available over over the counter in Europe, was originally derived from Justicia adhatoda.

4.4 Peristrophe Paniculata (FORSSK) Brummitt

FIG. 4.5: PERISTROPHE PANICULATA (FORSSK) BRUMMITT

Peristrophe paniculata is a traditional medicinal herb with several therapeutic uses. Its leaf has traditionally been used to treat eye & ear disorders, bacterial infections, and insect stings and bites. It's a dicotyledonous plant. Herbs that stand erect and have 6-angled, hispid stems. Leaves are simple, opposite, and oval, with a sharp apex and rounded or truncate bases. P. paniculata's ethanolic extract reduced the development of Escherichia coli, Bacillus cereus, & Staphylococcus aureus. The leaf and stem extract drastically inhibited Colletotrichum capsicum's radial growth. Peristrophe paniculata (Forssk) R. K. Brummitt. belongs to Acanthaceae family & is now known as Dicliptera paniculate (Forssk.) I. Darbysh. The plant is less woody, grows to a height of 60 - 180 cm, is a perennial with stems, & may be found practically anywhere in India, Afghanistan, or Africa. The leaves are oblong or elliptic ovate with conspicuous veins on 1 - 1.7 cm long petioles, thickly lineolate, appressed hairy, pubescent, primary nerves 4 - 6 pairs, and the basal section rounded to acute to acuminate at the tip. Petioles up to 6 - 15 cm long are often rough on the angles, more or less hairy, and 6-angled on stems and branches. Flowers are solitary, pink or purple, pedicellate, in loose panicles, with a tubular corolla, panicles axillary and terminal, trichotomously branching, considerably uneven, 7 - 15 mm long, with scarious borders. Under the calyx 2, there is a two-lipped creature, with a linear-spathulate upper lip and a bigger bottom lip. It is connected by two stamens, and the neck appears white with dark purple patterns.

4.4.1 Classification

KINGDOM: PLANTAE
PHYLUM: TRACHEOPHYTA
CLASS: MAGNOLIOPSIA
ORDER: LAMIALES
FAMILY: ACANTHACEAE
GENUS: PERISTROPHE
SPECIES: PANICULATE FORSSK) R. K. BRUMMITT.
LOCAL NAMES/COMMON NAMES
HINDI: KALI AGHEDI ATRIAL, ITRELAL MASI, NASBHANGA
SANSKRIT: KAKAJANGHA

4.4.2 Traditional uses of Peristrophe Paniculata

Traditionally, entire plants have been employed in many medical systems. In Ayurveda, it is used to make Aragvadhadi kwatha churna. The root is used to treat pruritus, worms, leucorrhoea, internal hemorrhage, ulcers, wounds, bone fractures and sprains, skin problems, and sleeplessness. The leaf extract has been utilized by Indian tribes to cure fever, cold, and cough, as well as ear and eye problems (mucilage), liver illnesses, rheumatism, gout, antinematodes, and pesticides. It has antibacterial effects (tuberculostatic), anti-venom action, and is beneficial in psychological diseases. The 50% hydroethanolic and aqueous extract has strong anti-inflammatory, antibacterial, wound healing, and analgesic properties, according to Rathi et al. (2003).

4.4.3 Pharmacological Activity of Peristrophe Paniculata

Chemical composition: During the chemical examination, 14-methyltritriacont-14-en-15-ol & 35-hydroxynonatriacontanal were discovered in dried aerial parts. Essential Oil: In vitro activity against different strains of Mycobacterium tub

4.5. Alternantherasessilis (L.) R. BR. EX DC.

FIG. 4.6: ALTERNANTHERA SESSILIS (L.) R. BR. EX DC.

The plant thrives in tropical and subtropical regions of the Old World. It has been brought into the Southern United States, although its origins in South and Central America are unclear. This plant has become a weed in several parts of the southern United States. It is

commonly found in moist or damp environments (but not always, especially in high moisture areas where it may be a garden weed).

Alternanthera sessilis is a multi-branched terrestrial, annual, or perennial. Alternanthera is derivedfrom two Greek words: "alternans" (alternating) & "anthera" (anther), which relate to the alternations of pseudosaminodes and stamen. The stems are flat and 1–10 dm long, and they may be found across India. It originated in Brazil but is now widely distributed over tropical Africa, southern and eastern Asia, and Australia. The stems are prostrate, cylindric, seldom ascending, frequently rooted at nodes, villous in lines, and transverse at nodes. The leaves are simple, sometimes obovate, but mainly elliptic, opposite, and decussate, about 0.3–3 cm broad and 1–15 cm long. The petioles are indistinct & 1 - 5 mm long. The inflorescences are white, sessile spikes on the leaf axils that measure 1 cm across and have segments up to 2.5 mm in length. The blooms are supported by a white scarious bract, sessile spikes, bract, and bracteoles that are dazzling white and 0.7 - 1.5 mm long. The perianth is made up of tepals that are all equal, clearly mucronate, and have a somewhat denticulate edge. Sepals are similar in form and are 2.5 - 3 mm length. The stamens are five times the number of sepals. The fruit is dark brown with a lighter border, having an obcordate to orbicular-obcordate utricle that is 2 to 3 mm in length. The seeds are lens-shaped, with a brilliant brown testa that is somewhat reticulate and is 0.5 to 1 mm in diameter.

4.5.1. Classification

KINGDOM: PLANTAE
PHYLUM: SPERMATOPHYTA
CLASS: DICOTYLEDONAE
ORDER: CARYOPHYLLALES
FAMILY: AMARANTHACEAE
GENUS: ALTERNANTHERA
SPECIES: SESSILIS (L.) R. BR. EX DC.
LOCAL NAMES/COMMON NAMES: “DWARF COPPERLEAF" OR “SESSILE JOY WEED"
ASSAMESE: MATIKADURI
TAMIL: PONNANGANNI
KANNADA: HONNAGONE

4.5.2. Traditional uses of Alternanthera Sessilis

The leaves, stems, & seeds are used in traditional medicine to treat cane vulgaris, a prevalent skin disease found in India, Sri Lanka, & China. In India's Bargarh area, tribals use the herb to cure bleeding dysentery. People in Assam are utilized to cure jaundice and other diseases (Bhuyan et al., 2018).

Different populations in Karnataka utilize plant to cure ulcers, cuts, & wounds, whereas Irula tribals in Kalavai, Vellore district, Tamil Nadu, use it to treat headaches, hepatitis, & asthama (Sravani et al., 2017).

A. sessilis is used for the treatment of headaches & vertigo in Nigeria, gastrointestinal problems in India and Sri Lanka, hepatitis, bronchitis, and asthma in
Taiwan.

4.5.3. Pharmacology Activity of Alternanthera Sessilis

Gayathri et al. (2006) employed an aqueous leaf extract of A. sessilis to treat biliousness,dyspepsia associatedwith slow liver, chronic liver congestion, acute & chronic pyelitis, cystitis, and gonorrhea in mice. Sivakumar & Sunnathi (2016) tested the phytochemical screening & antimicrobial activity of ethanolic leaf extracts of A. sessilis and A. philoxeroides against four gram-positive bacterial species (Staphylococcus aureus, Staphylococcus heamolyticus, Enterococcus faecalis, Bacillus subtilis), four gram-negative bacterial species (Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, & Proteus mirabilis), as well as one fungus (Capsicum albicans).

4.5.4. Uses

The plant grows wild, but it is also grown for food, herbal medicine, and decorative purposes. Alternanthera reineckii, an aquarium plant, is commonly confused as A. sessilis.

In certain regions of Southeast Asia, foliage and young shoots are used as vegetables. In Karnataka, Andhra Pradesh, Tamil Nadu, and Sri Lanka, the leaves, blooms, and delicate

stalks are used as vegetables. The plants are finely shredded and stir-fried with grated coconut and spices to make a salad-like dish that is typically consumed with rice.

The leavesare crisp, slightly more so than spinach grown in temperate climates, & not slimy. Some cultivars taste somewhat bitter. Because of the presence of oxalates, they must be steamed or boiled before consumption in considerable quantities. It may be eaten on its own as a green or substituted for spinach in other meals. According to accounts, Brazilians usually eat it raw in salads with oil and/or vinegar, tomato plants, and onion, although the literature recommends boiling it. The vegetable can be substituted for spinach in quiches, pies, curries, dals, pasta sauces, lasagna, or dinners and stir-fries late in the cooking process in order to provide a nutty taste.

As a herbal medicine, plant possesses diuretic, cooling, tonic, & laxative qualities. It is used to treat dysuria and hemorrhoids. The herb is also said to be good for the eyes and is utilized in manufacture of medicinal hair oils & kajal.

4.6. Acalypha Indica L

FIG. 4.7: ACALYPHA INDICA L.

Acalypha indica is an upright, generally simple-stemmed annual medicinal plant that grows in moist, temperate, and tropical regions. It is said to have originated in India, Indochina,

Ethiopia, and the Nigeria region of southern Africa, which includes South Africa. They may reach an average height of one meter. The leaves are broadly oval, base cuneate / rounded to briefly attenuate, and glabrous thin, measuring up to 1.2 cm - 6.5cm by 1 cm - 4 cm. Theleaf edge is 5-veined at the base, with 4 to 5 pairs oflateral veins and a petiole ranging in length from 0.02 to 12.00 cm. The leaves have a sharp apex, toothed and membranous borders, and sparse short hairs on both sides. Within one month of germination, the stem begins to develop woody and becomes excessively thickly hairy. The little male blooms on the top section are whitegreen in color. Flower spikes are numerous, upright, lax, and elongated, with clusters and auxiliary spikes up to 2.5 - 6 cm long. The nerve bract is roughly 6 to 8 mm in diameter. The stem is striated and pubescent. The fruit can measure up to 1.5 - 2 mm.

4.6.1. Classification

KINGDOM:	PLANTAE
PHYLUM:	TRACHEOPHYTA
CLASS:	EQUISETOPSIDA
ORDER:	MALPIGHIALES
FAMILY:	EUPHORBIACEAE
GENUS:	ACALYPHA
SPECIES:	INDICA L.
LOCAL NAMES/COMMON NAMES	
HINDI:	KUPPU
SANSKRIT:	KHOKALI

4.6.2. Traditional use of Acalypha Indica

A. indica's leaves, stem root, and whole plant can all be employed, however the leaves (64%) are the most commonly used in various medical systems. A. indica is utilized for a variety of medicinal uses, including anthelmintics, asthma, diarrhea, laxatives, rheumatoid arthritis, syphilitic ulcers, and wound healing (Zahidin et al. 2017). In Taiwan, leaf portions are used to treat asthma.

4.6.3. Pharmacology Studies of Acalypha Indica

Mineral concentration of Acalypha indica is highest in iron, followed by copper, nickel-zinc, and chromium. Patients with mineral deficits have benefitted. The ethanolic leaf extract includes phenolic components such as corilagin, geraniin, glucogallin, and chebulagic acid, which are beneficial antioxidants (Chekuri et al., 2020).

Seebaluck et al. (2015) reported that leaf extract is useful to cure jaundice. A. indica extract (leaves, root, and entire plant) has analgesic, anthelmintic,anti-cancer, anti-bacterial, anti-fungal, antiobesity, anti-inflammatory, antioxidant, and anti-ulcer properties, among others (Chekuri et al., 2020).

4.6.4. USES

The herb has several traditional therapeutic applications. In Madagascar, crushed plants are used to treat skin parasites. In Mauritius, crushed leaf sap is mixed with salt or a plant decoction to cure scabies and other skin problems. In the Seychelles and Réunion, root infusions or decoctions are used to cure asthma and cleanse the liver and kidneys. The root infusion is also used to treat intestinal parasites and stomach pains. The leaf sap serves as an emetic. In Réunion, a solution of Tylophora indica roots is used as an emetic in cases of poisoning. On Réunion and Madagascar, leaf infusions are used as purgatives and vermifuges. In East Africa, sap from the leaves is used to cure eye conditions. Leaf powder is used to heal wounds that have been infected by maggots. The Indian Pharmacopoeia includes Acalypha indica as an a stimulant for the management of asthma and pneumonia. It was once categorized in the British Pharmacopeia.

4.7. Cocculushirsutus (L.) W.THEOB.

FIG. 4.8 COCCULUSHIRSUTUS (L.) W. THEOB.

Cocculus hirsutus Linn., Diels is a climbing shrubthat may reach a heightof 3 meters and is found intropical & subtropical regions of India, South China, & Africa. Capsicum hirsutus flowers range from white to yellowish, while the fruits are dark purple and measure 4 to 8 mm in diameter. Capsicum hirsutus roots are used in Ayurveda to eliminate "Kapha & Vata," reduce bile urethral discharges, and nourish the blood while experiencing a burning feeling. It is alsoused as a refrigerant and laxativein cases of chronicrheumatism, venereal infections, fever, & syphiliticcachexia. The alcoholic extract of Capsicum hirsutus roots possesses strong analgesic, antiinflammatory, hypoglycemic, and cardiotonic properties. The herb is well-known and has long been utilized as medicine by local tribal people to cure avariety of ailments.

4.7.1 Classification

KINGDOM:	PLANTAE
PHYLUM:	TRACHEOPHYTA
CLASS:	MAGNOLIOPSIA
ORDER:	LAMIALES
FAMILY:	MENISPERMACEAE
GENUS:	COCCULUS

SPECIES: HIRSUTUS (L.), W. TEOB.

LOCAL NAMES/COMMON NAMES

HINDI: JALJAMINI/JALYAMINI

SANSKRIT: PATALGARUDI, BROOM CREEPER

4.7.2. Traditional uses of Cocculus Hirsutus

The Koyas use the plant's leaf paste and juice combined with sesame oil on their heads for cooling and to their bodies for heat reduction. The plant paste is applied to the navel area to relieve stomach discomfort and cure blood dysentery. The leaves are used to cure prurigo, bladder issues, fever, leucorrhoea, gonorrhea, cuts, wounds, and other skin conditions. The leaves and stems can be used to treat gastrointestinal issues and conjunctivitis. It has been claimed that the leaf powder is used orally to treat dysentery & diarrhea. (Gairola et al. 2013).

The roots are bitter,alterative, & laxative, & are used to treat fever,skin irritation, rheumatism,gout, and syphiliticcachexia, while the stem & root extracts are sedative, hypotensive, cardiotonic, and spasmolytic (Logesh et al., 2020).

4.7.3. Pharmacological activity of Capsicum Hirsutus

Jethvaet al. (2020) employed an aqueous extract to demonstrate anti-mycobacterial activity, whereas Gupta et al. (2018) used an ethanolextract of Capsicum hirsutus leaves to combat M. tuberculosis. Devi et al. (2019) and Nayak and Singhai (2003) tested antibacterial activity of aqueous extract, ethanol, and methanol of Capsicum hirsutus leaves against seven clinical bacterial isolates. Devi et al. (2017) tested the antifungal activity of an aqueous leaf extract on Sclerotium rolfsii, Rhizopus arrhizus, and Fusarium solani fungus strains.

Brahmam & Sunita (2018) investigated in vitro antimalarial efficacy of several root extracts of Capsicum hirsutus against 2 Plasmodium falciparum strains. De Wet et al. (2009) demonstrated anticancer activity in cancer cell lines using a crude alkaloidal extract of Capsicum hirsutus rhizomes. Arunabha and Satish (2015) studied the immunomodulatory efficacy of a combination of Capsicum hirsutus leaves & Sesbania grandiflora flowers in mice.

Rastogi et al. (2008) investigated immunostimulatory properties of an aqueous & ethanolic extract of aerial portions of Capsicum hirsutus in rats. Badole et al. (2006) investigated the antihyperglycemic effect of an aqueous extract of Capsicum hirsutus leaves in alloxan-induced diabetic rats. Sangameswaran and Jayakar (2007) investigated the antidiabetic properties of Capsicum hirsutus in streptozotocin-induced diabetic rats, & found that oral treatment of the methanolic extract reduced blood glucose levels.

4.8. Mitragynaparvifolia (ROXB.)KORTH.

FIG. 4.9: MITRAGYNAPARVIFOLIA (ROXB.) KORTH.

Mitragynaparvifolia (Roxb). Korth is a tree that is native to India & Sri Lanka but may also be found in tropical & subtropical parts of Africa & Asia. Korthals named genus Mitragyna after the form of the species' stigmas, which resembled a bishop's mitre. It is a deciduous tree with a smooth and thin trunk or bark that may reach a height of 25 meters. When young, branchlets range from angular to subterete. The immature woody stalks have 10 - 12 simple, opposite, decussate leaves. Stipules, or the base of the leaf stem, are foliaceous, with a keeled back, interpetiolar, and caduceus. Petioles are glabrous and canaliculate in cross section, measuring upto 1 - 4 cm long. The lamina iselliptic-obovate to orbiculate, ovate, with an acute to attenuate to subcordate base and an abruptly acuminate apex with a blunt tip. The border is whole, coriaceous, glabrous, and occasionally acute. It can measure up to 16 × 10 cm. The midrib is flat above, with secondary nerves in 6 - 10 pairs and domatia at the axils. Tertiary nerves are distributed in a remotely oblique reticulopercurrent pattern. Flowers are sessile, the inflorescence on the terminal head appears cream-white, and the calyx lobes are short. Fruits

are grouped in globose heads and capsules, each containing two follicular cocci. Seeds are many and winged.

4.8.1. Classification

KINGDOM: PLANTAE
PHYLUM: MAGNOLIOPHYTA
CLASS: MAGNOLIATAE
ORDER: RUBIALES
FAMILY: RUBIACEAE
GENUS: MITRAGYNA
SPECIES: PARVIFOLIA (ROXB.) KORTH.
LOCAL NAMES/COMMON NAMES
HINDI: KADDAM

4.8.2. Traditional uses of Mitragynaparvifolia

The bark & roots have traditionally been usedto cure a variety of diseases, including fever, colic, muscle discomfort, burning sensation, cough, edema, poisoning, gynecological issues, and worm removal. The fruit juice of M. parvifolia improves breast milk production in breastfeeding moms. It is known as lactodepurant. Ankit et al. (2009) found that leaves are used as a bandage to relieve pain and swelling, as well as to promote wound and ulcer healing. The Chenchus, Yerukalas, Yanadis, & Sugalis tribes of Gundur District in AndhraPradesh employ fresh leaf juice to treat jaundice. Mitragyna speciosa (Korth.) Havil, used for certain medicinal purposes, is known as "Kratom" in Thailand & "BiakBiak" inMalaysia.

4.8.3 Pharmacological Activity of Mitragyna Parvifolia

In rodents, the methanolicextract of M. parvifolialeaves demonstrated antiarthritic & antipyretic activity (Choudhary and Jain, 2016). Gupta et al.(2009) investigated anti-inflammatory & antinociceptive properties. They employed an ethanolic extract of M. parvifolia dried leaves in mice to produce paw edema with Carrageenan and the tail-flick technique. Vishal and Sanjay (2009) investigated the anxiolytic efficacy of the methanolic,

ethyl acetate extract, & alkaloid-rich fraction of M. parvifolia stem bark in mice using the elevated plus maze (EPM) & marble-burying test (MBT).

Kumar and Shreya (2011) investigated antimicrobial efficacy of ethanol, methanol, & water extracts of M. parvifolia barks against human pathogenic bacterial strains (Staphylococcus epidermidis, Bacillus subtilis, E. coli,Pseudomonas aeruginosa, Saccharomyces cerevisiae, Candida albicans) using an agar well diffusion assay. The distilled water, methanol, acetone, ethyl acetate, & hexane extractsof M. parvifolia leaves & dried bark were investigated for antioxidant capability, lipid peroxidation, & antiproliferative activity on HeLa cell lines. Ghatak, et al. (2014).

Kaushik et al. (2009) used an ethanolic extract of M. parvifolia leaves and discovered considerable anticonvulsant effectiveness in PTZ (pentylenetetrazole) and maximal electroshock-induced seizure in mice. Mitragynine, an indole-alkaloid, was isolated from Mitragyna leaves and shown antinociceptive activity.

Another Mitragyna species (M. speciosa) was tested for mutagenic and antimutagenic properties (anticancer activity) (Ghazali et al., 2011).

4.8.4. Uses

Tribals in Gundur District, Andhra Pradesh, employ Mitragyna parvifolia fresh leaf sap to cure jaundice. Its leaves are used to relieve pain and swelling, as well as to promote wound and ulcer healing. The stem bark is used to treat biliousness and muscle disorders by the people of Tumkur district, Karnataka, India. The tribals of Sonaghati in Sonbhadra district, Uttar Pradesh, cure fever using a decoction of M. parvifolia bark. The Valaiyans tribe, who dwell in the Sirumalai highlands of Madurai district, Western Ghats, Tamil Nadu, utilizes stem bark to relieve rheumatic pain. The bark and roots are used to treat fever, colic, muscular pain, burning sensation, poisoning, gynecological illnesses, cough, and edema, as well as aphrodisiac effects. Fruit juice raises the volume of breast milk in nursing mothers and works as a lactodepurant. This species is eaten by commander (Limenitis procris) caterpillars, which are a kind of brush-footed butterfly

CHAPTER 5

Result and Discussion

5.1 Percentage of Plant Extract Yield

Table 5.1 and Figure 5.1 indicate the percentage yields of crude plant extracts in various solvents. Except for Capsicum hirsutus, aqueous extracts produced the maximum yield of extract from the majority of the leaves (6 of 7). In the case of plant stems, only three plants produced the highest yield of aqueous extract compared to extracts in other solvents. Table 5.1 shows that for aqueous extracts, maximum yieldwas achieved in leaves of A. indica and A. vasica (52.71% and 32.25%, respectively), while the lowest yield was obtained in the stems of A. indica (10.00%) and M. parvifolia.

TABLE 5.1 Moisture Content and Percentage Crude Extract of Plants (Leaf and Stem)

EXTRACTS	*A.INDICA*		*A.VASICA*		*A.SESSILIS*		*C.HIRSUTUS*		*M.PARVIFOLIA*		*P.PANICULATA*		*T.BELLIRICA*	
	LEAF	STEM	LEAF	STEM	LEAF	STEM	LEAF	STEM	LEAF	STEM	LEAF	STEM	LEAF	STEM
MOISTURECONTENT%	77.98	71.16	39.01	55.65	69.85	57.64	66.66	71.11	67.91	82.97	56.15	32.08	54.48	55.22
AQUEOUSCRUDEEXTRACT%	52.71	10.01	32.25	25.01	24.24	19.24	17.31	12.15	16.45	10.01	17.54	22.06	26.84	17.11
ETHANOLICCRUDEEXTRACT%	33.01	8.74	5.97	30.11	15.01	15.84	19.01	13.54	12.25	19.5	14.75	29.44	18.11	3.95
METHANOLICCRUDEEXTRACT%	22.11	8.85	29.61	11.41	8.25	15.04	34.54	14.25	10.31	6.41	14.15	15.65	13.94	5.44
HEXANECRUDEEXTRACT%	0.77	1.74	0.86	1.55	2.26	1.24	3.05	4.31	1.65	4.03	3.22	2.27	0.51	0.6

The maximumyield of ethanolic extract was obtained in leaves of A. indica (33.00%) and the stem of A. vasica (30.11%), while the lowest yield was seen in the stem of T. bellirica (3.95%) and the leaves of A.vasica (5.97%). Theleaves of capsicum hirsutus (34.54%) and A. vasica (29.61%) yielded the most methanolic extract, whereas the stems of T. bellirica (5.44%) and M. parvifolia (6.41%) yielded the least amount.

The yield of hexane extract was the lowest of all the extracts. In case of hexane extracts, the maximum yield was recorded in the stem of M. parvifolia (4.03%) and the leaves of Capsicum hirsutus (3.05%), while the lowest yield was observed in the leaves (0.5%) and stem (0.7%) of T. bellirica. In the case of leaves, the maximum and lowest extract yields were obtained from A. indica leaves with water (52.71%) and T. bellirica leaves with hexane (0.5%) as the extracting solvent, respectively. In the case of stems, the maximum and lowest extract yields were achieved for A. vasica stem with ethanol (30.11%) and T. bellirica stem leaves with hexane (0.7%) as the extracting solvent, respectively.

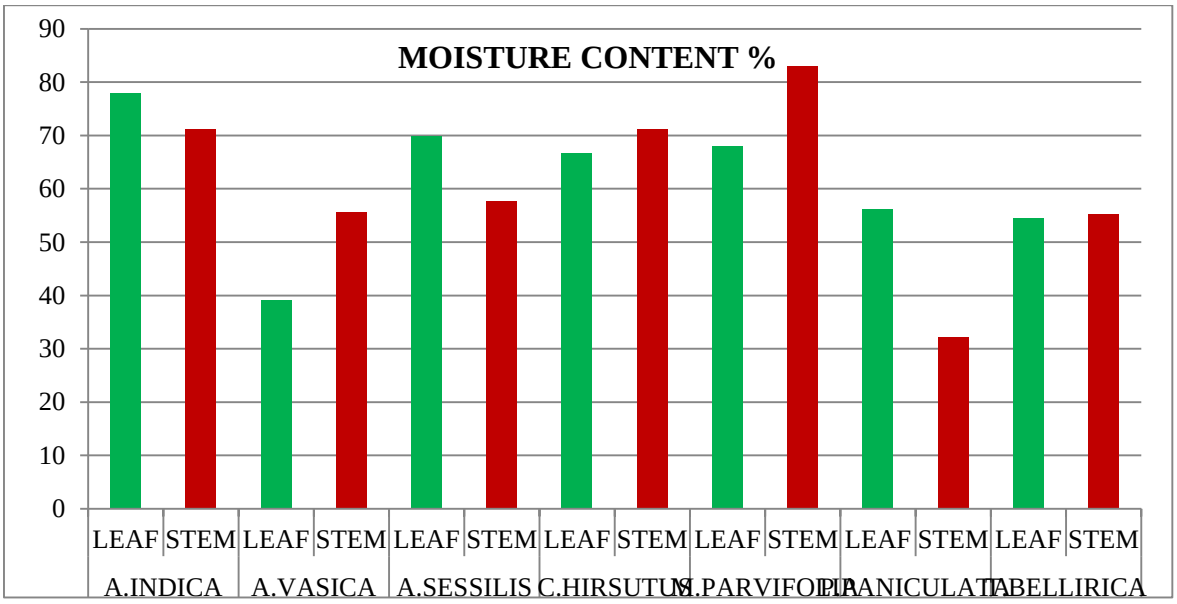


FIG. 5.1: MOISTURE CONTENT (%) OF PLANTS EXTRACT (LEAF AND STEM)

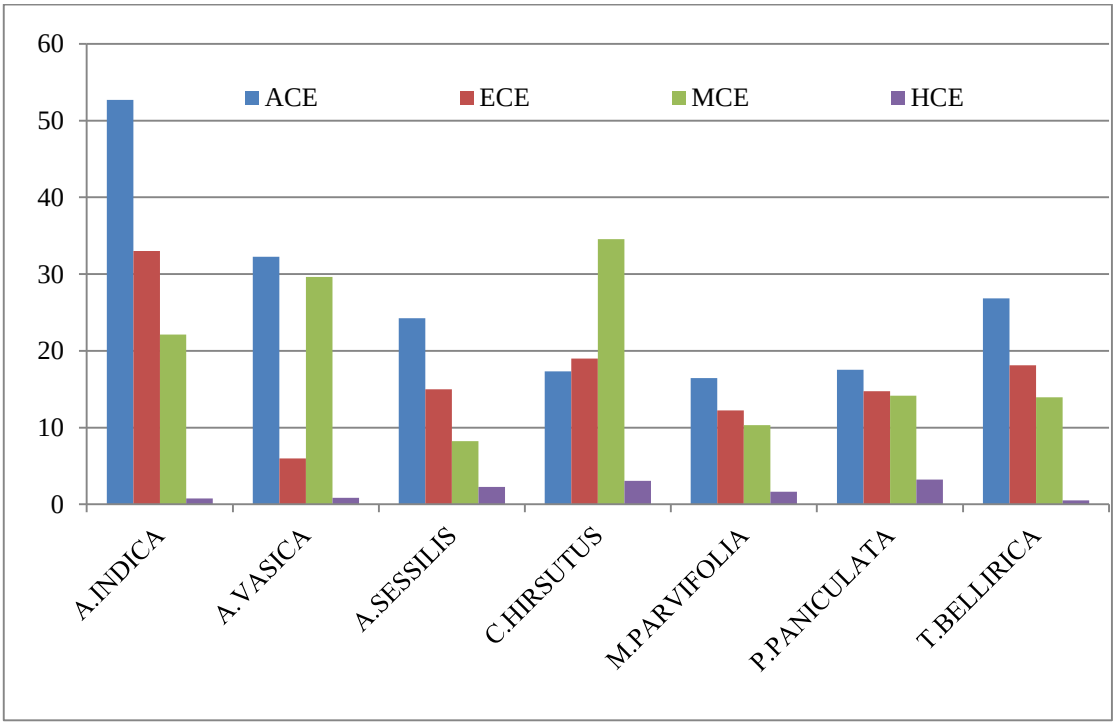
5.2. In-Vitro Bioassay

The leaves & stem extracts of seven plants (A. indica, A. vasica, A. sessilis, Capsicum hirsutus, M. parvifolia, P. paniculata, and T. bellirica) were evaluated against Capsicum capsici. The antifungalactivity of aqueous, 70% ethanolic, 70% methanolic, & hexane extracts

of all chosen plants were evaluated against Capsicum capsici. Table 5.2 presents the results of antifungal activity.

The findings reveal that both ethanolic and methanolic extracts of plants inhibited development of Capsicum capsici, with ethanolic extracts having a greater inhibitory impact than methanolic extracts. The aqueous and hexane extracts also slowed the development of Capsicum capsici, although only in a few cases.

The ethanolicextract of the leaves of all plants [except P.paniculata (stem), T. bellirica (stem) and capsicum hirsutus (leaf and stem)] exhibited fungal growth inhibition, with maximum inhibition (94.27 ± 0.17%) shown by A. indica stem, while the least inhibitory activity (41.41 ± 2.96%) was reported with ethanolic extract ofM. parvifolia leaves. The methanolicextract of all the seven-plant expressed antifungal activity except capsicum hirsutus stem. The maximuminhibition of fungal growth, among methanolic extracts, was observed in A. vasica leaves (82.18 ± 3.04%), while the stem of M. parvifolia showed minimum inhibition (27.48 ± .67%). The aqueous and hexane extracts of all plants were not effective in controlling growth of capsicum capsici. The aqueous extracts of only A. vasica (leaf & stem), A. indica (stem) & T. bellirica (stem) were found effective in inhibiting growth of capsicum capsici, while the hexane extract of only A. vasica (stem), P. paniculata (stem) and T. bellirica (leaf) expressed antifungal activity.



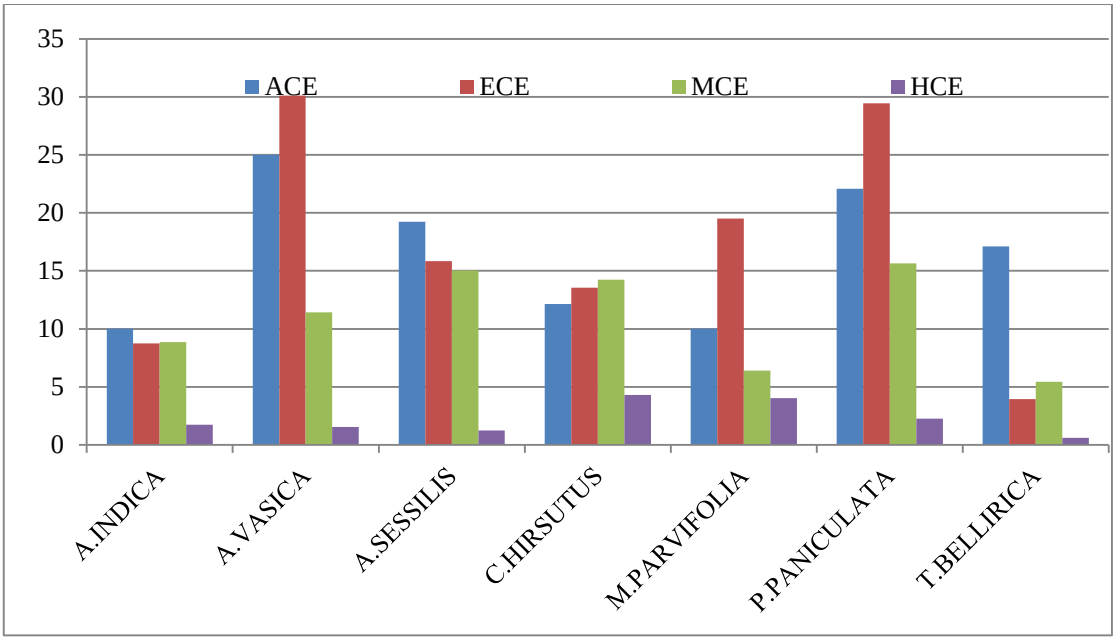


FIG. 5.2: LEAF (a) AND STEM (b) EXTRACT YIELD (%)

5.2.1 Antifungal Activity of Plant Extract

Extracts at various doses (1, 2, 3, 4, and 5 mg/ml) that decreased fungus radial development by more than 20% were tested for antifungal efficacy against capsicum capsici. Table 5.2 summarizes the effects of aqueous, ethanolic, and methanolic leaf & stem extracts at various concentrations on radial development of capsicum capsici.

TABLE 5.2: Effect Ofaqueous, Ethanolic and Methanolic Plants Extracts on Radial Growth (Percent) of Capsicum Capsici.

PLANTPARTSUSED	SOLVENTS			
	AQUEOUS	ETHANOL	METHANOL	HEXANE
*A.vasica*Leaf	27.63±1.46	93.66±0.16	82.18±3.04	-
*A.vasica*Stem	24.43±7.38	91.35±1.88	73.07±1.18	8.46±2.76
*A.indica*Leaf	-	91.25±1.87	72.86±0.42	-
*A.indica*Stem	21.91±1.38	94.27±0.17	77.45±3.65	-
*A.sessilis*Leaf	-	86.95±2.93	79.01±4.28	-
*A.sessilis*Stem	-	87.71±2.42	60.56±2.41	-
*P.paniculata*Leaf	-	88.83±2.55	43.88±4.41	-
*P.paniculata*Stem	-	-	55.31±4.85	7.25±2.42
*T.bellirica*Leaf	-	69.08±3.82	42.46±3.48	9.37±2.76
*T.bellirica*Stem	21.85±4.25	-	79.88±0.92	-

*M.parvifolia*Leaf	-	41.41±2.96	32.23±0.96	-
*M.parvifolia*Stem	-	53.88±2.98	27.48±0.69	-
*C.hirsutus*Leaf	-	-	48.46±1.99	-
*C.hirsutus*Stem	-	-	-	-

There was no significantdifference in capsicum capsici growth b/w aqueous leaves & A. indica stem extract. A. vasica and T. bellirica at all concentration. The maximum radial growth inhibition was exhibited 27.63 ± 1.46%, 21.96 ± 1.38% and 21.85 ± 4.25% at 5 mg/ml concentration of A. vasica (leaf), T. bellirica (stem) and A. indica (stem) over control (Table 5.3 & Fig.5.3).

TABLE 5.3. Effect of Aqueous Plant Extracts on Percentage Growth Inhibition on Capsicum Capsici

CONCENTRATIONS (MG/ML)	***A.INDICA***		***A.VASICA***		***A.SESSILIS***		***M.PARVIFOLIA***		***P.PANICULATA***		***T.BELLIRICA***		***C.HIRSUTUS***	
	LEAF	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**
1	-	05.01 ±1.01^{c}	00.45 ±2.14^{c}	06.05 ±1.21^{c}	-	-	-	-	-	-	-	04.56± 0.55^{c}	-	-
2	-	08.24± 4.35^{c}	02.23 ±1.88^{c}	10.98 ±1.53^{c}	-	-	-	-	-	-	-	07.15± 2.85^{c}	-	-
3	-	08.66± 1.35^{c}	15.75 ±2.63^{b}	14.51 ±1.56^{b}	-	-	-	-	-	-	-	13.01± 1.02^{b}	-	-
4	-	18.46±2 .86^{b}	18.07 ±1.34^{b}	16.78 ±7.16^{b}	-	-	-	-	-	-	-	17.27± 6.25^{b}	-	-
5	-	21.96± 1.38^{a}	27.63 ±1.46^{a}	24.43 ±7.38^{a}	-	-	-	-	-	-	-	21.85± 4.25^{a}	-	-

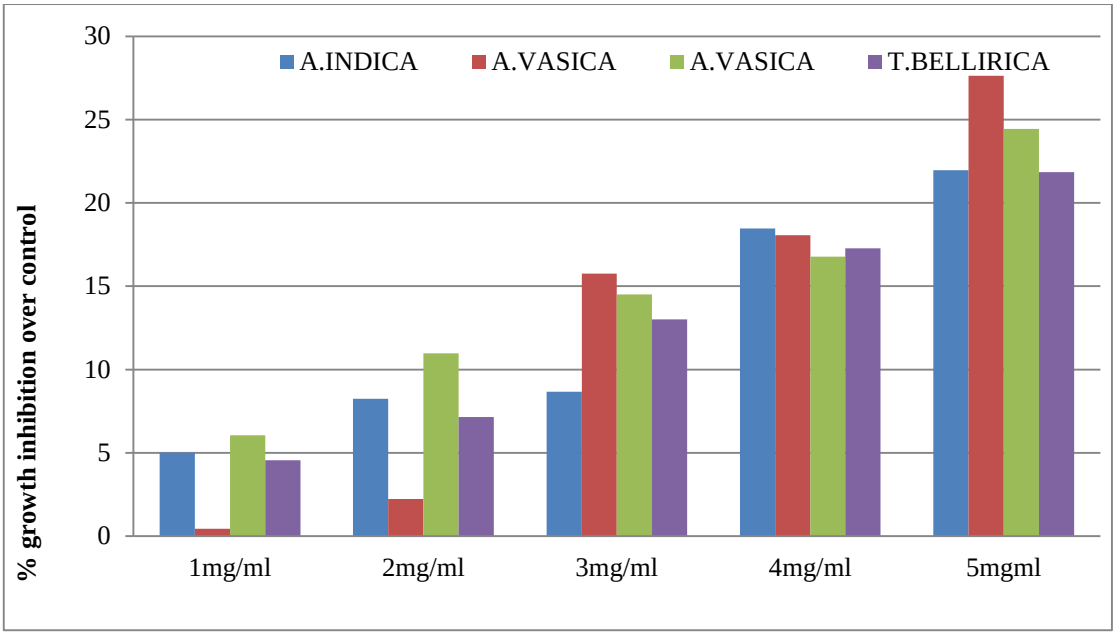


FIG. 5.3 Effect of Aqueous Extract on Percentage Growth Inhibition In Capsicum Capsici

Table 5.4 and Figure 5.4 illustrate the effects of ethanolic leaf (a) and stem (b) extracts on capsicum capsici at concentrationsranging from 1 to 5 mg/ml. The findings suggest that A. indica, A. vasica, and A. sessilis ethanolic extract had a larger percentage of growth inhibition than M. parvifolia, P. paniculata, and T. bellirica. The ethanolic stem extract of A. indica showed the highest radial inhibition (91.70 ± 2.49%), followed by A. vasica stem extract (82.72 ± 2.69%) at a dosage of 2 mg/mL. The ethanolic stem extract of T. bellirica at a dosage of 2 mg/ml showed the lowest growthinhibition (24.86 ± 2.11%). P. paniculata expressed 54.18 ± 3.12% and 43.7 ± 5.32% in ethanolic leaf and stem extracts, respectively. Eachvalue is given as mean of triplicates, & columns with same alphabeticalletters do not differ substantially ($p < 0.05$). - There is no impediment in radial development.

At a dosage of 3 mg/ml, A.indica and A. sessilis demonstrated over 80% growth inhibition. The ethanolic extract of A. indica and A. sessilis (leaf and stem) at 3 mg/ml concentration suppressed it by 82.07 ± 3.01%, 94.54 ± 0.84%, 81.83 ± 0.89%, and 80.53 ± 0.22%, respectively. In contrast, ethanolic extracts of M. parvifolia and T. bellirica (leaf and stem) showed less than 50% suppression of radial development. The ethanolic leaf and stem extract of M.parvifolia and T. bellirica at a concentration of 3 mg/ml showed 36.71 ± 4.32%, 44.42 ± 5.20%, 48.58 ± 6.48%, and 40.32 ± 2.82%, respectively.

At a dosage of 4 mg/ml, the ethanolic extract modestly increased radial growth inhibition. However, there was asignificant difference in ethanolic leaf extract of P. paniculata at 4 mg/ml concentration vs 3 mg/ml.

At a dosage of 5 mg/ml, ethanolic stem extract of A. indica was shown to be the most efficient in preventing radial growth of capsicum capsici when compared to the other ethanolic extracts tested. At a dosage of five mg/ml, A. indica inhibited capsicum capsici growth by 94.28 ± 1.09%. The ethanolic leaf & stem extracts of A. vasica inhibited growth diameter by 93.34 ± 1.89% and 91.34 ± 1.89 percent, respectively.

Each valueis given as mean of triplicates, & columns with same alphabetical letters do not differ substantially ($p < 0.05$). - exhibits no inhibition of radial development.

Overall, result revealed that among all concentration of plant extract of leaves and stem, 5 mg/ml concentration of leaves and stem extract were more effective against capsicum capsici whereas M. parvifolia was found less effective {41.40 ± 2.97% and 53.89 ± 2.99% (leaf and stem)} in inhibiting the growth.

TABLE 5.4 EFFECT OF ETHANOLIC PLANT EXTRACT (LEAF AND STEM) ON PERCENTAGE GROWTH INHIBITION IN CAPSICUM CAPSICI.

CONCENTRATIONS(MG/ML)	*A.INDICA*		*A.VASICA*		*A.SESSILIS*		*M.PARVIFOLIA*		*P.PANICULATA*		*T.BELLIRICA*		*C.HIRSUTUS*	
	LEAF	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**
1	74.01 ±2.85^{b}	81.60 ±3.22^{b}	42.53 ±5.00^{c}	80.12 ±2.24^{b}	39.53 ±2.36^{b}	62.23 ±1.15^{c}	22.88 ±4.61^{b}	24.38 ±2.86^{c}	49.86 ±5.04	37.27 ±4.84	31.25 ±0.46^{d}	28.93 ±8.81^{d}	-	-
2	77.14 ±0.33^{b}	91.70 ±2.41^{a}	76.78 ±6.89^{b}	82.72 ±2.69^{b}	80.45 ±3.36^{a}	74.16 ±3.04^{b}	35.75 ±1.6^{a}	36.59 ±0.97^{c}	54.18 ±3.12	43.7 ±5.32	24.86 ±2.11^{d}	55.84 ±2.17^{c}	-	-
3	82.07 ±3.01^{a}	94.54 ±0.84^{a}	76.35 ±5.29^{b}	84.75 ±0.75^{b}	81.83 ±0.89^{a}	80.53 ±0.22^{ab}	36.71 ±4.3^{a}	44.42 ±5.20^{b}	56.05 ±2.15	48.58 ±6.48	40.32 ±2.82^{c}	62.18 ±1.41^{b}	-	-
4	84.99 ±1.26^{a}	94.74 ±1.09^{a}	93.97 ±1.02^{a}	85.09 ±2.27^{b}	82.01 ±1.06^{a}	86.17 ±3.14a	40.15 ±2.3^{a}	46.53 ±0.48^{ab}	88.22 ±1.00	58.92 ±2.89	55.70 ±2.98^{b}	69.52 ±1.26^{ab}	-	-
5	91.26 ±1.88^{a}	94.28 ±0.18^{a}	93.65 ±0.17^{a}	91.34 ±1.89^{a}	86.96 ±2.92^{a}	87.70 ±2.41^{a}	41.40 ±2.9^{a}	53.89 ±2.99^{a}	88.22 ±2.54	69.03 ±4.4	69.09 ±3.81^{a}	74.27 ±1.45^{a}	-	-

Each value isexpressed as mean of triplicates, & column sharing same alphabetical letters are not significantly different ($p \leq 0.05$).

– represents no inhibition in radial growth

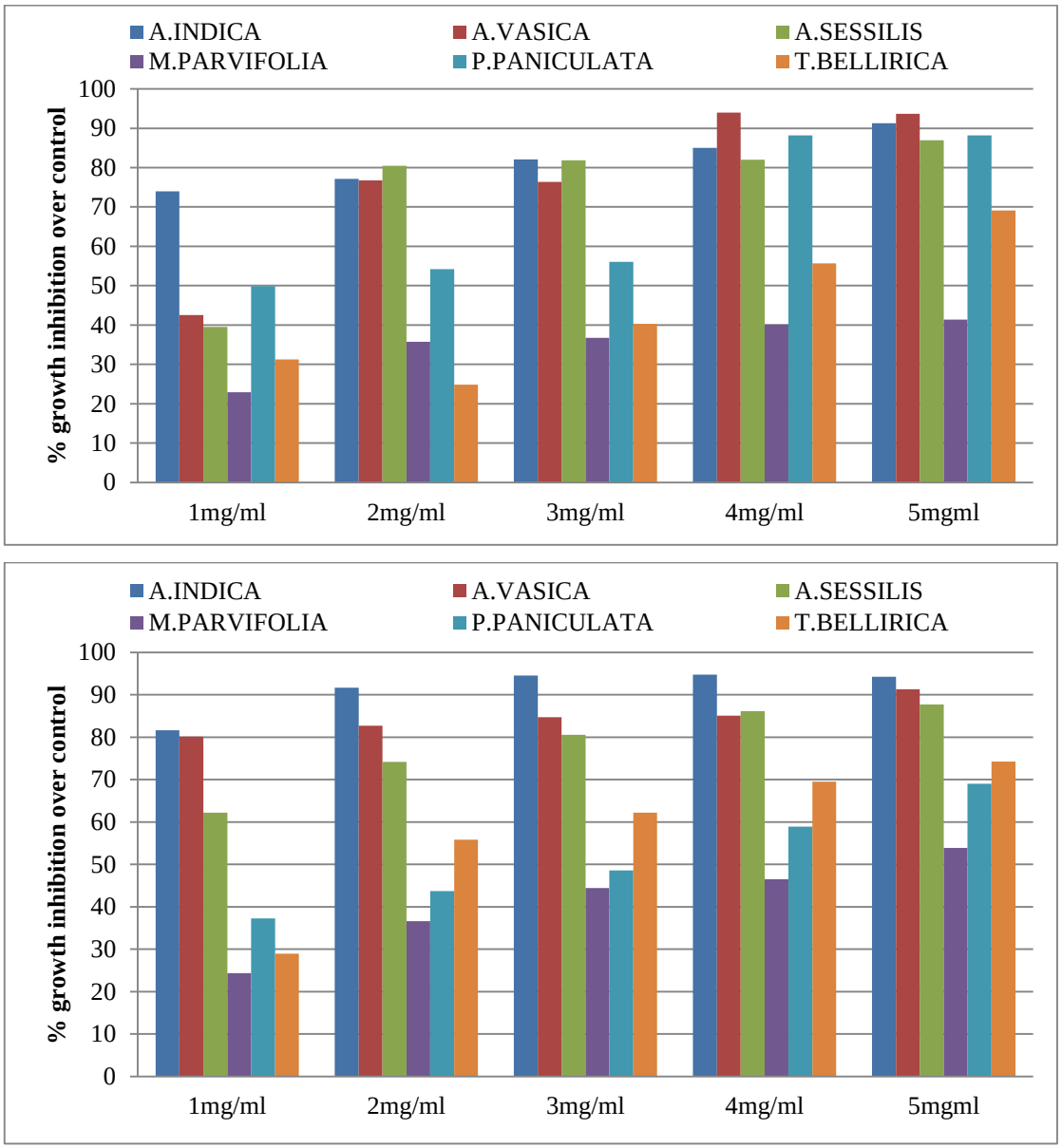


FIG. 5.4: EFFECT OF ETHANOLIC LEAF (a) & STEM (b) EXTRACTS ON % GROWTH INHIBITION IN CAPSICUM CAPSICI.

Table 5.5 and Figure 5.5 demonstrate the influence of methanolic plant extracts on radial development in capsicum capsici. At aconcentration of 1 mg/ml, methanolic extracts of M. parvifolia, P. paniculata, T. bellirica (leaf and stem), and capsicum hirsutus (leaf) had no effect on capsicum capsici growth, as did aqueous extracts. Growth inhibition was (16.69 ± 2.89%, 8.97 ± 3.99%), (19.21 ± 8.19%, 16.28 ± 8.9%), (28.73 ± 2.29%, 27.87 ± 2.67%), and 14.37 ± 4.77%, respectively, compared tocontrol.

At a dosage of 2 mg/ml, methanolicextracts of A. indica (leaf & stem) and A. vasica leaf extract inhibited radial growth of capsicum capsici by 71.29 ± 11.50%, 66.74 ± 5.93%, and 64.78 ± 10.25% respectively. At 2 mg/ml concentrations of A.vasica stem, leaf, & stem of A. sessilis, M. parvifolia, P. paniculata, T. bellirica, and leaf of capsicum, radial growth inhibition decreased by 45.34 ± 11.34%, (37.05 ± 2.45%, 32.33 ± 0.51%), (23.59 ± 5.56%, 20.79 ± 3.23%), (20.02 ± 2.5%, 17.78 ± 5.48%), (29.80 ± 1.17%, 25.34 ± 3.87%), and 29.02 ± 1.

TABLE 5.5 EFFECT OFMETHANOLIC PLANT EXTRACT (LEAF & STEM) ON % GROWTH INHIBITION ON CAPSICUM CAPSICI.

ONCENTRATIONS(MG/ML)	*A.INDICA*		*A.VASICA*		*A.SESSILIS*		*M.PARVIFOLIA*		*P.PANICULATA*		*T.BELLIRICA*		*C.HIRSUTUS*	
	LEAF	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**	**LEAF**	**STEM**
1	56.89 ±13.16^{b}	58.72 ±4.00^{b}	58.06 ±4.29^{b}	49.94 ±11.40^{b}	32.88 ±3.68^{c}	27.75 ±3.37^{c}	16.69 ±2.89^{b}	8.97 ±3.99^{b}	19.21 ±8.19^{d}	16.28 ±8.9^{c}	28.73 ±2.29^{b}	27.87 ±2.67^{c}	14.37 ±4.77^{c}	-
2	71.29 ±11.50^{a}	66.74 ±5.93^{b}	64.78 ±10.25^{a}	45.34 ±11.34^{b}	37.05 ±2.45^{c}	32.33 ±0.51^{bc}	23.59 ±5.56^{ab}	20.79 ±3.23^{a}	20.02 ±2.5^{cd}	17.7 ±5.4^{c}	29.80 ±1.17^{b}	25.34 ±3.87^{c}	29.02 ±1.56^{b}	-
3	78.19 ±5.10^{a}	77.79 ±1.77^{a}	64.54 ±5.6^{ab}	68.08 ±9.61^{a}	69.58 ±0.69^{b}	41.61 ±7.83^{b}	26.10 ±4.41^{ab}	22.91 ±1.33^{a}	25.43 ±1.06^{c}	43.96 ±2.4^{b}	30.97 ±2.89^{b}	52.45 ±2.39^{b}	43.96 ±2.46^{a}	-
4	80.78 ±1.24^{a}	72.15 ±2.15^{a}	67.45 ±1.66^{a}	66.44 ±8.52^{a}	75.00 ±1.06^{ab}	54.97 ±1.38^{a}	28.21 ±2.62^{ab}	26.73^{a} ±0.49	33.28 ±1.74^{b}	48.94 ±0.7^{a}	35.58 ±1.31^{ab}	72.83 ±1.27^{a}	46.66 ±1.97^{a}	-
5	82.19 ±3.03^{a}	73.08 ±1.19^{a}	72.87 ±0.41^{a}	74.44 ±3.64^{a}	79.00 ±4.29^{a}	60.55 ±2.40^{a}	32.22 ±0.97^{a}	27.49 ±0.68^{a}	43.89 ±2.77^{a}	55.3 ±4.8^{a}	42.47 ±3.49^{a}	79.89 ±0.91^{a}	48.45 ±1.98^{a}	-

Each value isexpressed as mean of triplicates, & column sharing same alphabetical letters are notsignificantly different ($p \leq 0.05$).

– represents no inhibition in radial growth.

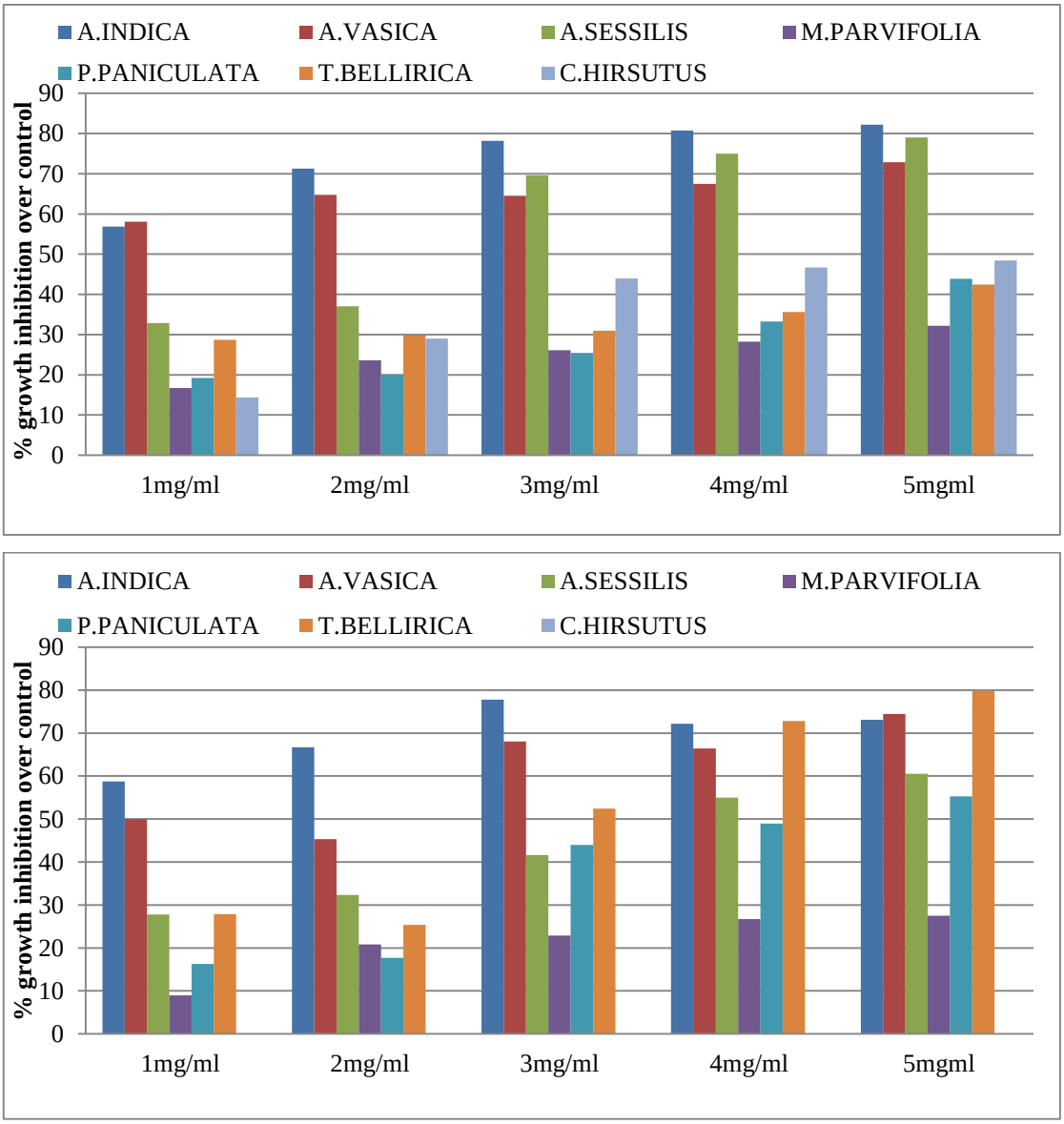


FIG. 5.5 EFFECT OF METHANOLIC LEAF (a) & STEM (b) EXTRACTS ON % GROWTH INHIBITION IN CAPSICUM CAPSICI.

3mg/ml concentration of methanolic extract of A. indica, A.vasica and A. sessilis (leaves & stem) were found effective (78.19 ± 5.10%, 77.79 ± 1.77%), (64.54 ± 5.60%, 68.08 ± 6.91%) and (69.58 ± 0.69%, 41.61 ± 7.83%) in inhibiting radial growth of capsicum capsici than M. parvifolia, P. paniculata, T. bellirica and capsicum hirsutus.

Finally, methanolic extracts of leaves at a dosage of 4 mg/ml were shown to be more efficient than stem extracts in inhibiting capsicum capsici's radial growth. A. indica, A.vasica, and A. sessilis leaf extracts showed considerable radial growth (80.78 ± 1.24%, 67.45 ± 2.66%, and 75.00 ± 1.06%, respectively). Methanolic stem extractsof A. indica, A.vasica, and A. sessilis were less efficacious than leaves at a concentration of 4 mg/mL. Stem extracts of A.indica, A. vasica, and A. sessilis inhibited radial growth by 72.15 ± 2.15%, 66.44 ± 8.52%, and 54.97 ± 1.38%, respectively. At a concentration of 4 mg/ml, methanolic stem extractof T. bellirica inhibited capsicum capsici growth more effectively (72.83 ± 1.27%) than leaf extract (35.58 ± 1.31%).

The methanolic (leaf and stem) extract had the greatest effect on capsicum capsici growth at a dosage of 5 mg/ml. The methanolic leaf extractof A. indica showed highest growth inhibition (82.19 ± 3.03%), followed by A. sessilis (79.00 ± 4.29%) and A. vasica (72.87 ± 0.41%). In methanolic stem extract, T. bellirica showed the strongest growth inhibition (79.89 ± 0.91%) compared to A. v asica (74.44 ± 3.64%) and A. vasica (73.08 ± 1.19%).

5.2.2. Inhibitory Concentration

The minimum inhibitory concentration (MIC) of A. indica, A.vasica, A. sessilis, & P. paniculata against capsicum capsici in an ethanolic extract of leaves was 5 mg/ml (Table 5.6).

TABLE 5.6 MIC & IC50 OF PLANT EXTRACTS (MG/ML).

EXTRACTS	***A.INDICA***		***A.VASICA***		***A.SESSILIS***		***M.PARVIFOLIA***		***P.PANICULATA***		***T.BELLIRICA***		***C.HIRSUTUS***	
	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC
EL	0.5	5	1.5	5	1.5	5	-	-	2	5	4	-	-	-
ES	0.5	2	0.5	5	1	5	5	-	4	-	-	-	-	-
ML	1	-	1.5	-	2.5	-	-	-	-	-	-	-	5	-
MS	1	-	2.5	-	4	-	-	-	5	-	3	-	-	-

Data are expressed in replication (n = 3) of mean ± standard error. – represents no inhibition in radial growth. Where, EL = Ethanolic leaf, ES =
Ethanolic stem, ML = Methanolic leaf, MS = Methanolic stem

M. parvifolia, T. bellirica, and capsicum hirsutus did not have MICs with ethanolic extracts. A. vasica and A. sessilis had MICs of 5 mg/ml in an ethanolic extract of stem. In

contrast, 2 mg/ml was recorded for A. indica extract. The MIC for the methanolic extract of leaves & stem was reported by all plant extracts. The half inhibitory concentrations (IC50) were also measured and differed from extract to extract. The IC50 for A. indica leaf extract was reported tobe 1 mg/ml. The IC50 of methanolic stem extract was observed as rising as 2.5, 3, 4, and 5 mg/ml by A. vasica, T. bellirica, A. sessilis, and P. paniculata.

5.2.3. Inhibition of Conidia Germination

Table 5.7 shows the impact of ethanolic leaf & stem extracts of A. indica, A. vasica, A.sessilis, M. parvifolia, P. paniculata, T. bellirica, and capsicum hirsutus on capsicum capsici conidia germination. The MIC and IC50 of ethanolic leaf and stem extracts were determined to prevent capsicum capsici conidia germination. At MIC concentrations, ethanolic leaf extract of A. vasica significantly inhibited capsicum capsici conidia germination.

TABLE 5.7: EFFECT OF PLANT EXTRA CTSON CONIDIA GERMINATION % INHIBITION

EXTRACTS	***A.INDICA* (MG/ML)**		***A.VASICA* (MG/ML)**		***A.SESSILIS* (MG/ML)**		***M.PARVIFOLIA* (MG/ML)**		***P.PANICULATE* (MG/ML)**		***T.BELLIRICA* (MG/ML)**		***C.HIRSUTUS* (MG/ML)**	
	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC	IC50	MIC
EL	36.53 ± 4.98	87.5 ±0.93	72.0 ±6.43	90.38 ±1.22	48.06 ± 1.66	77.88 ± 0.96	-	-	10.55 ± 1.66	39.4 ± 1.66	7.67 ±1.75	-	-	-
ES	39.42 ± 2.99	75.96 ±1.5	59.61 ± 1.24	83.65 ±2.1	21.13 ± 0.96	54.79 ± 1.92	47.10 ± 0.96	-	-	-	-	-	-	-
ML	49.0 ± 0.49	-	52.88 ±1.36	-	5.75 ± 0.96	-	-	-	-	-	-	-	36.48 ± 0.75	-
MS	47.0 ±5.4	-	46.15 ± 7.74	-	34.60 ± 0.96	-	-	-	12.25 ± 2.12	-	18.25 ±2.54	-	-	-

The MIC of ethanolic leaf extracts from A. vasica and A. indica inhibited conidia germination by 90.36 ± 1.22% and 87.5 ± 0.93%, respectively. Ethanolic leaf extract of A. sessilis inhibited conidia germination of capsicum capsici by 77.88 ± 0.96% at MIC. The MIC of ethanolic stem extract was also shown to be efficient at inhibiting conidia germination. The MIC of A. vasica, A. indica, and A. sessilis showed a reduction of 83.65 ± 2.10%, 75.96 ± 1.5%, and 54.79 ± 1.92%, respectively.

The half inhibitory concentration (IC50) of plant extract was also determined to prevent capsicum capsici conidia germination. At this percentage of ethanolic stem extract, conidia germination inhibition was 59.61 ± 1.24%, 47.10 ± 0.96%, 39.42 ± 2.99%, and 21.13 ± 0.96% in the capsicum capsici by the A. vasica, M. parvifolia, A. indica, and A. sessilis. capsicum capsici conidia germination was inhibited by A. vasica ethanolic leaf extract at an IC50 of 72.00 ± 6.43%, followed by 48.06 ± 1.66 (A. sessilis) and 36.53 ± 4.98% (A. indica).

The methanolic extract's IC50 was also shown to be efficient in inhibiting capsicum capsici conidia germination. The IC50 of methanolic leaf extract of A. vasica inhibited conidia germination by 52.88 ± 1.36%, followed by A. indica (49.00 ± 0.49%), capsicum hirsutus (36.48 ± 0.75%), and A. sessilis (5.75 ± 0.96%). capsicum capsici was inhibited by 47.00 ± 5.4%, 46.15 ± 0.96%, and 34.60 ± 0.96% methanolic stem extracts ofA. indica, A. vasica, and A. sessilis, respectively.

Overall, the MIC of A. indica, A.vasica, and A. sessilis ethanolic leaf extract was shown to bemore efficient in inhibiting conidia germination than stem extract. The MIC of P. paniculata prevented 39.4 ± 1.66% conidia germination. Furthermore, the methanolic extract was shown to have a higher IC50 for inhibiting capsicum capsici conidia germination.

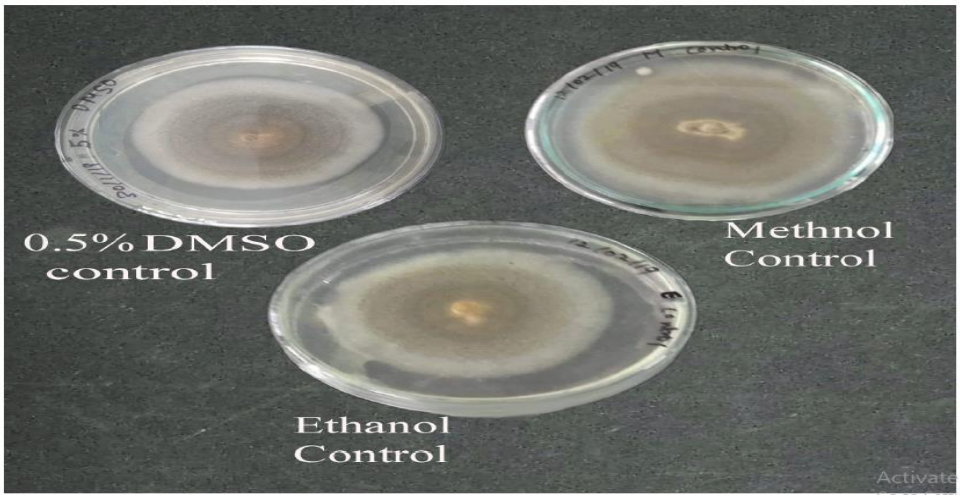


FIG. 5.6 EFFECT OF 0.5% DMSO (A), METHANOL CONTROL (B) AND ETHANOL CONTROL (C) ON RADIAL GROWTH OF CAPSICUM CAPSICI

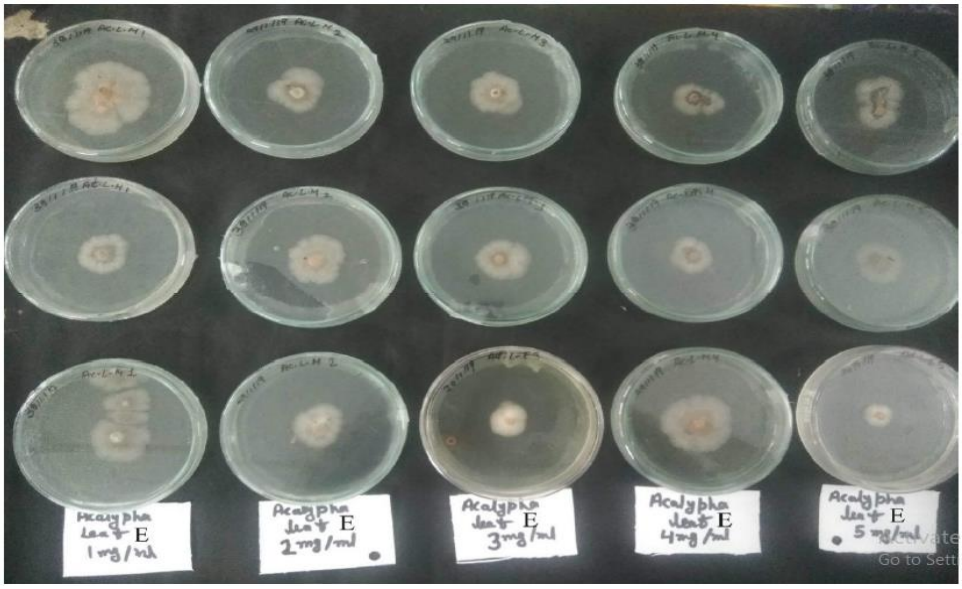


FIG. 5.7 IN-VITRO ANTIFUNGAL EFFECTOF ETHANOLIC LEAF EXTRACT OF A. INDICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/ML CONCENTRATIONS.

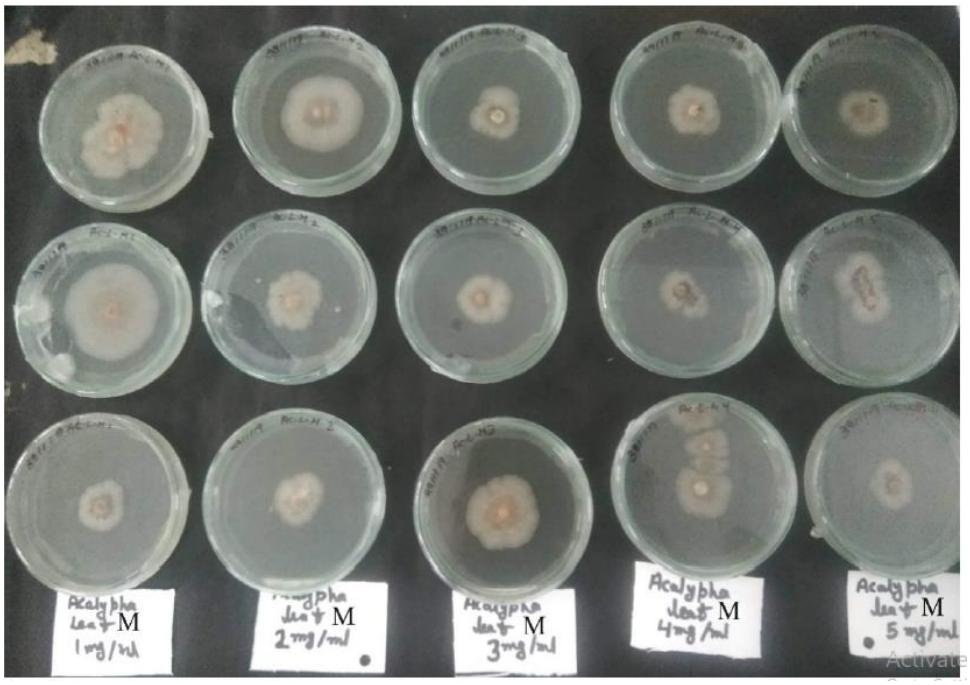


FIG. 5.8 IN-VITRO ANTIFUNGAL EFFECTOF METHANOLIC LEAF EXTRACT OF A. INDICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

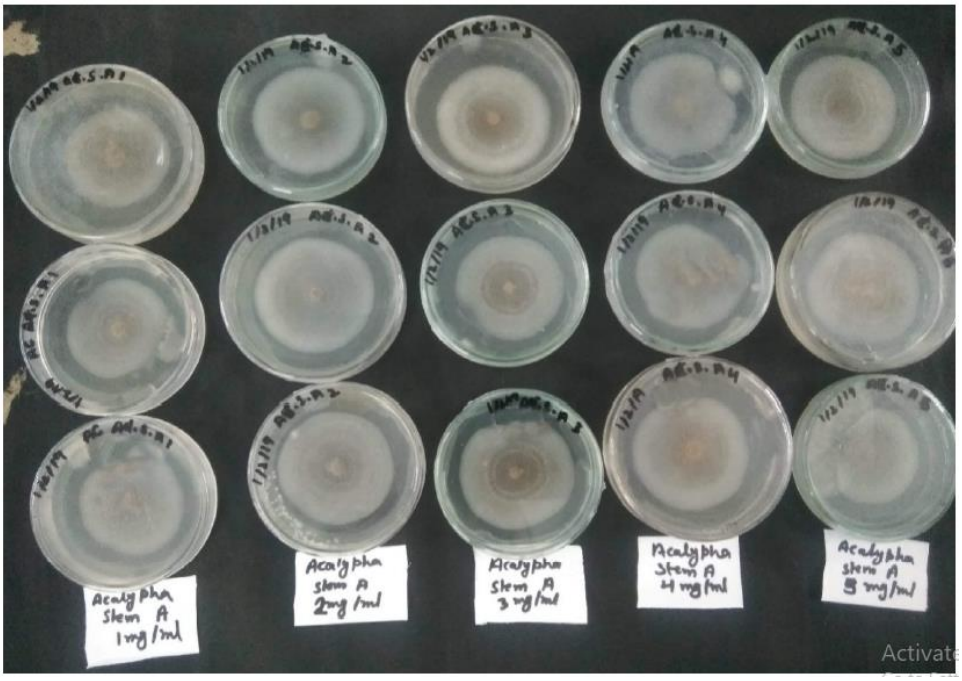


FIG. 5.9 IN-VITRO ANTIFUNGAL EFFECT OFAQUEOUS STEM EXTRACT OF A. INDICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

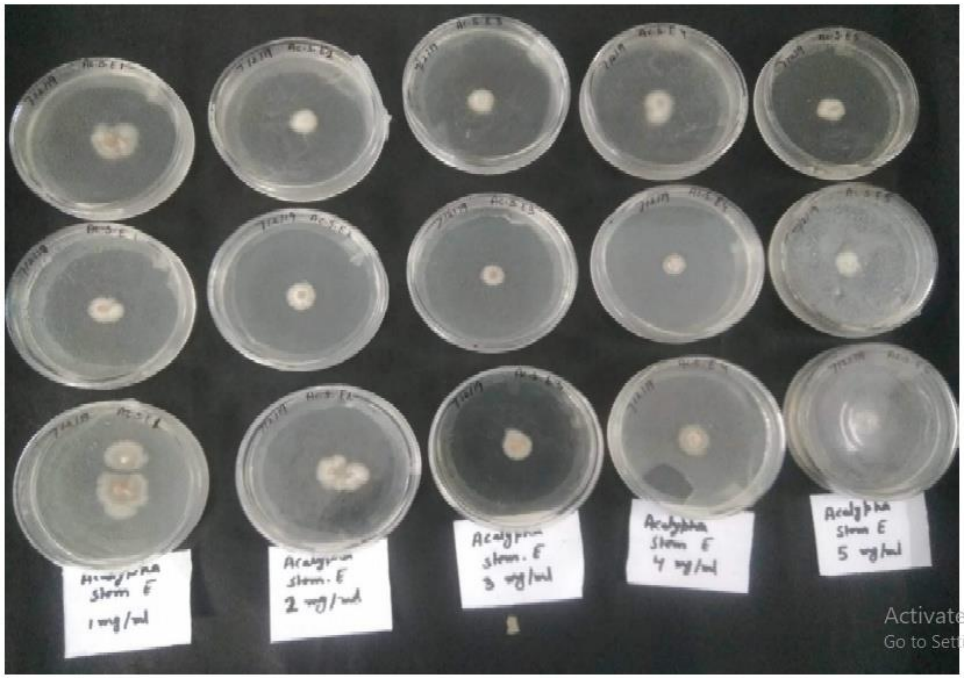


FIG. 5.10 IN-VITRO ANTIFUNGAL EFFECT OF ETHANOLIC STEM EXTRACTOF A. INDICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

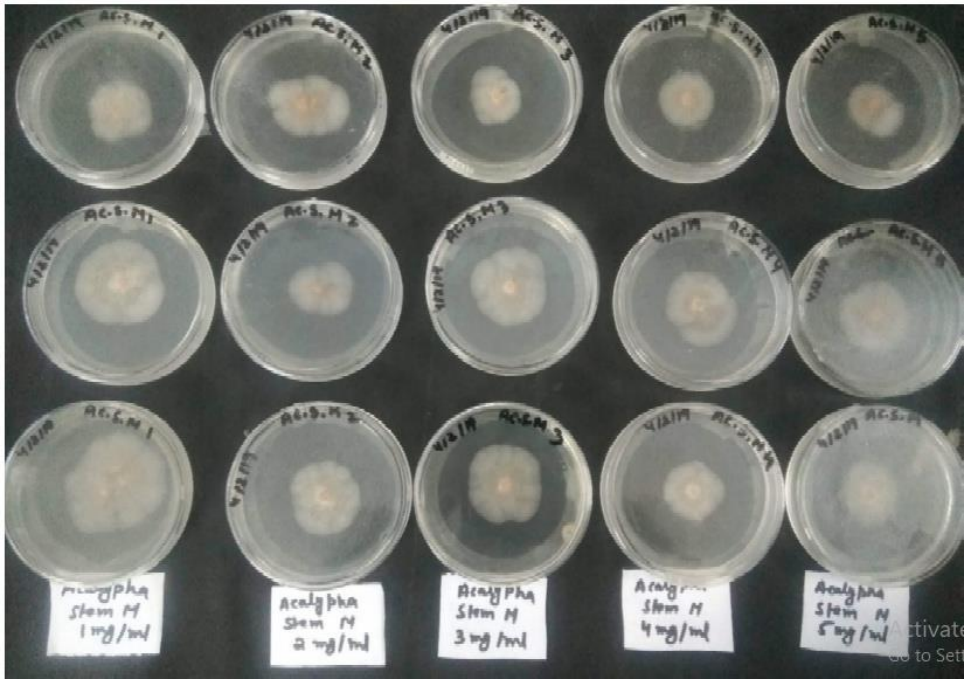


FIG. 5.11 IN-VITRO ANTIFUNGAL EFFECT OFMETHANOLIC STEM EXTRACTOF A. INDICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

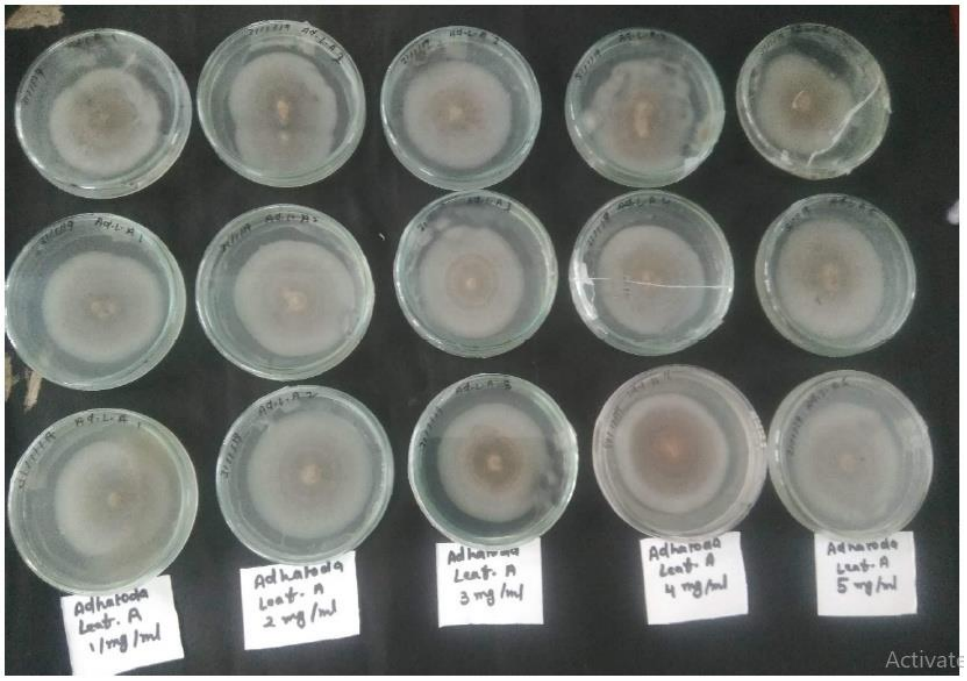


FIG. 5.12 IN-VITRO ANTIFUNGAL EFFECT OFAQUEOUS LEAF EXTRACTOF A. VASICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

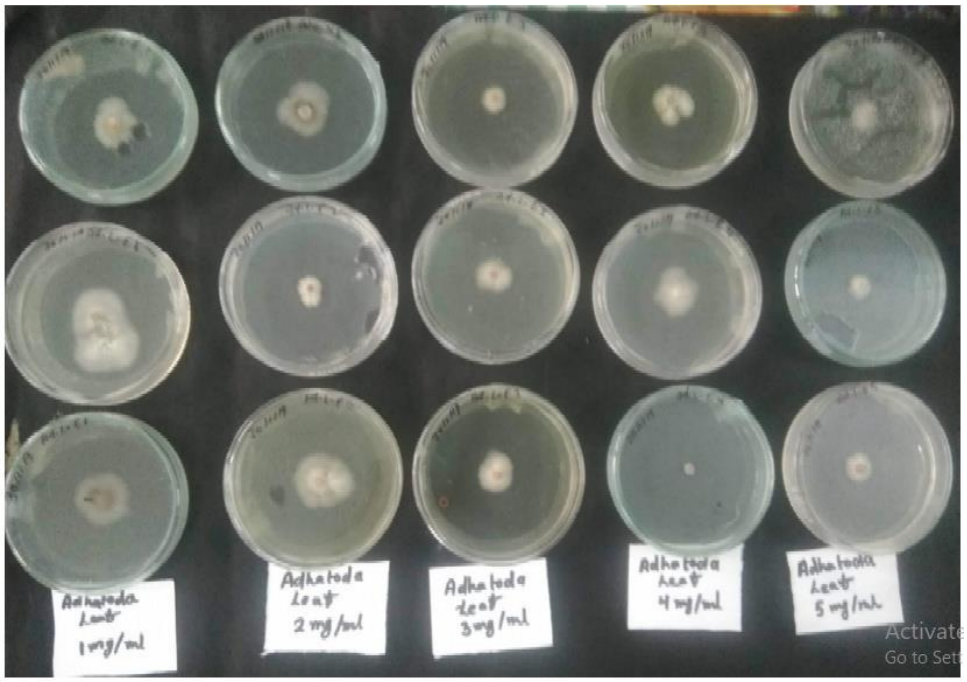


FIG. 5.13 IN-VITRO ANTIFUNGAL EFFECTOF ETHANOLIC LEAF EXTRACTOF A. VASICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

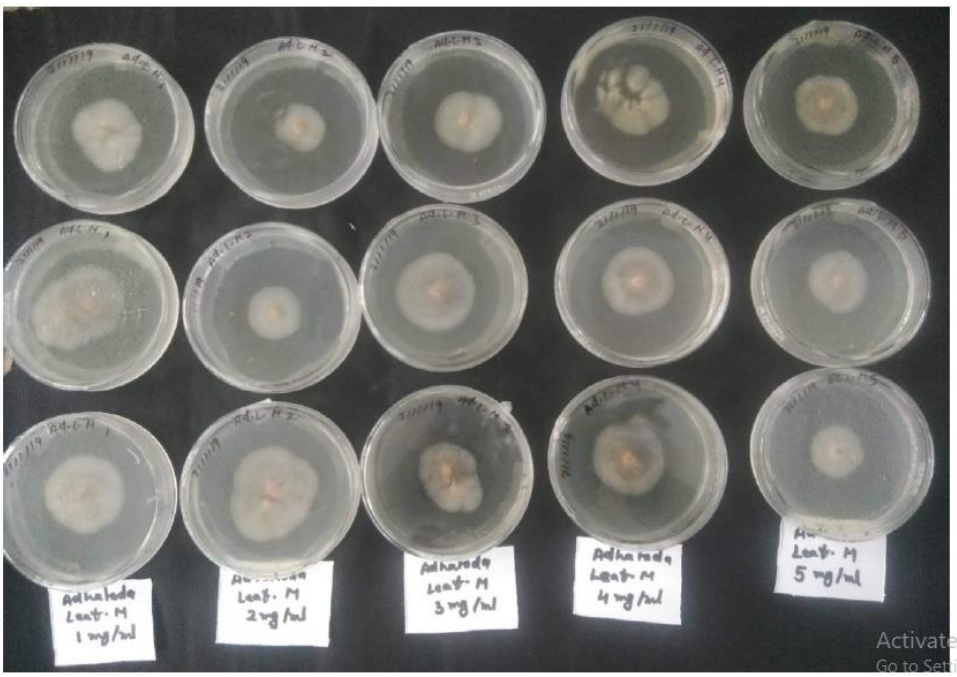


FIG. 5.14 IN-VITRO ANTIFUNGAL EFFECTOF METHANOLIC LEAF EXTRACTOF A. VASICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

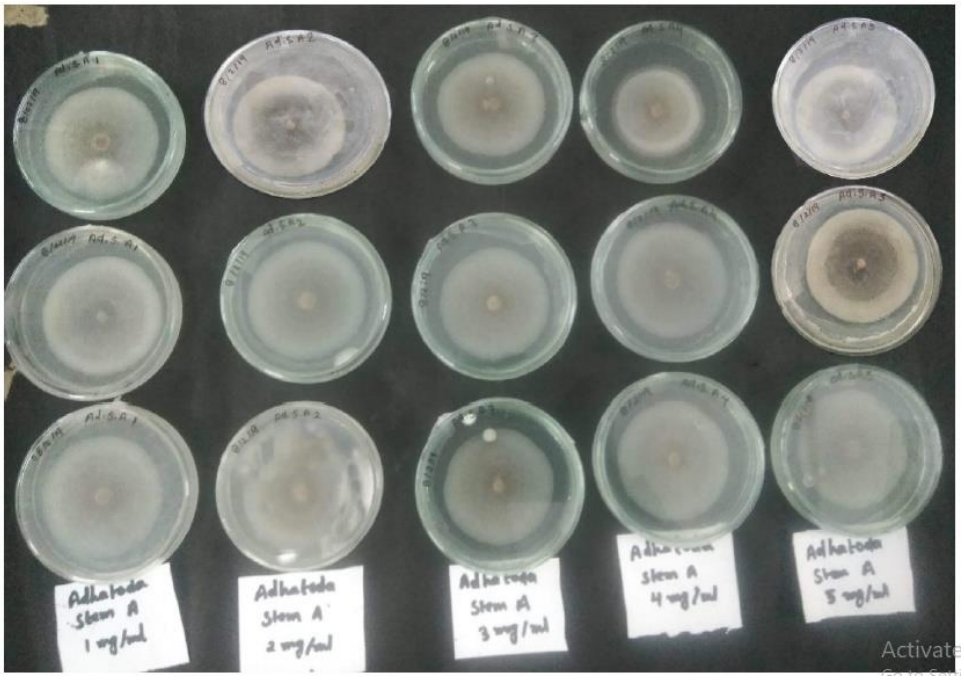


FIG. 5.15 IN-VITRO ANTIFUNGAL EFFECT OFAQUEOUS STEM EXTRACTOF A. VASICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

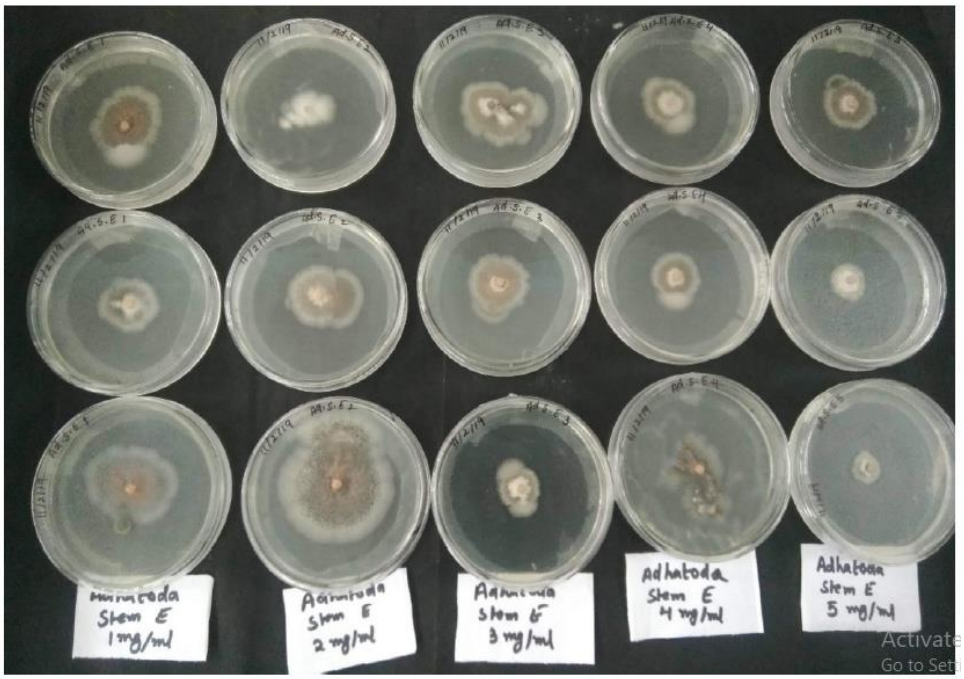


FIG. 5.16 IN-VITRO ANTIFUNGAL EFFECT OFETHANOLIC STEM EXTRACT OF A.VASICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

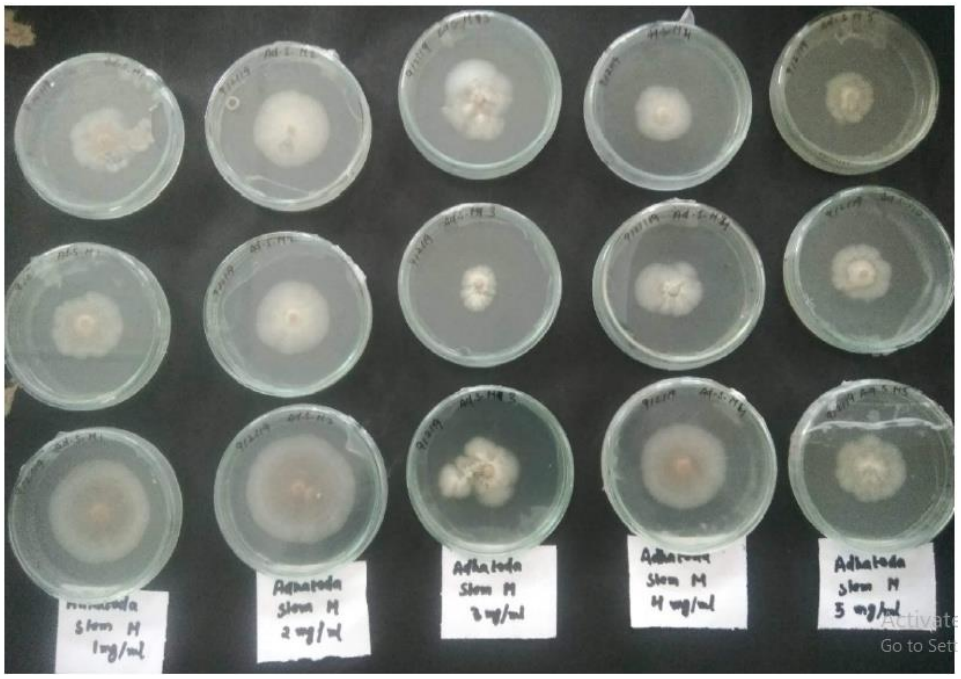


FIG. 5.17 IN-VITROANTIFUNGAL EFFECT OF METHANOLIC STEM EXTRACT OF A. VASICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

FIG. 5.18 IN-VITRO ANTIFUNGAL EFFECTOF ETHANOLIC LEAF EXTRACTOF A. SESSILIS ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

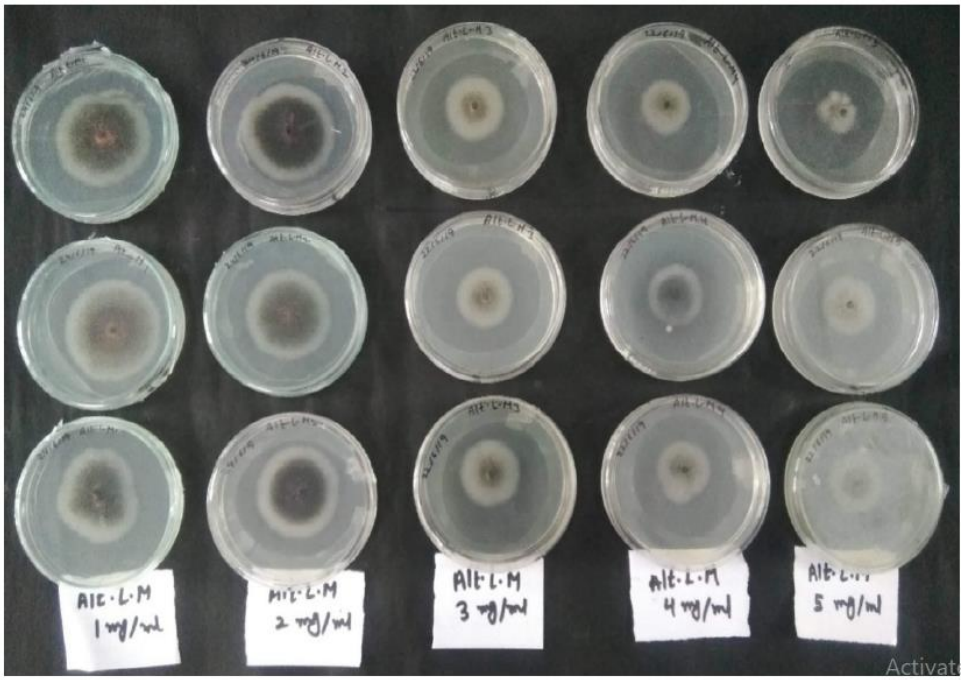


FIG. 5.19 IN-VITRO ANTIFUNGAL EFFECTOF METHANOLIC LEAF EXTRACTOF A. SESSILIS ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

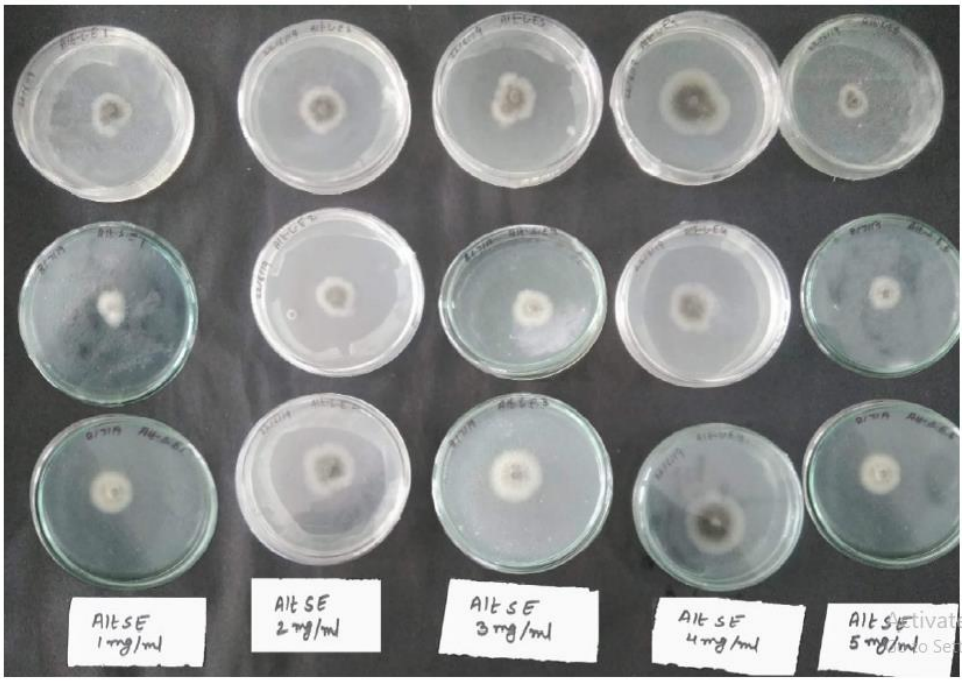


FIG. 5.20 IN-VITRO ANTIFUNGAL EFFECT OFETHANOLIC STEM EXTRACT OF A. SESSILIS ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

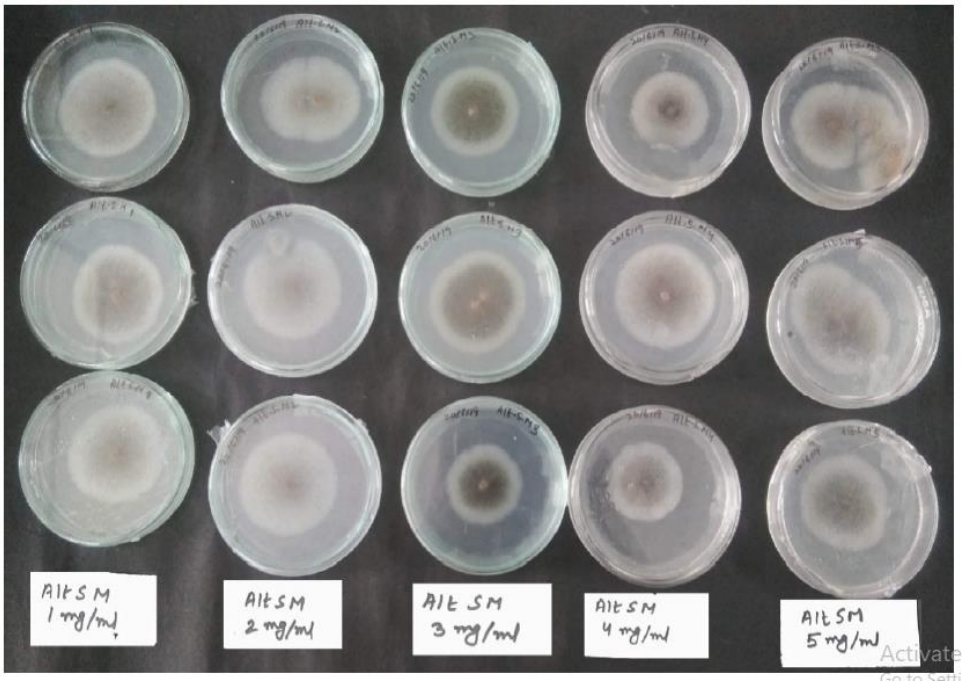


FIG. 5.21 IN-VITROANTIFUNGAL EFFECT OF METHANOLIC STEM EXTRACTOF A. SESSILIS ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

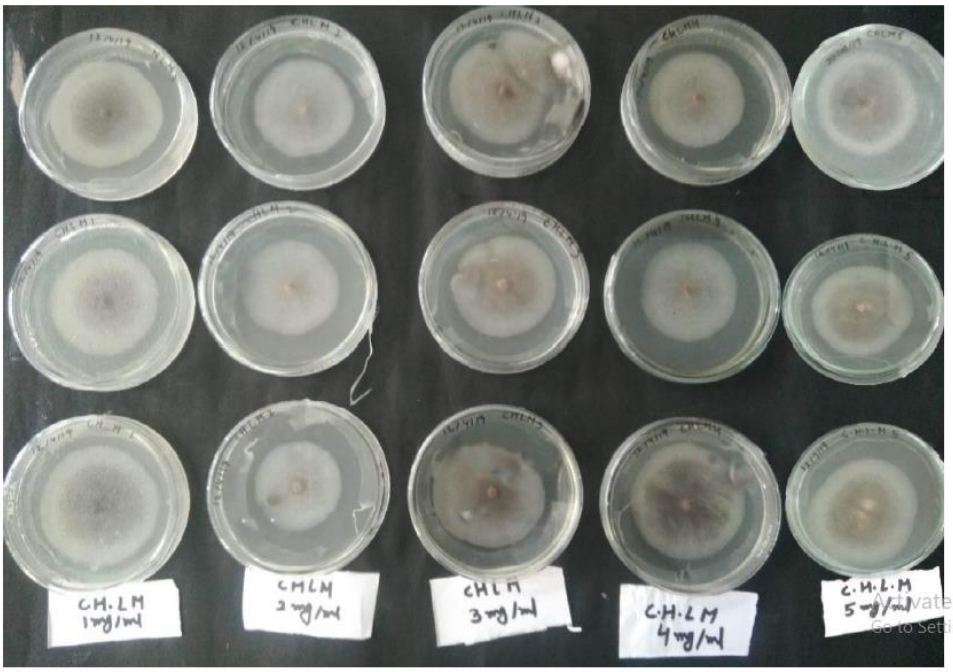


FIG. 5.22 IN-VITRO ANTIFUNGAL EFFECTOF METHANOLIC LEAF EXTRACTOF CAPSICUM HIRSUTUS ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

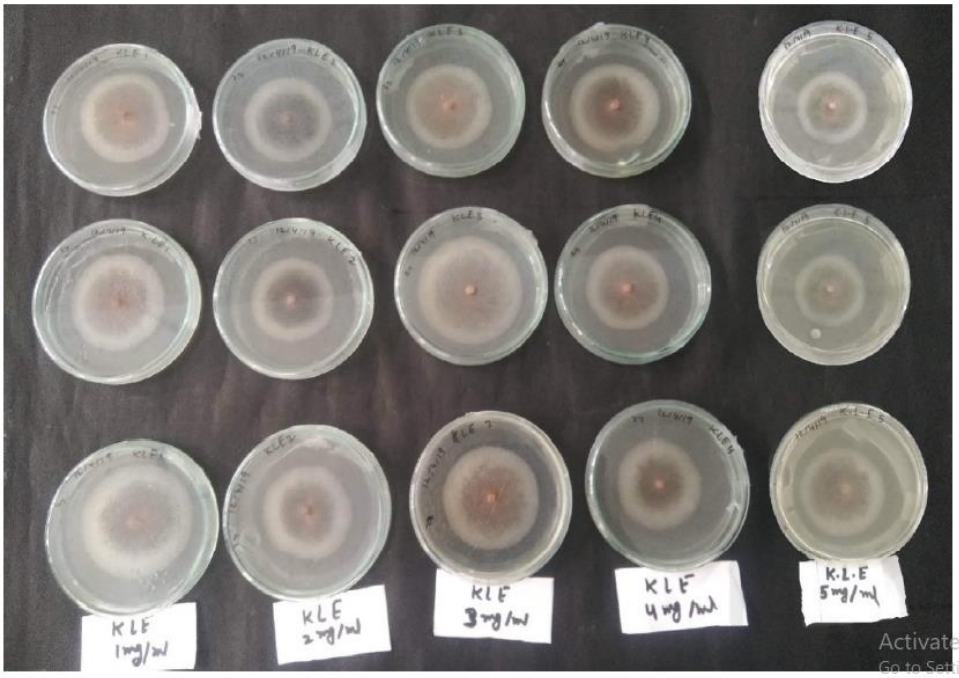


FIG. 5.23 IN-VITRO ANTIFUNGAL EFFECTOF ETHANOLIC LEAF EXTRACTOF M. PARVIFOLIA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

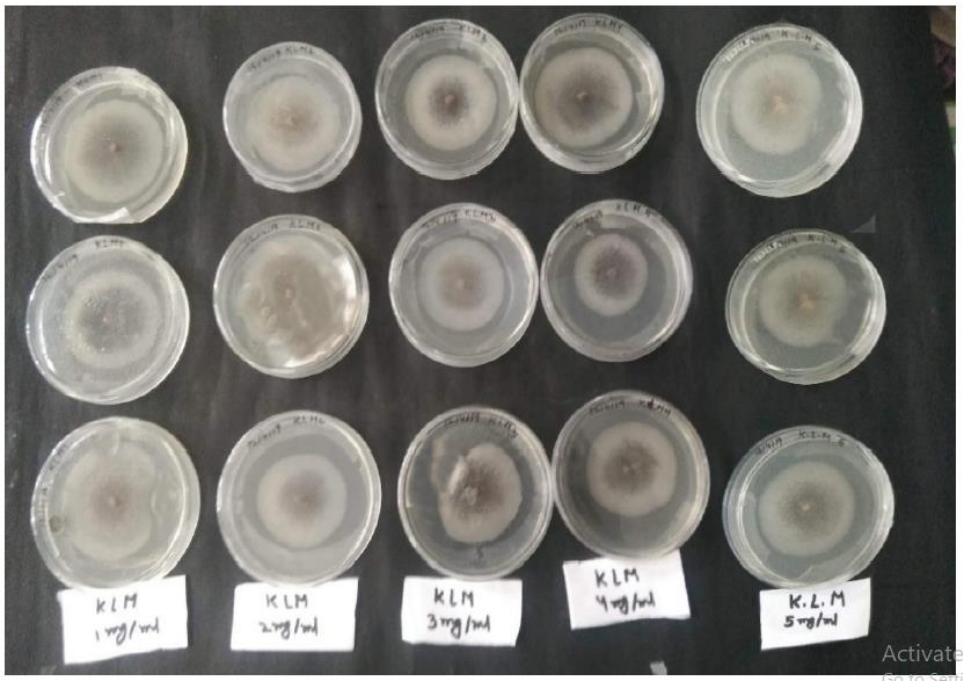


FIG. 5.24 IN-VITRO ANTIFUNGAL EFFECTOF METHANOLIC LEAF EXTRACTOF M. PARVIFOLIA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

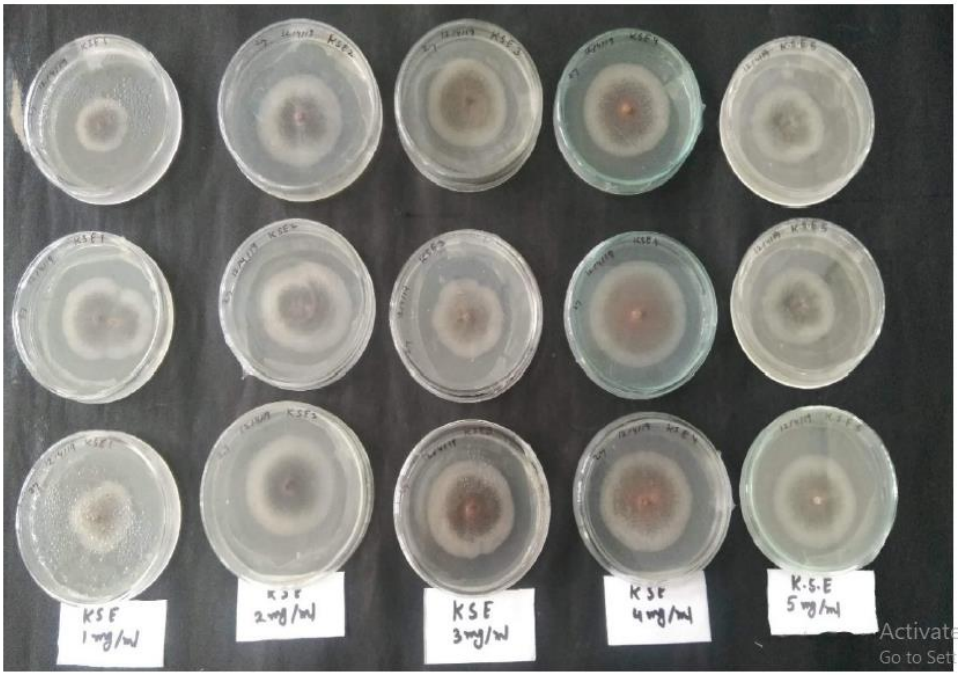


FIG. 5.25 IN-VITRO ANTIFUNGAL EFFECT OFETHANOLIC STEM EXTRACT OF M. PARVIFOLIA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

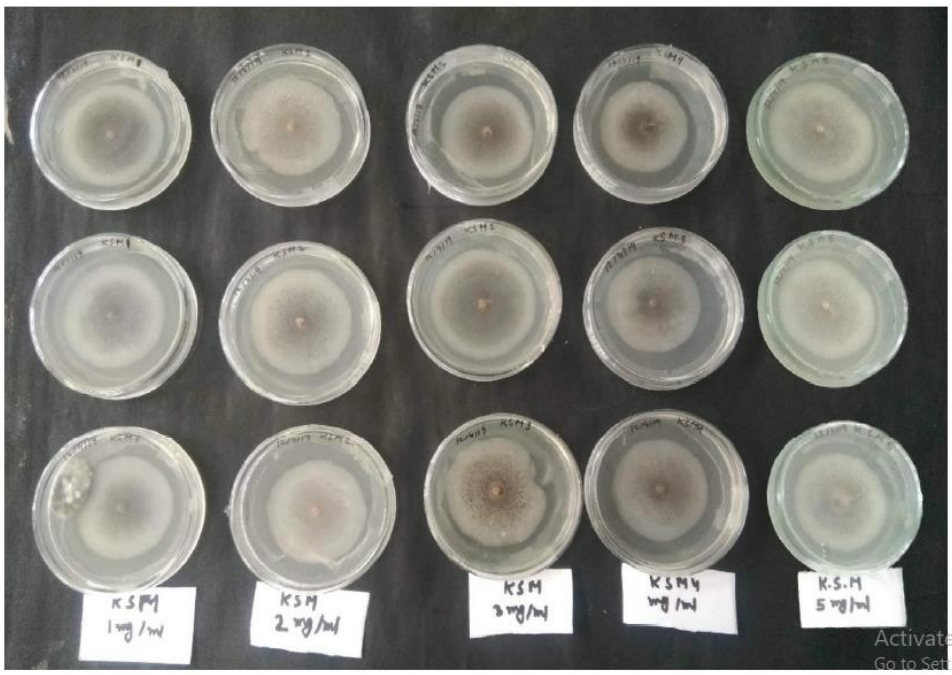


FIG. 5.26 IN-VITROANTIFUNGAL EFFECT OF METHANOLIC STEM EXTRACTOF M. PARVIFOLIA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5MG/ML CONCENTRATIONS

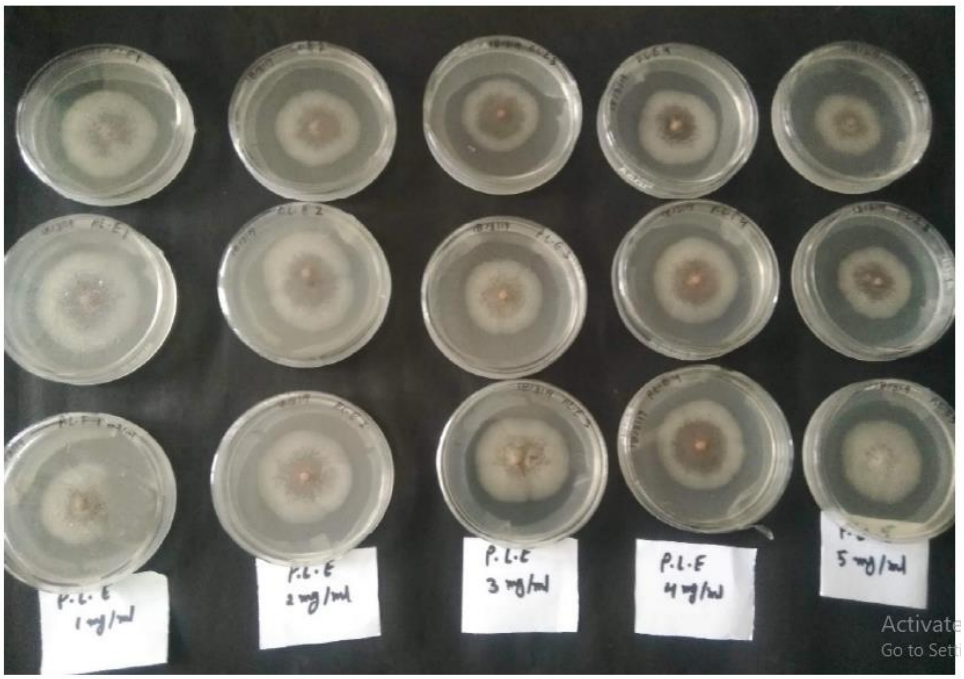


FIG. 5.27 IN-VITRO ANTIFUNGAL EFFECTOF ETHANOLIC LEAF EXTRACTOF P. PANICULATA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

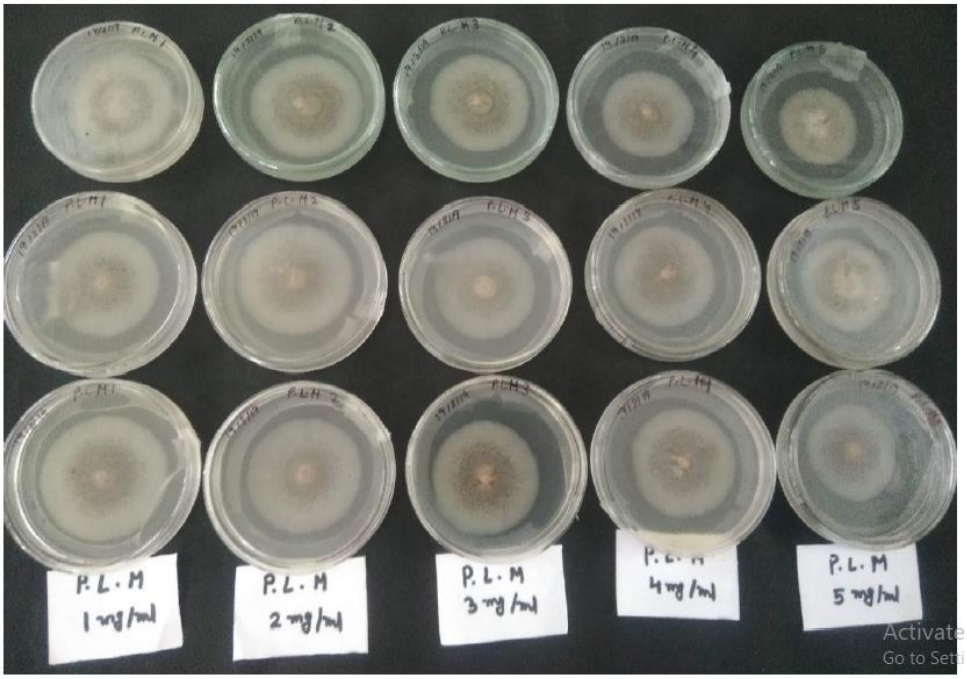


FIG. 5.28 IN-VITRO ANTIFUNGAL EFFECTOF METHANOLIC LEAF EXTRACTOF P. PANICULATA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

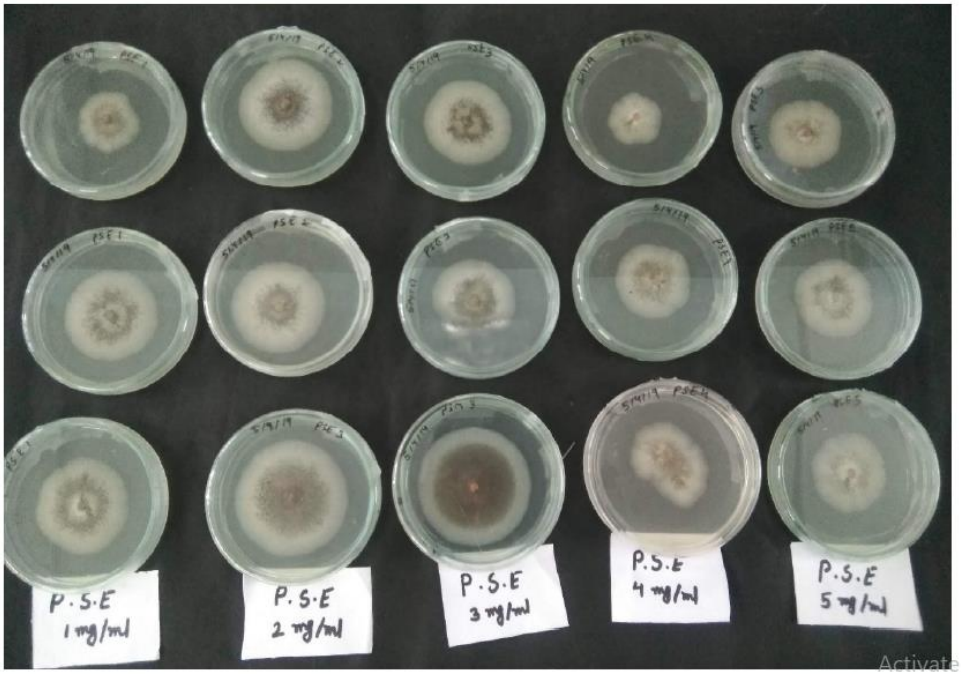


FIG. 5.29 IN-VITRO ANTIFUNGAL EFFECT OFETHANOLIC STEM EXTRACTOF P. PANICULATA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

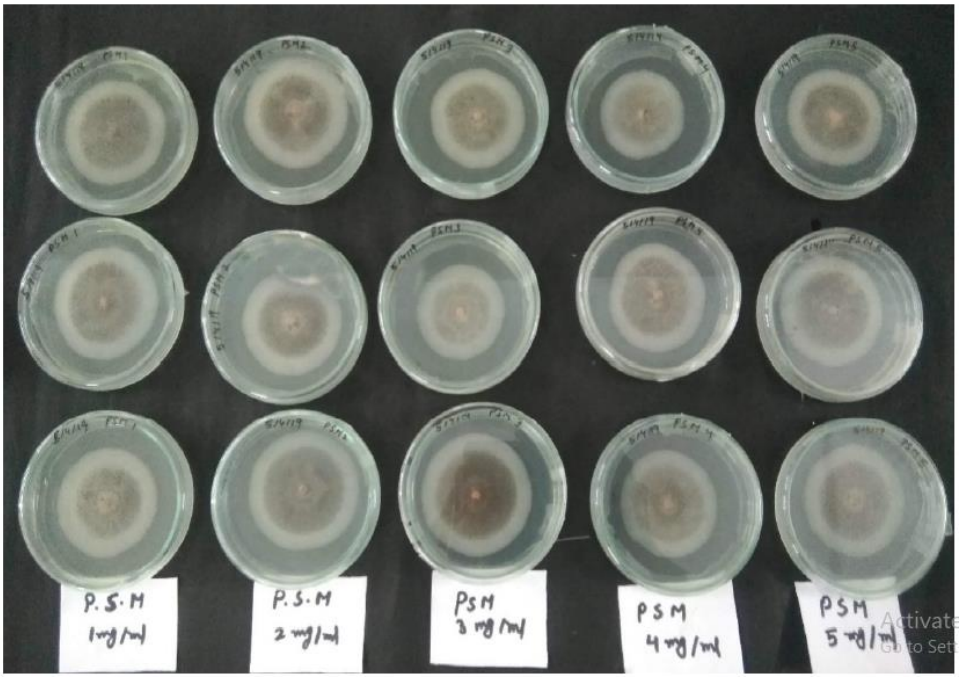


FIG. 5.30 IN-VITROANTIFUNGAL EFFECT OF METHANOLIC STEM EXTRACT OF P. PANICULATA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

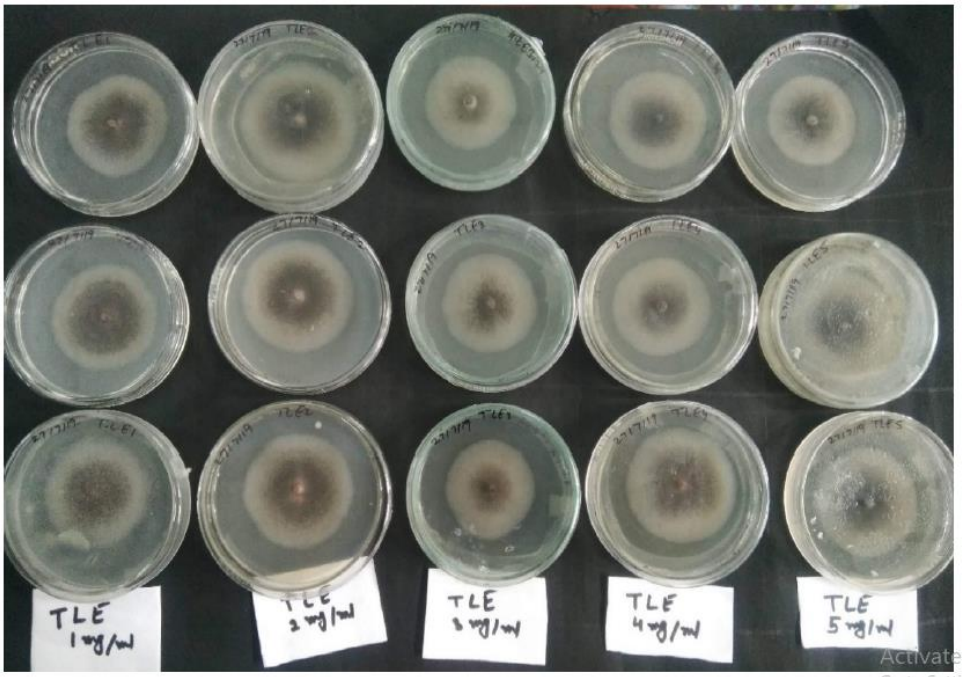


FIG. 5.31 IN-VITRO ANTIFUNGAL EFFECTOF ETHANOLIC LEAF EXTRACTOF T. BELLIRICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

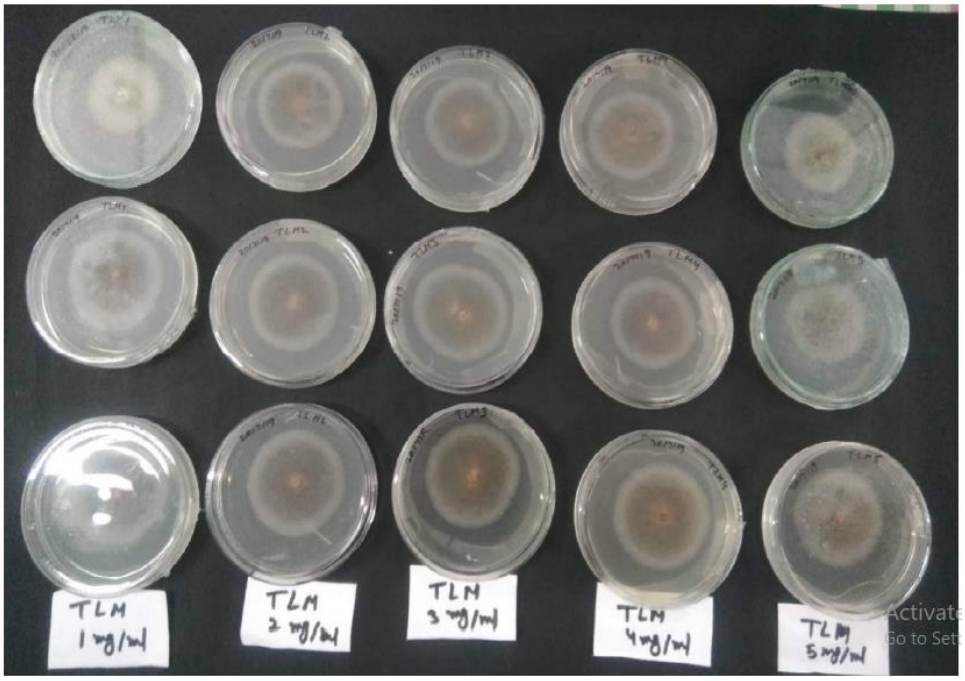


FIG. 5.32 IN-VITRO ANTIFUNGAL EFFECTOF METHANOLIC LEAF EXTRACTOF T. BELLIRICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS.

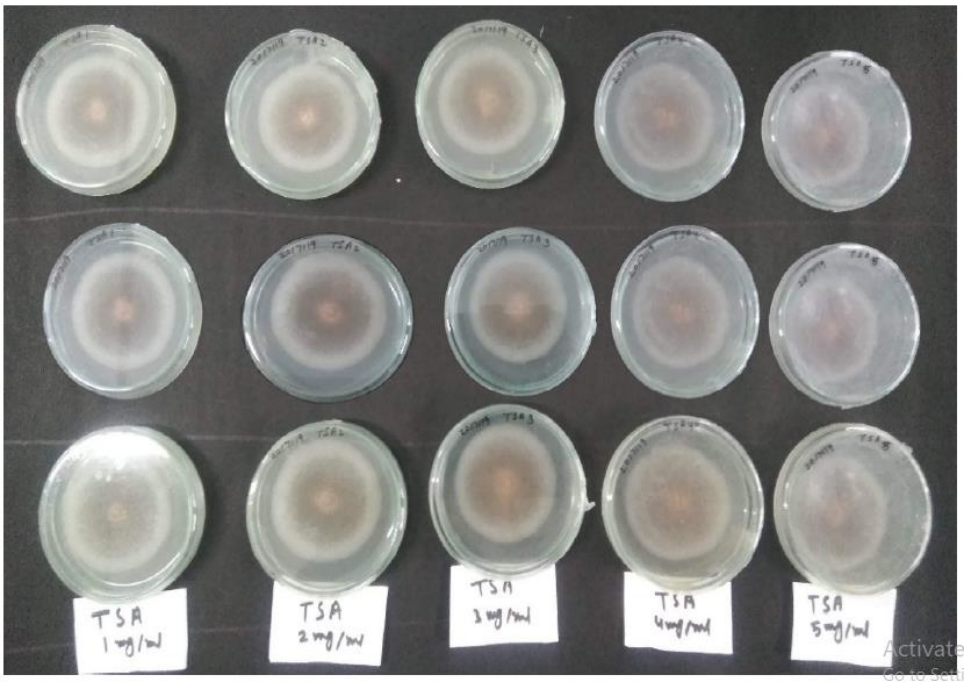


FIG. 5.33 IN-VITRO ANTIFUNGAL EFFECT OFAQUEOUS STEM EXTRACTOF T. BELLIRICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

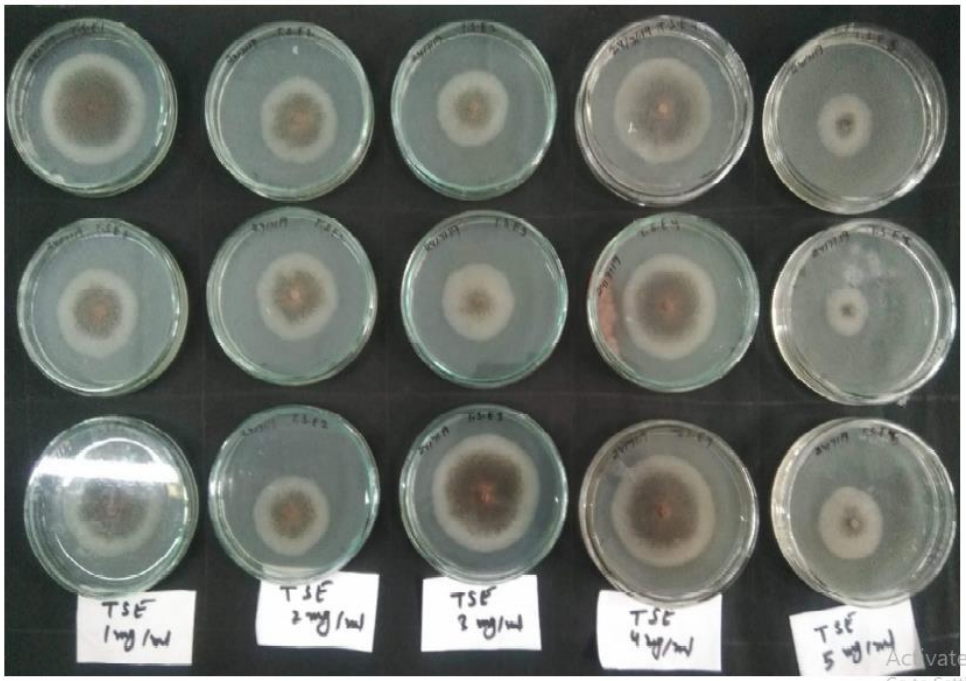


FIG. 5.34 IN-VITRO ANTIFUNGAL EFFECT OFETHANOLIC STEM EXTRACTOF T. BELLIRICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND 5 MG/MLCONCENTRATIONS

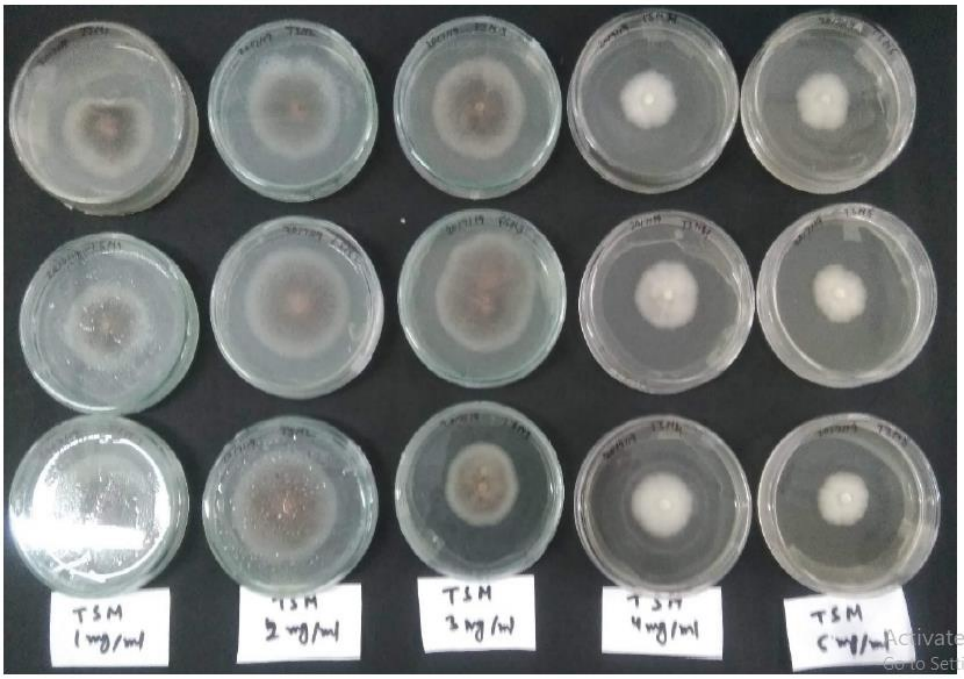


FIG. 5.35 IN-VITROANTIFUNGAL EFFECT OF METHANOLIC STEM EXTRACT OF T. BELLIRICA ON CAPSICUM CAPSICI RADIAL GROWTH AT 1, 2, 3, 4 AND FIVE MG/ML CONCENTRATIONS.

5.3. Effect of Heat and Trypsin on the Extract Efficacy

To study the stability of extracts against capsicum capsici. The Ethanolic and methanolic plant extracts were treated with temperatures (50 and 100 °C) and trypsin.

5.3.1. Heat Stability

Efficacy of all plant extract inradial growth inhibition of capsicum capsici are summarized in table 5.8. Significant difference was reported in growth inhibition of capsicum capsici, when extract was treated at 50 and 100 °C. Inhibition of radial growth of fungus after treatment with extracts at 50 °C and 100 °C has been shown in table 5.8. Ethanolic leaf and stem extract of A. indica exhibited 87.19 ± 2.60% and 91.70 ± 1.97% inhibition against the capsicum capsici, respectively. While the same extracts exhibited 64.39 ± 2.00% and 61.53 ± 0.90% inhibition of growth when heated at 100 °C (Fig. 5.36).

TABLE 5.8 ANTIFUNGAL ACTIVITY OF DIFFERENT EXTRACTS AT DIFFERENT TEMPERATURES

EXTRACTS	50˚C		100˚C	
	LEAF	STEM	LEAF	STEM
*A.vasica*ethanolic	86.58±1.66^{ab}	90.84±0.37^{a}	82.87±1.32^{a}	69.18±1.85^{bc}
*A.indica*ethanolic	87.19±2.60^{a}	91.70±1.97^{a}	64.39±2.00^{c}	61.53±0.90^{bc}
*A.sessilis*ethanolic	95.42±0.69^{a}	87.12±1.47^{a}	78.25±2.49^{b}	90.80±1.61^{a}
*P.paniculata*ethanolic	84.50±1.24^{b}	-	92.75±2.28^{a}	-
*A.vasica*methanolic	72.60±3.22^{c}	64.60±1.42^{b}	48.65±4.61^{e}	60.15±0.78^{bc}
*A.indica*methanolic	72.58±1.92^{c}	57.42±1.18^{c}	56.89±0.75^{d}	77.96±0.59^{b}
*A.sessilis*methanolic	77.96±0.59^{bc}	55.48±0.23^{c}	49.59±0.92^{e}	40.89±1.25^{c}

Similar result was observed in methanolic leaf extract heated at 50 °C of A. indica against the fungus. 72.58 ± 1.92% inhibition was observed with the extract heated at 50 °C, while 56.89 ± 0.75% inhibition of capsicum capsici was observed by the same extract heated at 100 °C (Fig. 5.36).

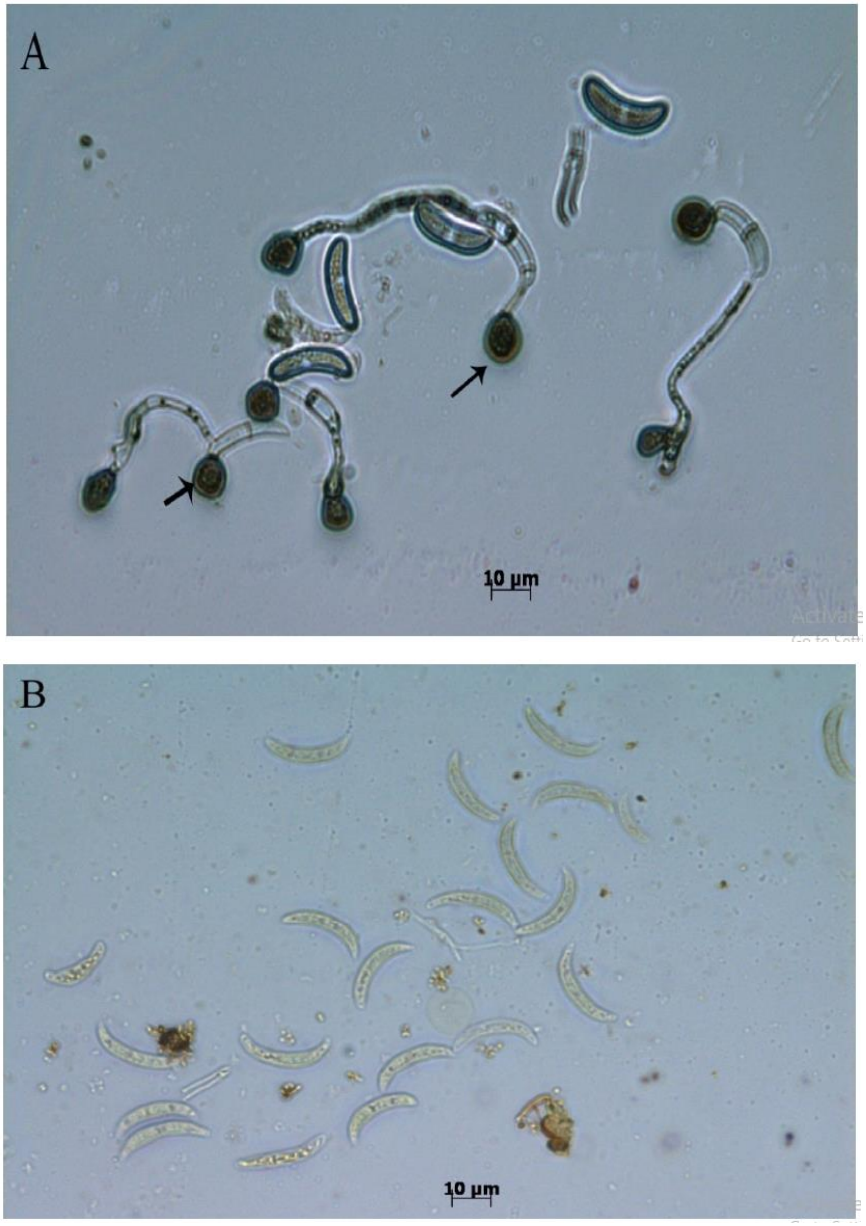


FIG. 5.36. EFFECT OFETHANOLIC LEAF EXTRACTOF A. VASICA (5 MG/ ML) ON CONIDIAL GERMINATION AFTER 48 HOURS (B). WHERE (A) REPRESENT CONTROL. BLACK ARROW INDICATED APPRESSORIA FORMATION

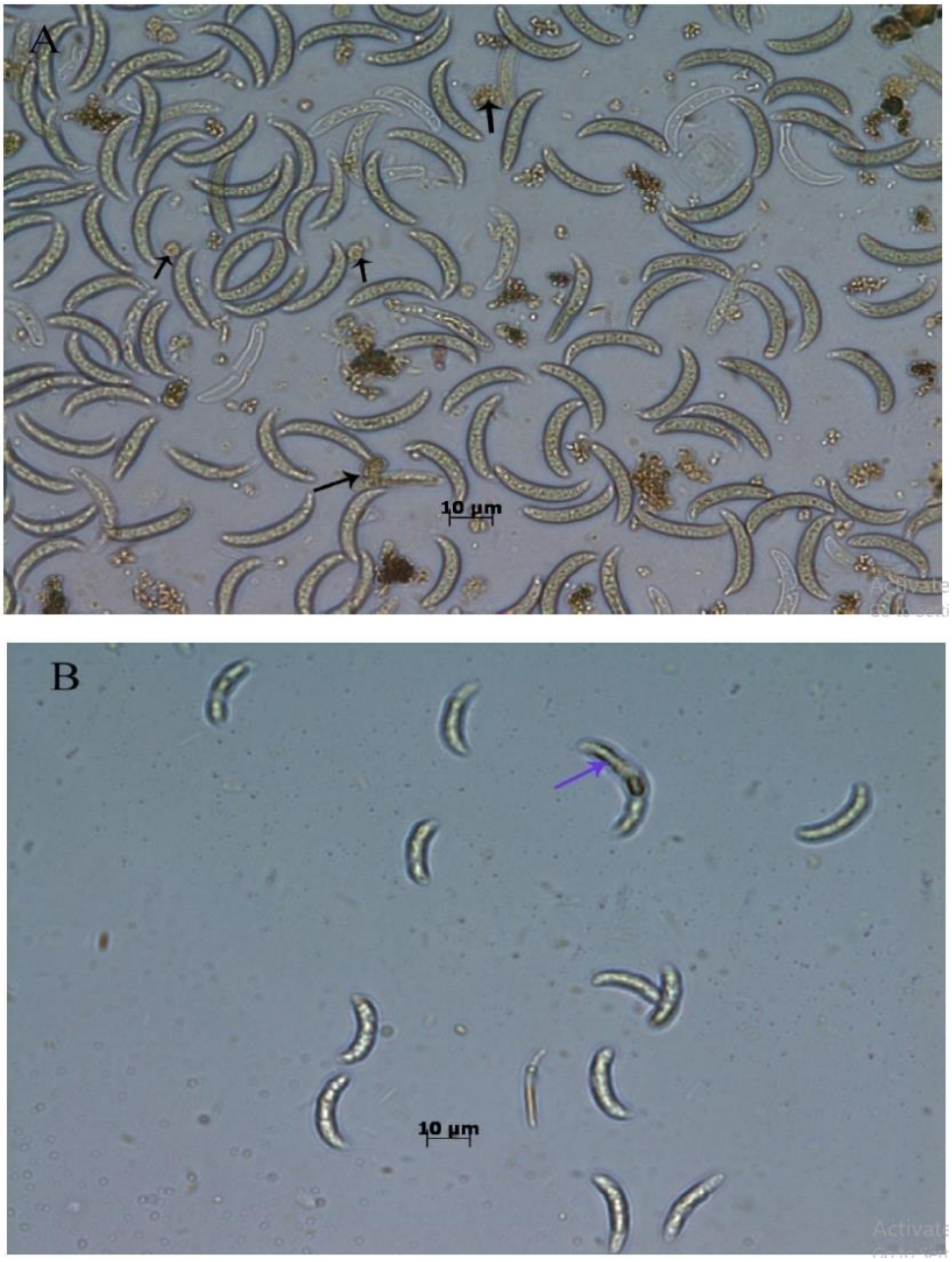


FIG. 5.37 EFFECT OF A.VASICA EXTRACTS ON CONIDIAL GERMINATION AFTER 48 HOURS. WHERE METHANOLIC LEAF EXTRACT (A) ETHANOLIC STEM EXTRACT (0.5 MG /ML) (B). BLACK ARROW INDICATED APPRESSORIA FORMATION AND BLUE ARROW INDICATE GERM TUBE FORMATION

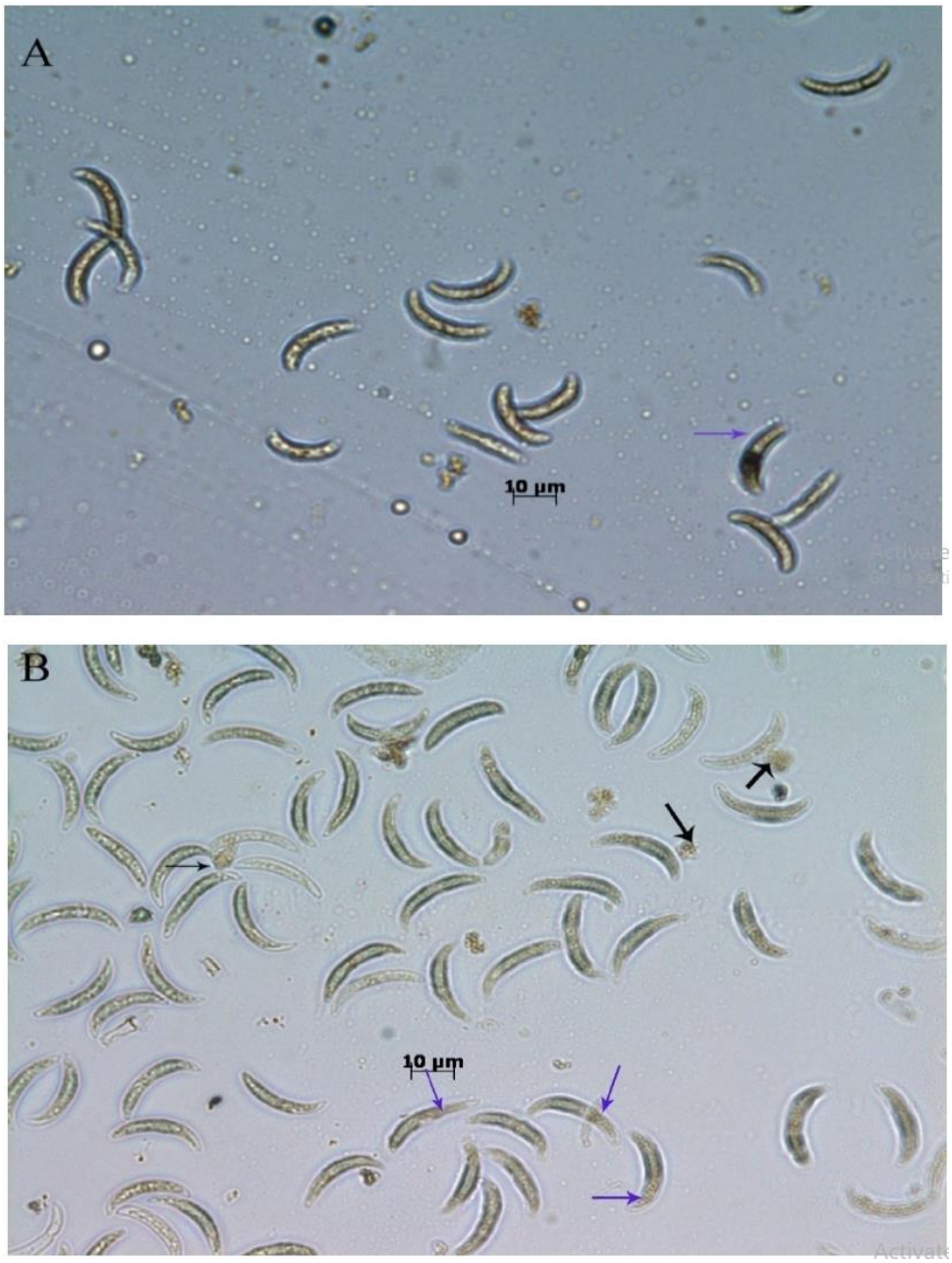


FIG. 5.38 EFFECT OF A. VASICA EXTRACTS ON CONIDIAL GERMINATION AFTER 48 HOURS. WHERE ETHANOLIC STEM EXTRACT (5 MG/ ML) (A) METHANOLIC STEM EXTRACT (B). BLACK ARROW INDICATED APPRESSORIA FORMATION AND BLUE ARROW INDICATE GERM TUBE FORMATION

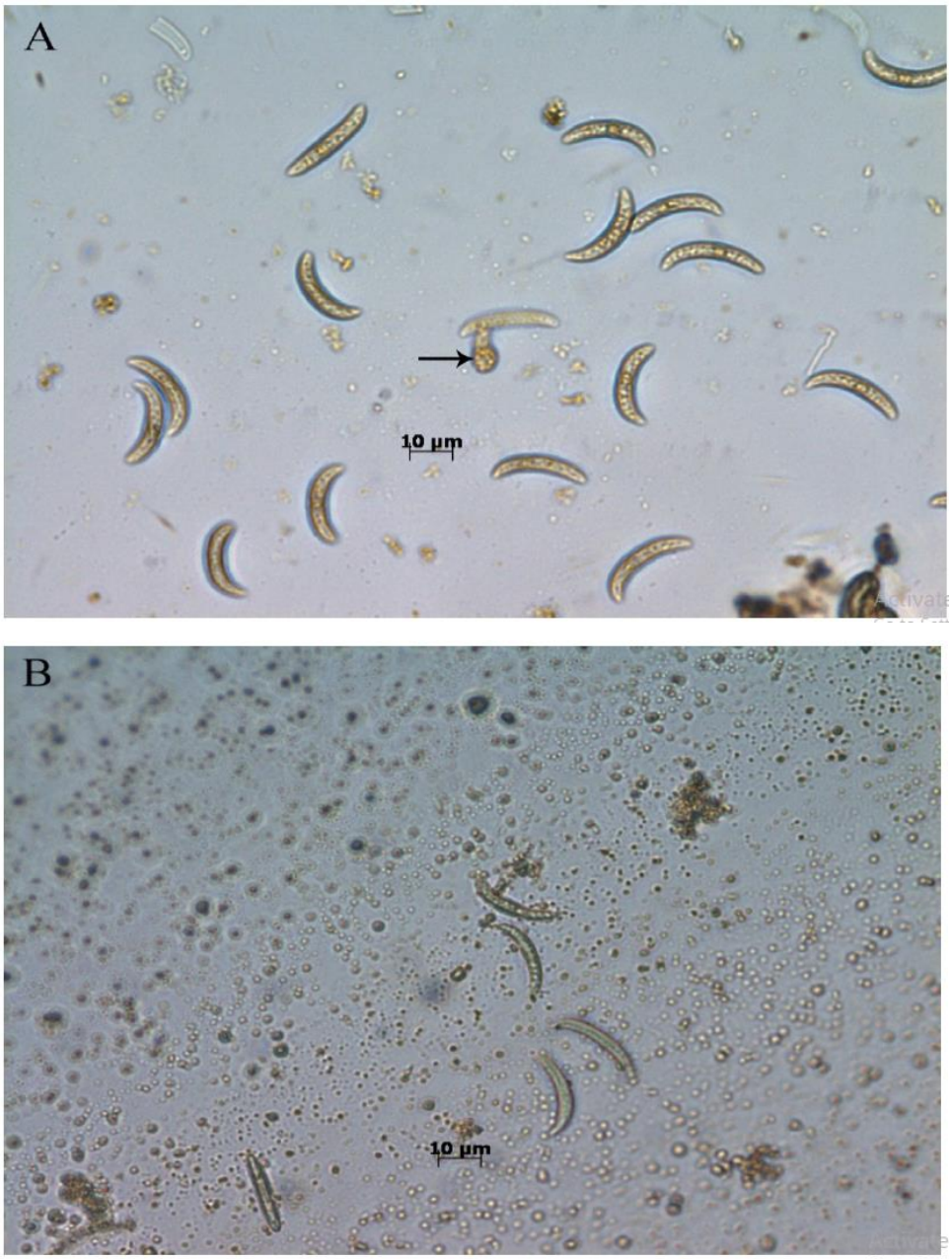


FIG. 5.39 EFFECT OF A. INDICA EXTRACTS ON CONIDIAL GERMINATION AFTER 48 H. WHERE ETHANOLIC LEAF EXTRACT (0.5 MG/ML) (A) ETHANOLIC LEAF EXTRACT (5 MG/ML) (B). BLACK ARROW INDICATED APPRESSORIA FORMATION AND BLUE ARROW INDICATE GERM TUBE FORMATION

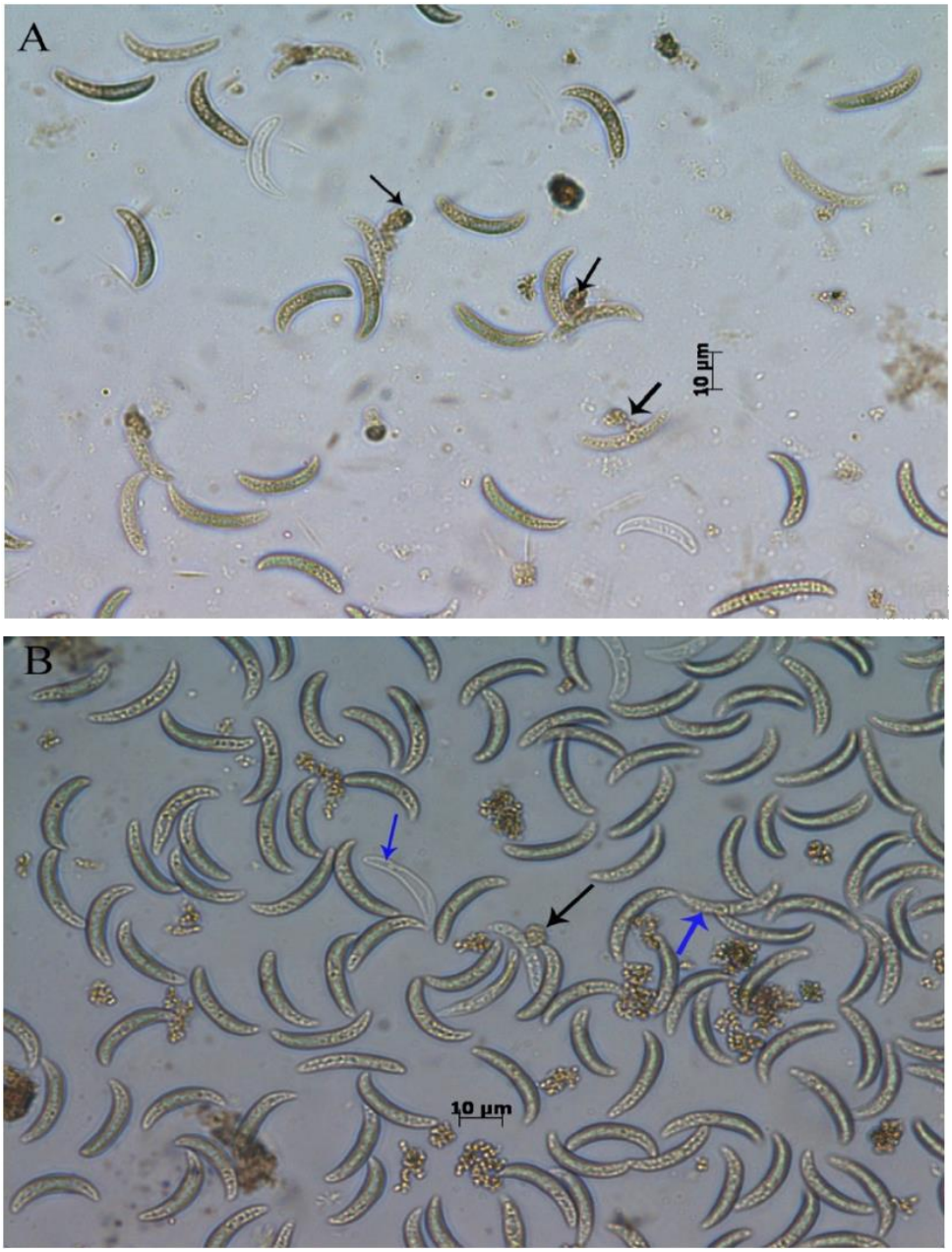


FIG. 5.40 EFFECT OF A. INDICA EXTRACTS ON CONIDIAL GERMINATION AFTER 48 H.WHERE METHANOLIC LEAF EXTRACT (A) ETHANOLIC STEM EXTRACT (0.5 MG/ML) (B). BLACK ARROW INDICATED APPRESSORIA FORMATION AND BLUE ARROW INDICATE GERM TUBE FORMATION

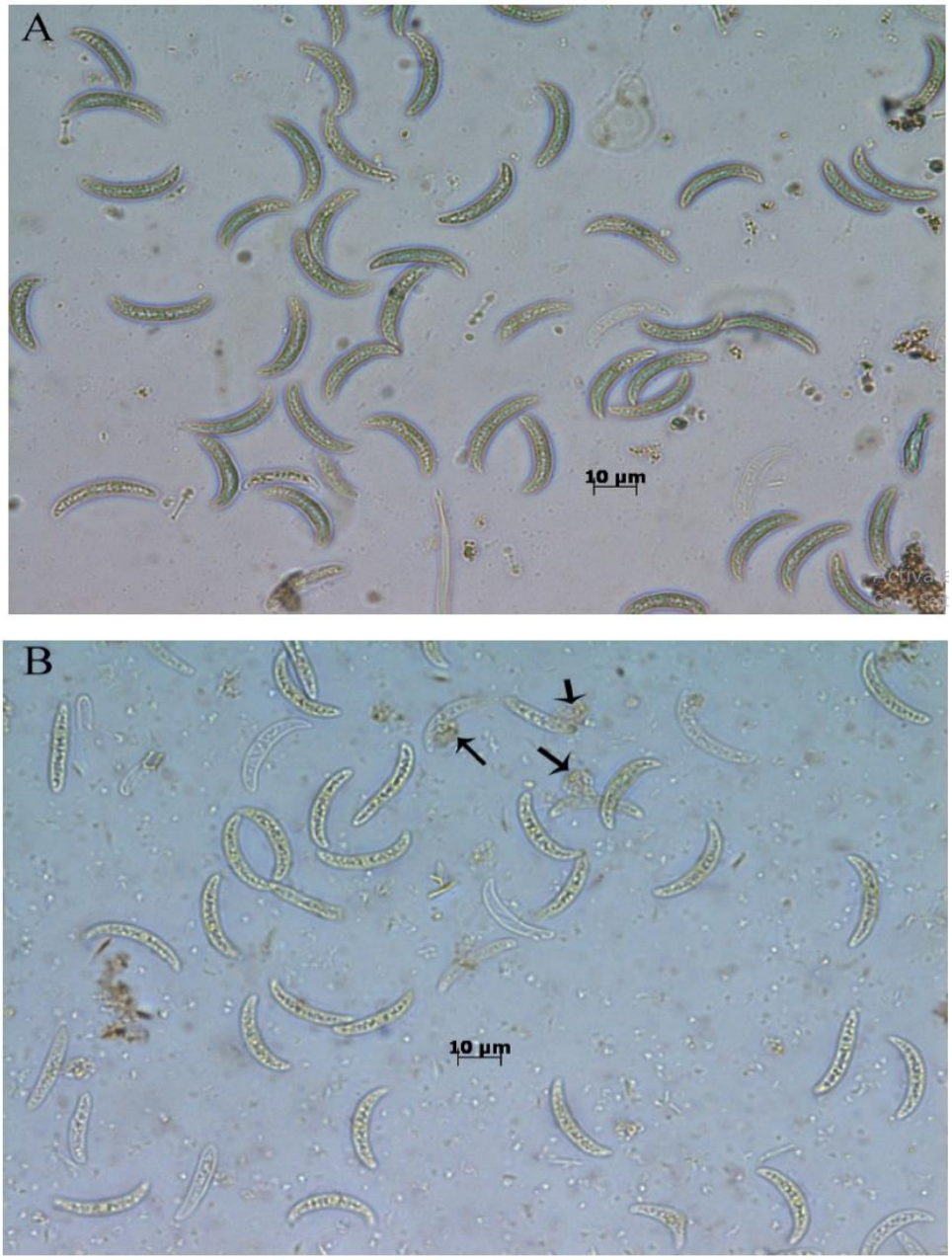


FIG. 5.41 EFFECT OF A. INDICA EXTRACTS ON CONIDIAL GERMINATION AFTER 48 H. WHERE ETHANOLIC STEM EXTRACT (4 MG/ML) (A) METHANOLIC STEM EXTRACT (B). BLACK ARROW INDICATED APPRESSORIA FORMATION

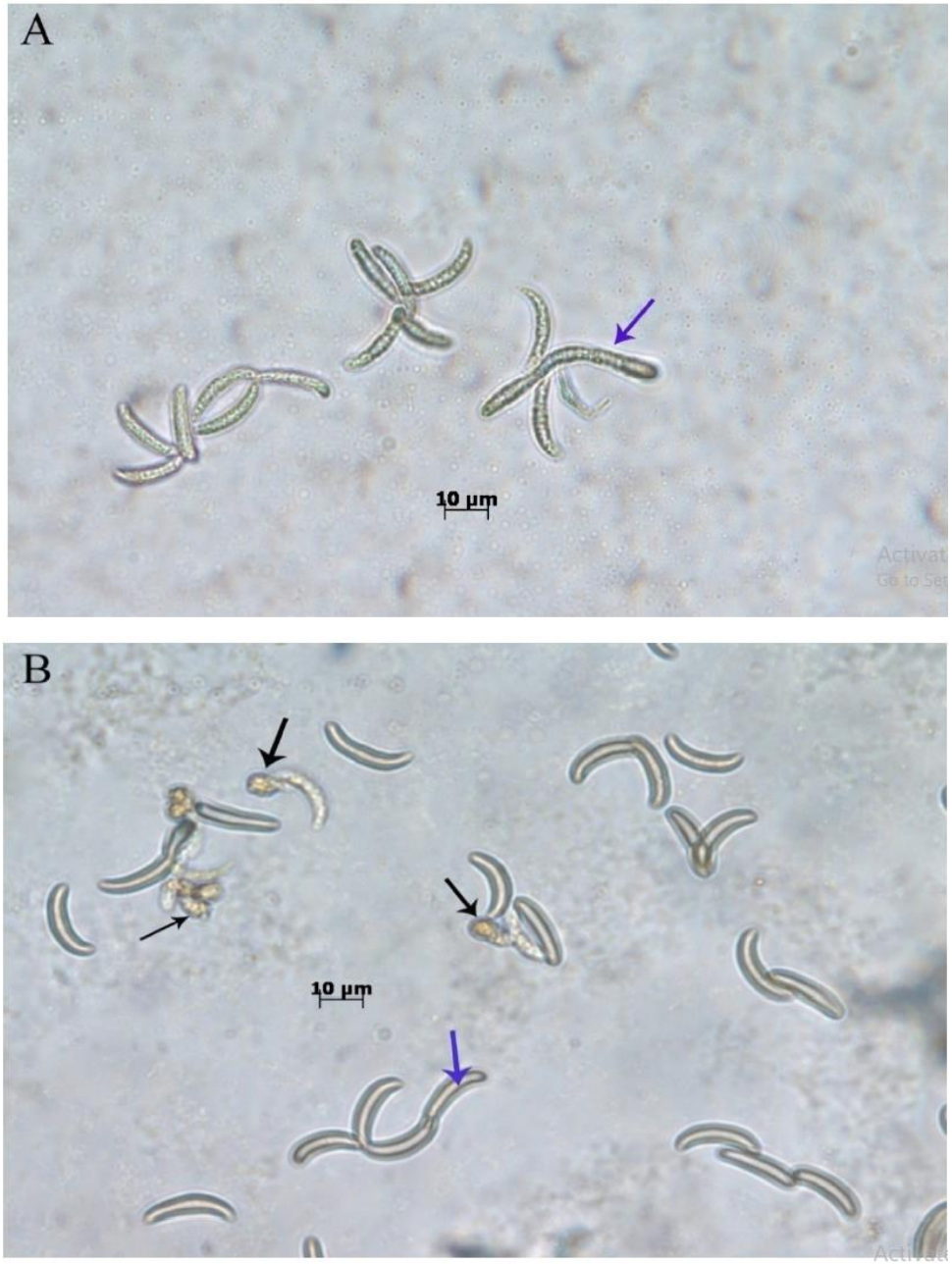


FIG. 5.42 EFFECT OF A. SESSILIS ETHANOLIC LEAF (A) AND STEM (B) EXTRACT ON CONIDIAL GERMINATION AFTER 48 H AT 5 MG /ML. BLACK ARROW INDICATED APPRESSORIA FORMATION AND BLUE ARROW INDICATE GERM TUBE FORMATION

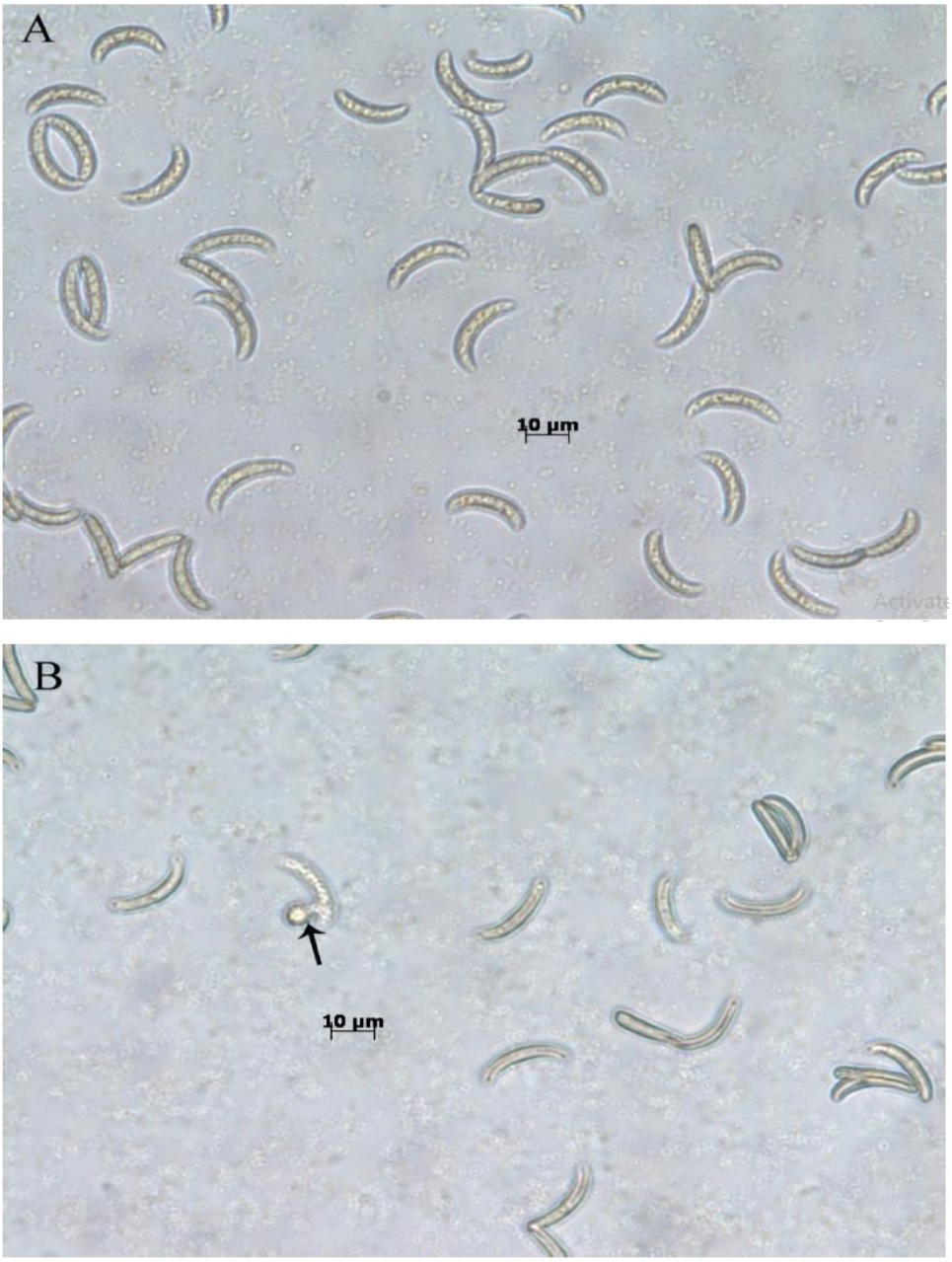


FIG. 5.43 EFFECT OF A. SESSILIS EXTRACTS METHANOIC LEAF (A) AND STEM EXTRACT (B) ON CONIDIAL GERMINATION AFTER 48 H AT 5 MG /ML. BLACK ARROW INDICATED APPRESSORIA FORMATION AND BLUE ARROW INDICATE GERM TUBE FORMATION

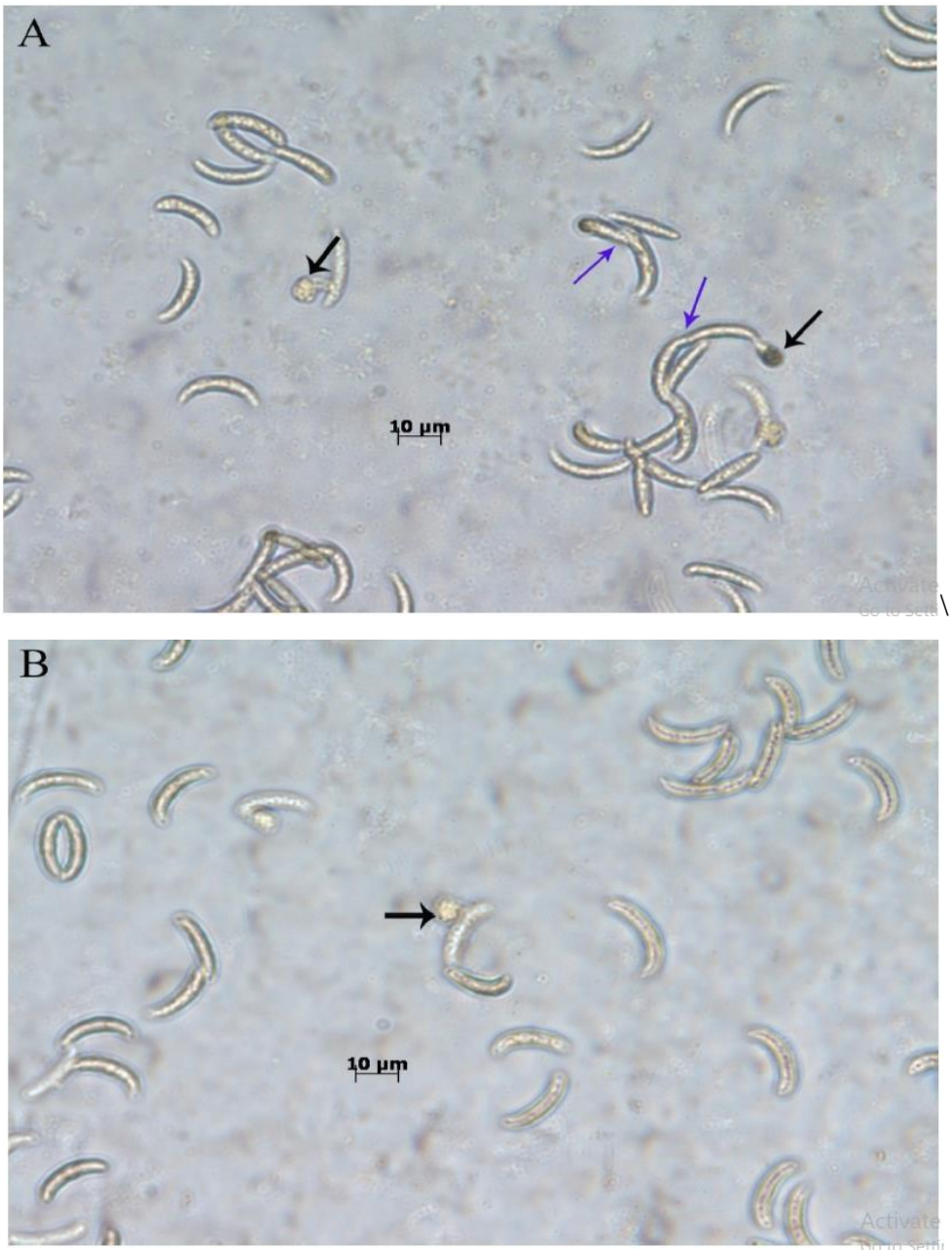


FIG. 5.44 EFFECT OF P. PANICULATA ETHANOIC LEAF (A) AND METHANOLIC LEAF (B) EXTRACTS ON CONIDIAL GERMINATION AFTER 48 H AT 5 MG /ML. BLACK ARROW INDICATED APPRESSORIA FORMATION AND BLUE ARROW INDICATE GERM TUBE FORMATION.

Each value isexpressed as mean of triplicates, & column sharing same alphabetical letters arenot significantlydifferent ($p \leq 0.05$). – represents no inhibition in radial growth.

Heating an ethanol extract of A. vasica leaves to 50°C and 100°C inhibited capsicum capsici growth by 86.58 ± 1.66% and 82.87 ± 1.32%, respectively. A.vasica's methanolic leaf

extract inhibited capsicum capsici growth less (72.60 ± 3.22% and 48.65 ± 4.6%) than the ethanolic extract at 50 and 100 °C, respectively (Table 5.8). Heating methanolic stem extract to 100°C did not modify its growth inhibition, which was 64.60 ± 1.42% and 60.15 ± 0.78% at 50°C and 100°C, respectively (Fig. 5.48).

Heating an ethanolic leaf extract of A. sessilis at 50°C inhibited capsicum capsici growth by 95.42 ± 0.69%, whereas heating at 100°C inhibited the fungus growth by 78.25 ± 2.49%. Heating the ethanolic and methanolic extracts of A. sessilis stem had a paradoxical impact; growth inhibition of capsicum capsici was observed to be higher when the extracts were heated at 100 °C compared to 50 °C. The growth inhibition was 87.12 ± 1.47% and 90.80 ± 1.61%, respectively, using the ethanolic stem extract of A. sessilis, heated at 50 and 100 °C (Fig. 5.49).

Heating methanolic extract of A. indica to 50°C & 100°C inhibited capsicum capsici growth by 57.42 ± 1.18% and 77.96 ± 0.59%, respectively. A similar result was seen in an ethanolic leaf extract of P. paniculata after heating at 50°C and 100°C. At 50°C and 100°C, capsicum capsici showed 84.50 ±1.24% and 92.75 ±2.28% inhibition, respectively (Fig. 5.51).

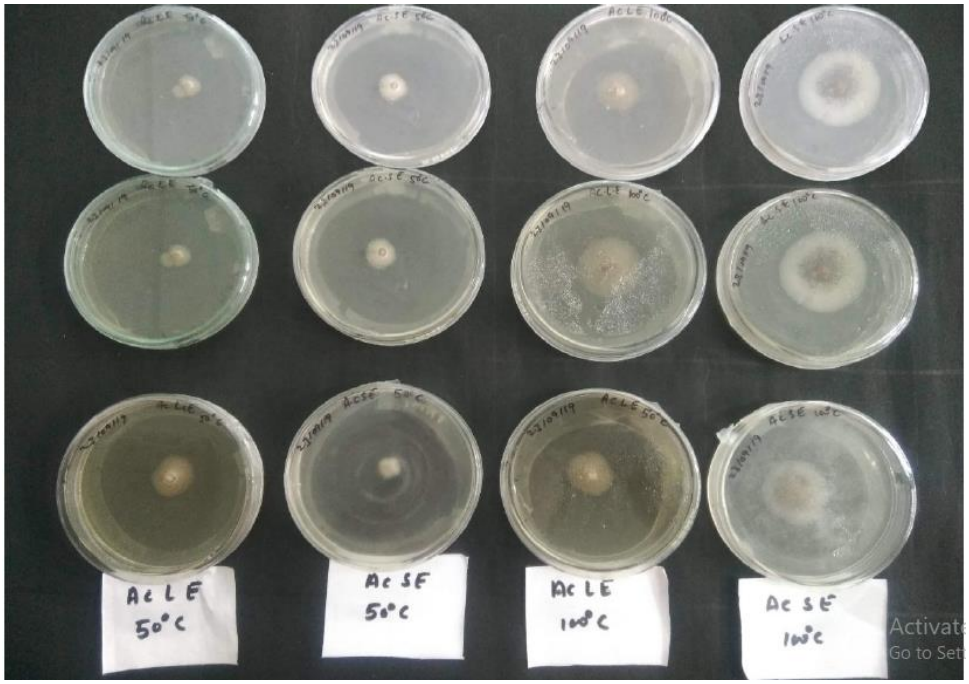


FIG. 5.45 HEAT TREATMENT (50, 100 °C) OF ETHANOLICEXTRACT OF A. INDICIA AND ITS EFFECTON RADIAL GROWTH OF CAPSICUM CAPSICI

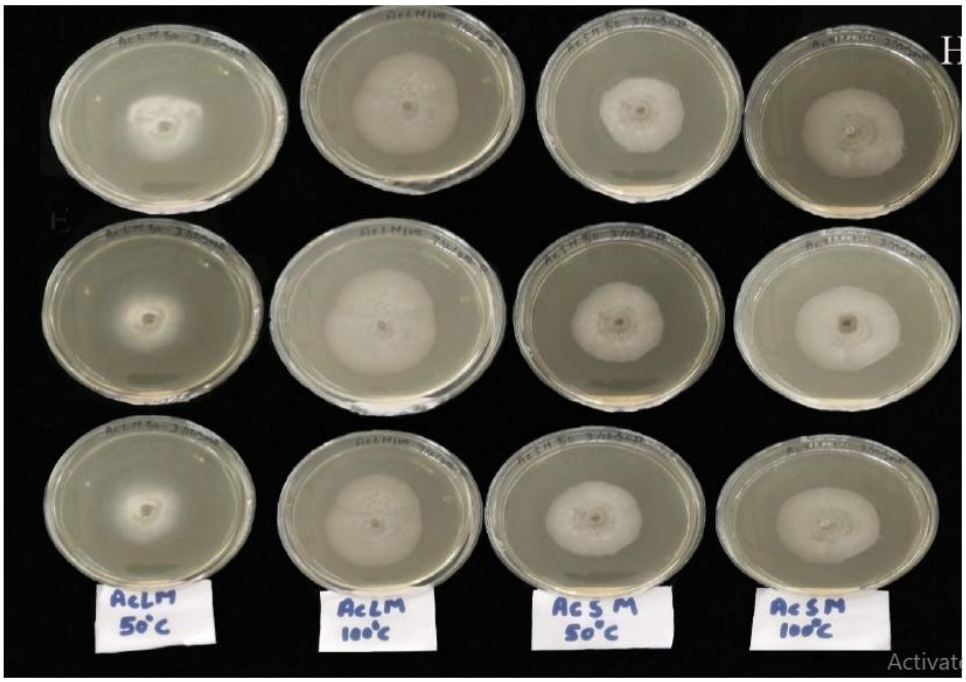


FIG. 5.46 HEAT TREATMENT (50, 100 °C) OF METHANOLICEXTRACT OF A. INDICA AND ITS EFFECT ON RADIAL GROWTHOF CAPSICUM CAPSICI

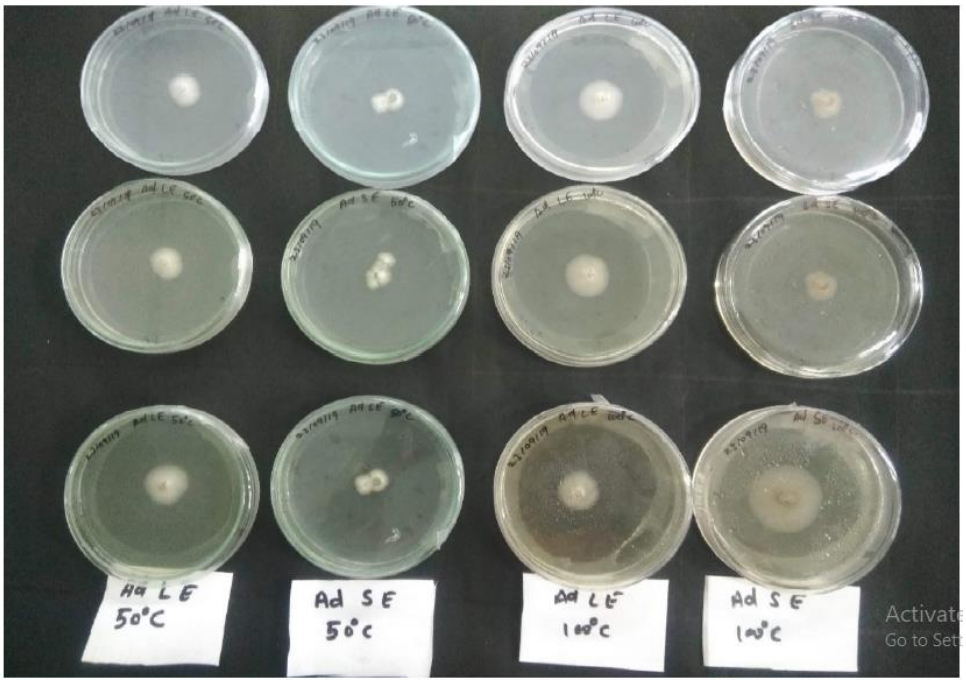


FIG. 5.47 HEAT TREATMENT (50, 100 °C) OF ETHANOLICEXTRACT OF A. VASICA AND ITSEFFECT ON RADIAL GROWTH OF CAPSICUM CAPSICI

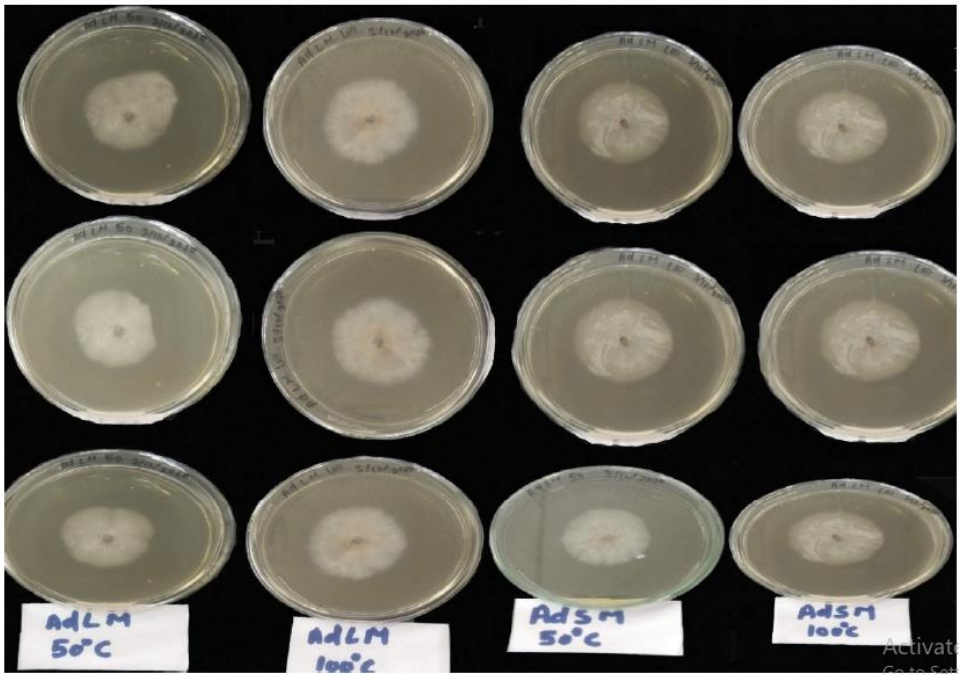


FIG. 5.48 HEAT TREATMENT (50, 100 °C) OF METHANOLICEXTRACT OF A. VASICA AND ITS EFFECTON RADIAL GROWTH OF CAPSICUM CAPSICI

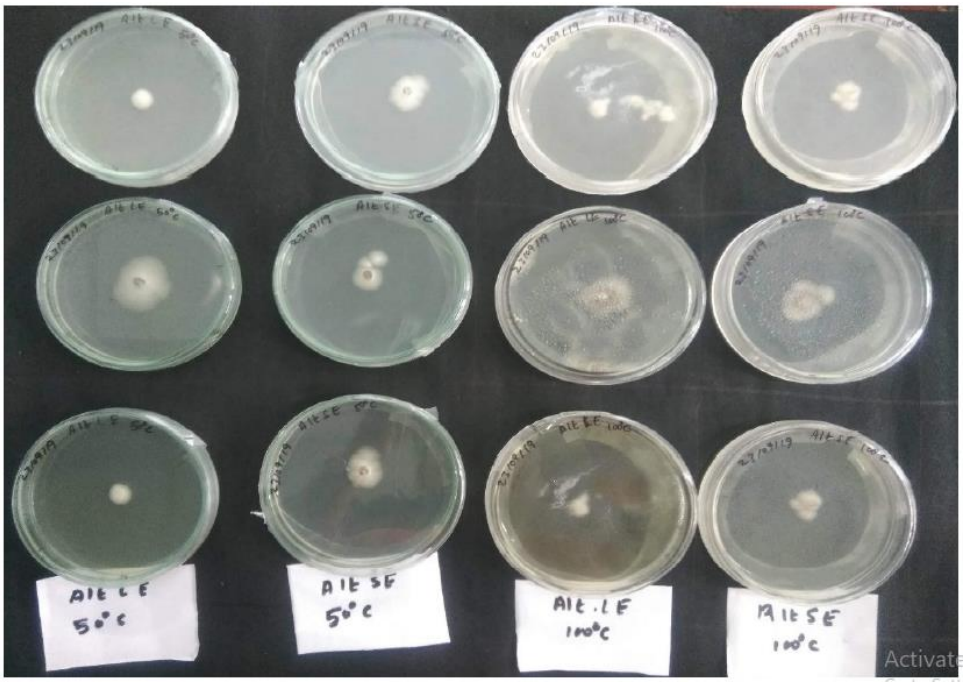


FIG. 5.49 HEAT TREATMENT (50, 100 °C) OF ETHANOLIC EXTRACTOF A. SESSILIS AND ITS EFFECTON RADIAL GROWTHOF CAPSICUM CAPSICI

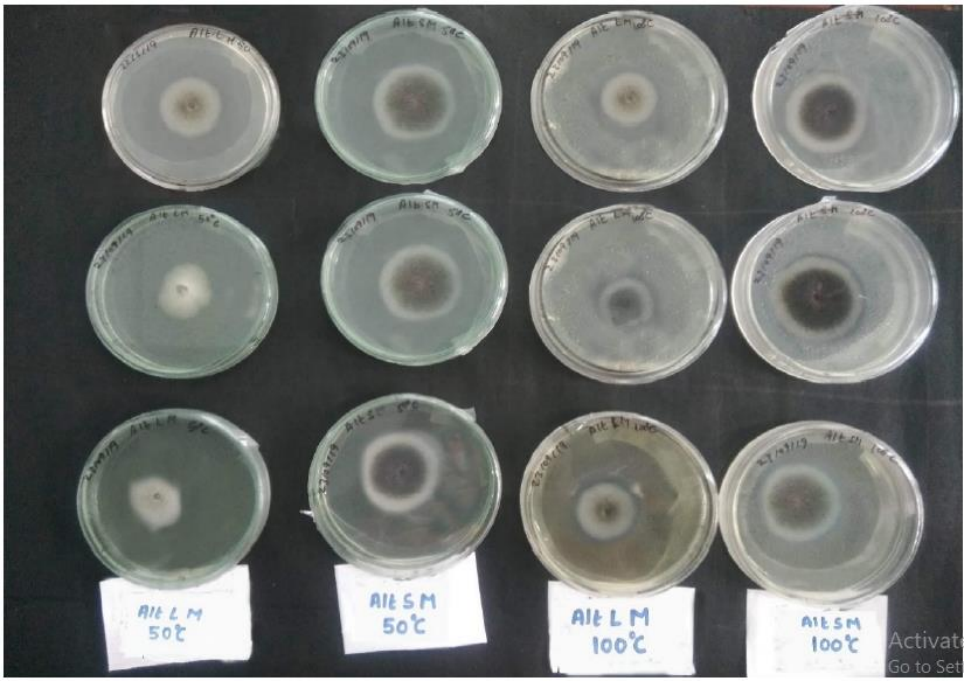


FIG. 5.50 HEAT TREATMENT (50, 100 °C) OF METHANOLICEXTRACT OF A. SESSILIS AND ITSEFFECT ON RADIAL GROWTH OF CAPSICUM CAPSICI

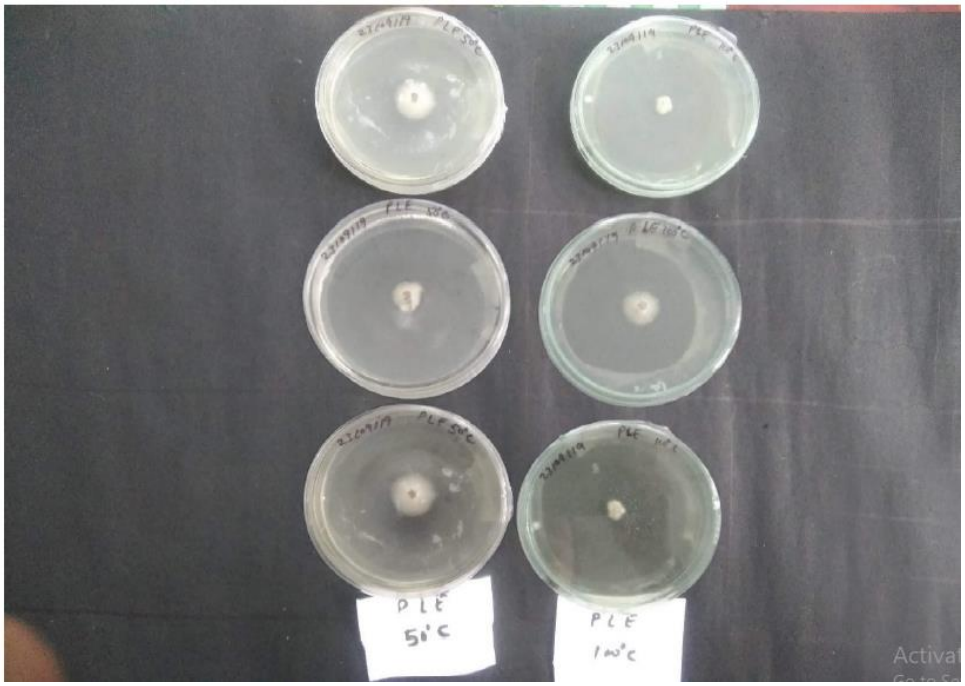
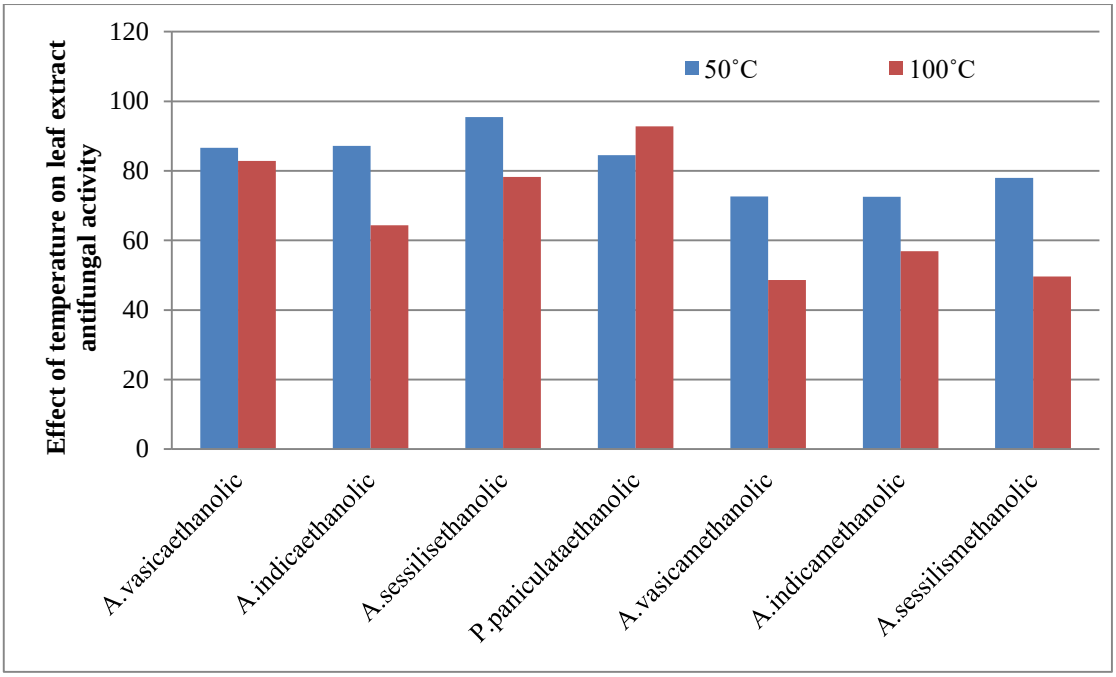


FIG. 5.51 HEAT TREATMENT (50, 100 °C) OF ETHANOLICEXTRACT OF P. PANICULATA AND ITS EFFECTON RADIAL GROWTH OF CAPSICUM CAPSICI



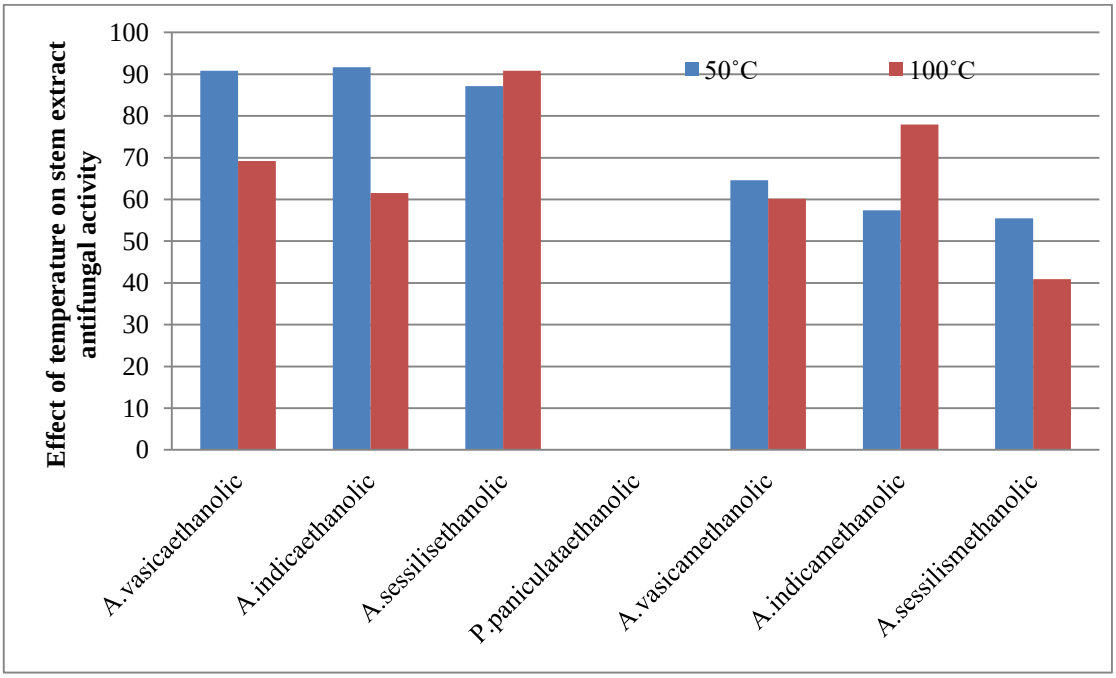


FIG. 5.52 EFFECT OF TEMPERATURE ON EXTRACT ACTIVITY AT 50 AND 100 °C. VARTICAL BARS REPRESENT ERROR BAR OF MEAN

5.3.2. Proteolytic Degradation of Extract

Table 5.9 compares the effects of trypsin, a proteolytic enzyme, on several plant extracts. The antifungal activity of ethanolic & methanolic (leaf & stem) extracts of A. indica

was increased following trypsin treatment as compared to untreated trypsin extract. It was decreased from 91.26 ± 1.88% and 82.19 ± 3.03% to 58.12 ± 3.74% and 48.90 ± 3.04% in ethanolic and methanolic leaf extracts, respectively. The ethanolic & methanolic stem extracts inhibited capsicum capsici growth by 94.28 ±0.18%, 73.08 ± 1.19%, 57.76 ± 1.13%, and 49.90 ± 2.13%, respectively.

Similarly, trypsin-treated ethanolic and methanolic extracts of A. sessilis, A. vasica, P. paniculata, & T. bellirica showed a reduction in capsicum capsici growth inhibition. The ethanolic leaf extracts of A. sessilis, A. vasica, & P. paniculata reduced capsicum capsici growth inhibition from 86.96 ±2.92%, 93.65 ±0.17%, 88.22 ±2.54%, and 69.09 ±3.81% to 68.11 ± 1.00%, 65.00 ± 1.26%, and 53.35 ± 1.15%, respectively. The methanolicleaf extract of A. vasica had noeffect on capsicum capsici inhibitory activities. A similar result was seen in both untreated and trypsin-treated methanolic extracts of A. vasica. P. paniculata extract caused an increase in inhibition in capsicum capsici.

In the case of ethanolic and methanolic stem extractsof A. sessilis, A.vasica, & P. paniculata, trypsin extract reduced growth inhibition in capsicum capsici. Ethanolic leaf extract of M. parvifolia increased capsicum capsici growth inhibition by 73.12 ± 0.75% compared to untreated trypsin extract (41.40 ± 2.97%).

TABLE 5.9 PROTEOLYTIC DEGRADATION OF EXTRACT AND RADIAL GROWTH OF FUNGUS.

EXTRACTS	ETHANOLIC		METHANOLIC	
	LEAF	STEM	LEAF	STEM
A.indica	58.12 ± 3.74^{c}	57.76 ± 1.13^{b}	48.90 ± 3.04^{b}	49.90 ± 2.13^{cd}
A.vasica	65.00 ± 1.26^{ab}	58.51 ± 00.77^{b}	70.97 ± 0.56^{a}	49.71 ± 0.87^{cd}
A.sessilis	68.11 ± 1.00^{a}	55.02 ± 2.44^{b}	44.95 ± 0.44^{b}	45.11 ± 3.34^{d}
M.parvifolia	73.12 ± 0.75^{a}	77.57 ± 1.15^{a}	48.20 ± 0.68^{b}	61.00 ± 0.74^{b}
P.paniculata	53.35 ± 1.15^{c}	41.13 ± 1.38^{c}	50.92 ± 0.82^{b}	51.63 ± 1.23^{c}
T.bellirica	65.84 ± 3.60^{ab}	70.48 ± 2.89^{a}	-	75.82 ± 2.05^{a}

Each value is given as mean of triplicates, & columns with same alphabetical letters do not differ substantially ($p < 0.05$). - exhibits no inhibition of radial development.

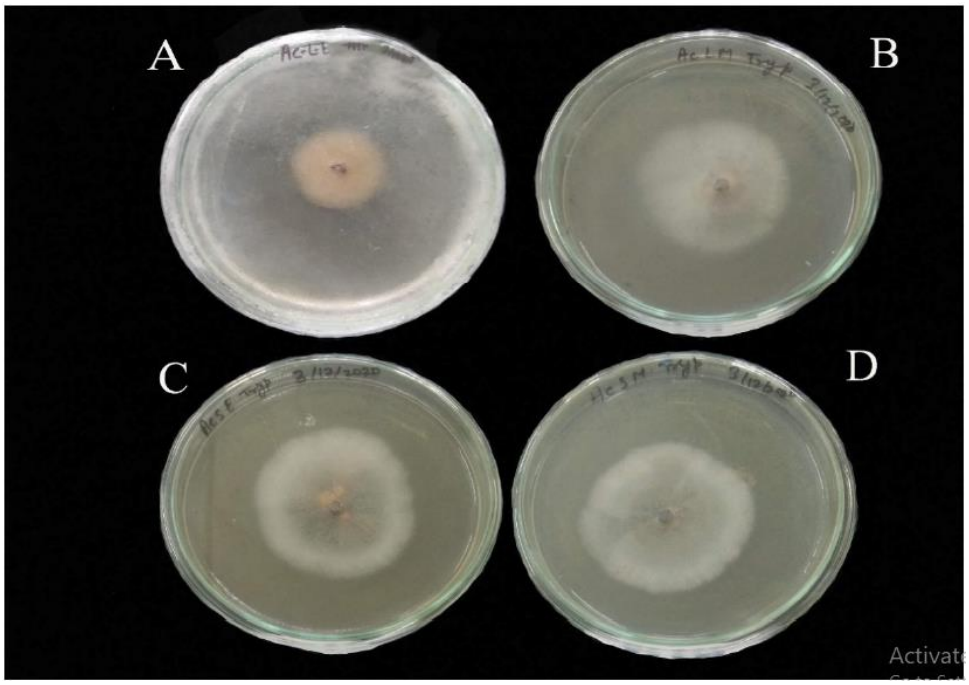


FIG. 5.52 EFFECT OF A. INDICA EXTRACT AFTER PROTEOLYTIC DEGRADATION ON RADIAL GROWTH OF CAPSICUM CAPSICI. WHERE ETHANOLIC LEAF (A) METHANOLIC LEAF (B) ETHANOLIC STEM (C) METHANOLIC STEM EXTRACT (D)

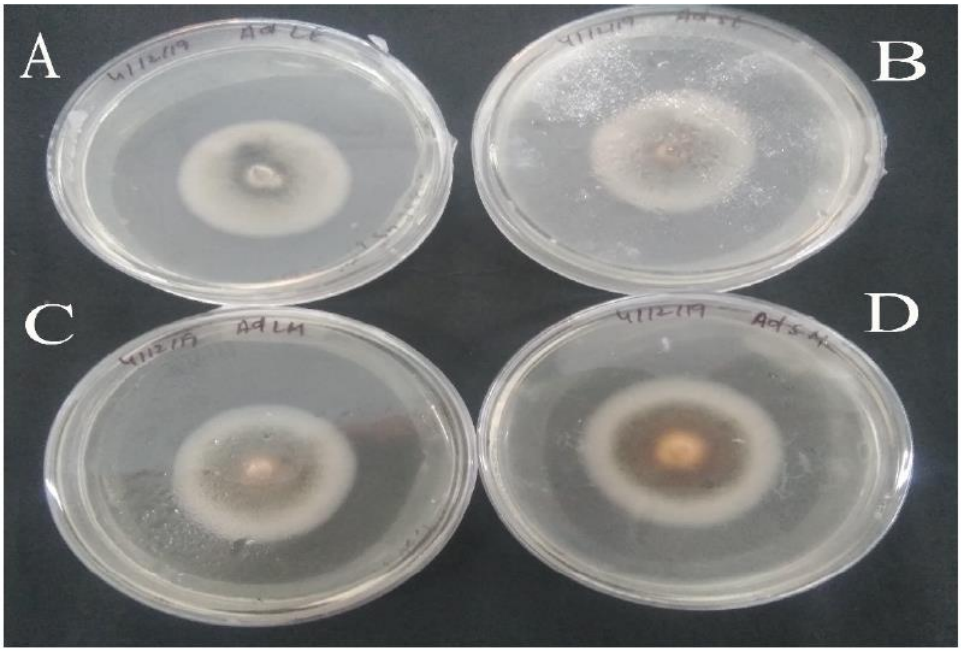


FIG. 5.54 EFFECT OF A. VASICA EXTRACT AFTER PROTEOLYTIC DEGRADATION ON RADIAL GROWTH OF CAPSICUM CAPSICI. WHERE ETHANOLIC LEAF (A) ETHANOLIC STEM (B) METHANOLIC LEAF (C) METHANOLIC STEM EXTRACT (D)

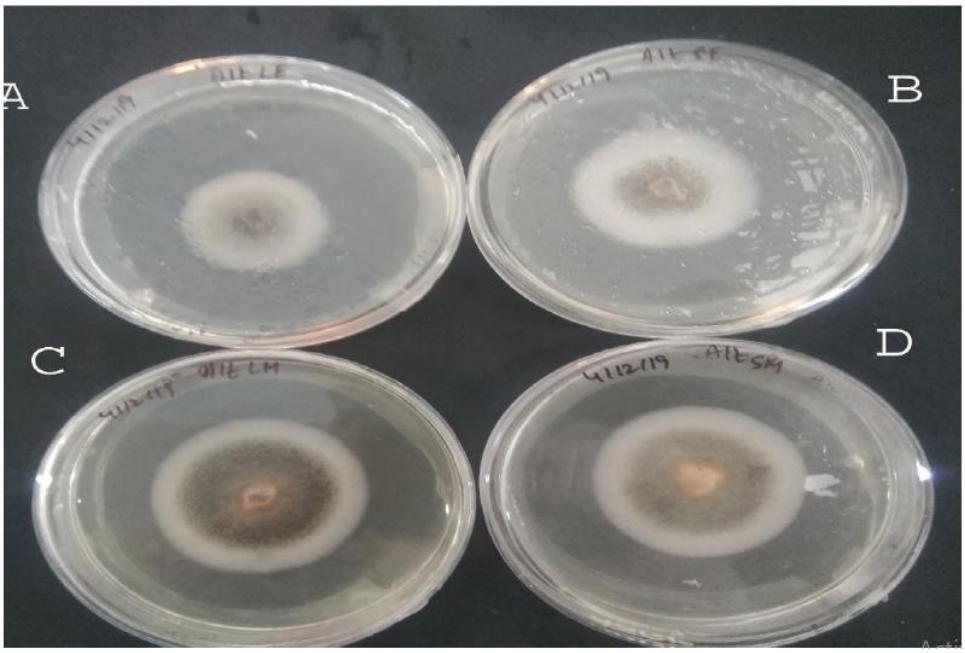


FIG. 5.55 EFFECT OF A. SESSILIS EXTRACT AFTER PROTEOLYTIC DEGRADATION ON RADIAL GROWTH OF CAPSICUM CAPSICI. WHERE ETHANOLIC LEAF (A) ETHANOLIC STEM (B) METHANOLIC LEAF (C) METHANOLIC STEM EXTRACT (D)

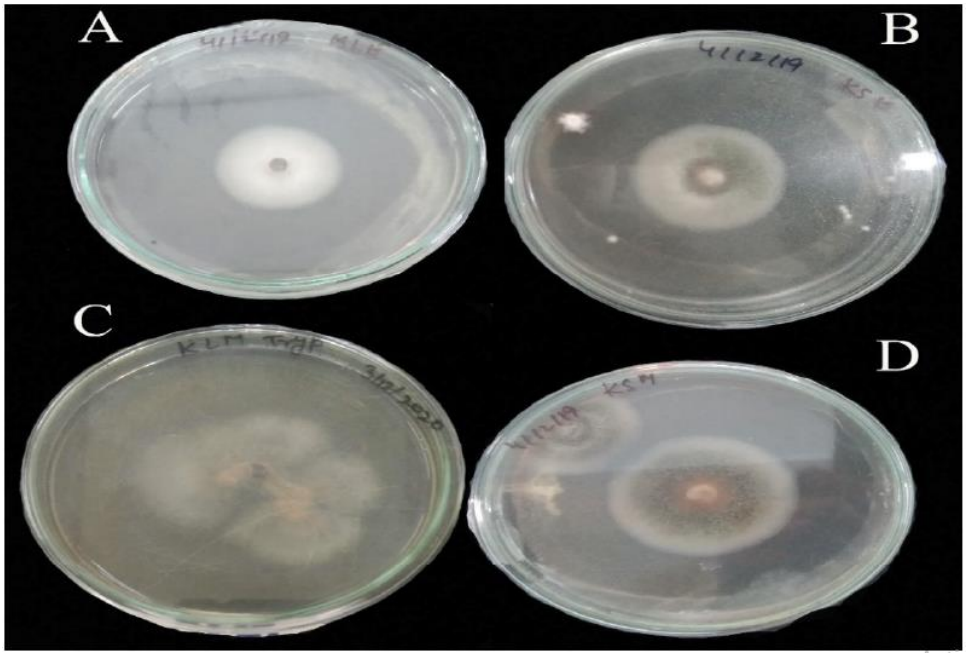


FIG. 5.56 EFFECT OF M. PARVIFOLIA EXTRACT AFTER PROTEOLYTIC DEGRADATION ON RADIAL GROWTH OF CAPSICUM CAPSICI. WHERE

ETHANOLIC LEAF (A) ETHANOLIC STEM (B) METHANOLIC LEAF (C) METHANOLIC STEM EXTRACT (D)

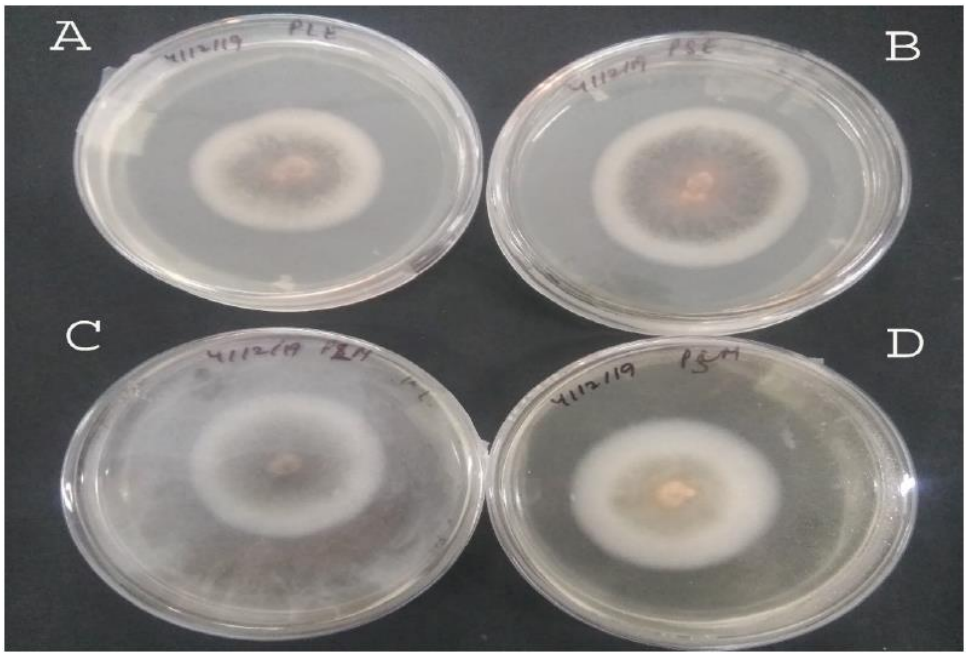


FIG. 5.57 EFFECT OF P. PANICULATA EXTRACT AFTER PROTEOLYTIC DEGRADATION ON RADIAL NGROWTH OF CAPSICUM CAPSICI. WHERE ETHANOLIC LEAF (A) ETHANOLIC STEM (B) METHANOLIC LEAF (C) METHANOLIC STEM EXTRACT (D)

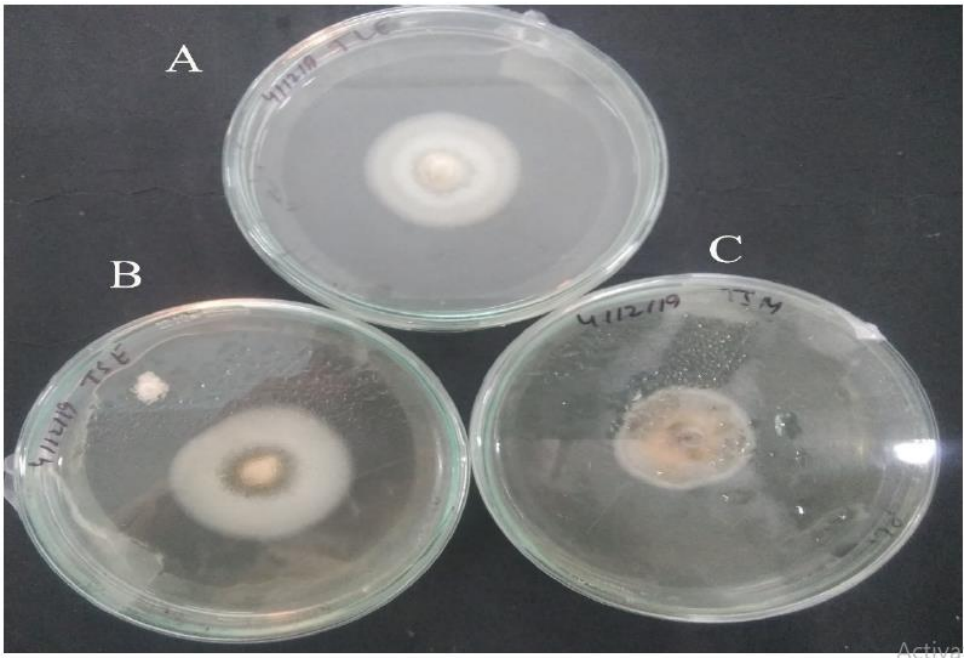


FIG. 5.58 EFFECT OF T. BELLIRICA EXTRACT AFTER PROTEOLYTIC DEGRADATION ON RADIAL GROWTH OF CAPSICUM CAPSICI. WHERE ETHANOLIC LEAF (A) ETHANOLIC STEM (B) METHANOLIC STEM EXTRACT (C)

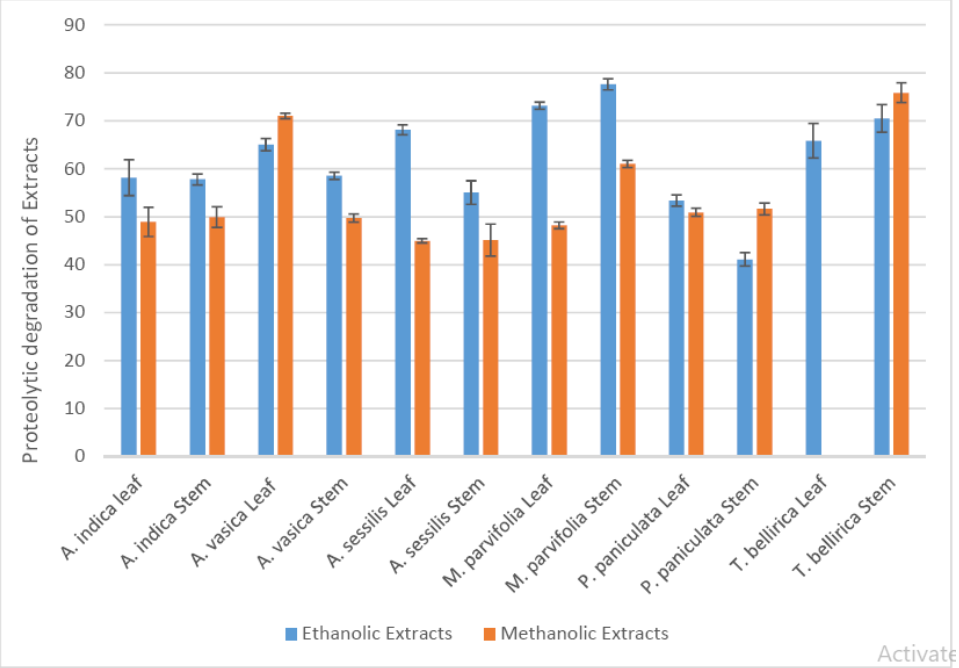


FIG. 5.59 EXTRACTS PROTEOLYTIC DEGRADATION EFFECT ON ANTIFUNGAL ACTIVITY. VARTICAL BARS REPRESENT ERROR BAR OF MEAN

5.4. In-Vivo Analysis in Chilli Fruit

Based on conidia germination inhibition, four ethanolic plant extracts were chosen for their in vivo impact on chilli. The MIC of A. sessilis, A. indica, A.vasica, & P. paniculata were chosen to investigate decay inhibition, illness severity, disease incidence, and defense enzymes.

Figure 60 (A) and table 5.10 illustrate the percentage of decay inhibition. Chilli decay inhibition was 64.12 ± 6.52% higher in positive controlcompared to negative control before inoculation at 25°C. The ethanolic leaf extract of A. sessilis showed the highest percentage of decay inhibition (89.79 ± 2.04% and 53.43 ± 3.32%) at 4°C and 25°C, respectively. At 25°C, ethanolic leaf extracts of A.indica, A. vasica, & P. paniculata showed decreased decay inhibition by 48.85 ± 3.32%, 48.09 ± 6.65%, and 46.57 ± 5.5%, respectively.

At 4 °C, leaf extract reduced the percentage decline inhibition of anthracnose in chili. Pre-inoculation with A. vasica leaf extract resulted in 79.59 ± 2.04% decay inhibition. The leaf extracts of A. indica and P. paniculata were shown to decrease chilli deterioration by 26.53 ± 10.6% and 14.28 ± 10.23% respectively.

The leaf extracts of A. vasica and A. sessilis were shown to be ineffective in inhibiting degradation following inoculation at 25 °C. The ethanolic leaf extractof A. indica inhibited decay in capsicum capsiciat the highest rate (29.76 ± 7.96%), followed by A. sessilis at 25.18 ± 8.50%, P. paniculata at 22.13 ± 4.76%, and A. Vasica at 21.37 ± 2.75%. The leaf extracts of A. vasica and A. sessilis were shown to be ineffective in inhibiting degradation following inoculation at 25 °C. The ethanolic leaf extract of A. indica inhibited decay in capsicum capsiciat the highest rate (29.76 ± 7.96%), followed by A. sessilis at 25.18 ± 8.50%, P. paniculata at 22.13 ± 4.76%, and A. Vasica at 21.37 ± 2.75%.

At 4 °C, ethanolic leaf extracts of A. indica, A.vasica, and A.sessilis were shown to be ineffective in inhibiting chilli deterioration, unlike at 25 °C. The inoculation of capsicum capsici on chilli resulted in decay inhibition of -46.93 ± 3.5%, -24.46 ± 10.15%, and 38.77 ± 7.06% in A. sessilis, A. indica, and A. vasica leaf extracts, respectively. After inoculating chilli with capsicum capsici, an ethanolic leaf extract of P. paniculata inhibited degradation by 60.45 ± 8.89% at 4 degrees Celsius.

At 25°C, ethanolic stem extract treatment of A. sessilis, A. indica, and P. paniculata inhibited anthracnose disease by 52.67 ± 2.01%, 45.80 ± 4.25%, and 46.57 ± 5.5%, respectively, compared to control group. The ethanolic stem extract of A. vasica shown lower decay inhibition (3.81 ± 2.64%). A. sessilis showed 50.37 ± 2.01% decay inhibition in ethanolic stem extract treatment following capsicum capsici inoculation in chilli, followed by 48.85 ± 1.52% (A. indica) and 48.09 ± 6.65% (A. vasica) at 25 °C. A. sessilis showed 50.37 ± 2.01% decay inhibition in ethanolic stem extract treatment following capsicum capsici inoculation in chilli, followed by 48.85 ± 1.52% (A. indica) and 48.09 ± 6.65% (A. vasica) at 25 °C.

TABLE 5.10. 15 Percentage Decay Inhibition and Disease Incidence in Chilli.

TREATMENTS	% OF DECAY INHIBITION		% OF DISEASE INCIDENCE	
	25°C	4°C	25°C	4°C
Negativecontrol			72.77^{a}	27.22^{d}
PositivecontrolBefore	64.12±6.52^{b}	79.59±4.08^{a}	26.11^{d}	5.55^{g}
PositivecontrolAfter	84.73±0.76^{a}	57.14±3.53^{ab}	11.11^{e}	11.66^{fg}
*A.vasica*LeafEthanolic Before	48.09±6.65^{bc}	79.59±2.04^{a}	37.77^{cd}	5.55^{g}
*A.vasica*LeafEthanolicAfter	21.37±2.75^{d}	38.77±7.06^{bc}	57.22^{b}	16.66^{ef}
*A.vasica*StemEthanolic Before	3.81±2.64^{e}	79.59±2.04^{a}	70.00^{a}	5.55^{g}
*A.vasica*StemEthanolicAfter	48.09±6.65^{bc}	22.44±7.35^{c}	37.77^{cd}	21.11^{de}
*A.indica*LeafEthanolic Before	48.85±3.32^{bc}	26.53±10.6^{bc}	51.11^{cd}	20.00^{def}
*A.indica*LeafEthanolicAfter	29.76±7.96^{d}	24.46±10.15 ^{d}	39.44^{b}	33.88^{bc}
*A.indica*StemEthanolic Before	45.80±4.25^{bc}	71.42±5.39^{a}	37.77^{c}	7.77^{g}
A.indica StemEthanolicAfter	48.85±1.52^{bc}	-50.45±8.95^{e}	37.22^{cd}	58.88^{a}
*A.sessilis*LeafEthanolic Before	53.43±3.32^{bc}	89.79±2.04^{a}	33.88^{cd}	2.77^{g}
*A.sessilis*Leaf EthanolicAfter	25.18±8.5^{d}	-46.93±3.53^{d}	54.44^{b}	40.00^{b}
*A.sessilis*StemEthanolic Before	52.67±2.01^{bc}	81.63±3.25^{a}	34.44^{cd}	5.00^{g}
*A.sessilis*StemEthanolic After	50.37±2.01^{bc}	-55.10±8.56^{d}	36.11^{cd}	42.22^{b}
*P.paniculata*LeafEthanolic Before	46.57±5.5^{c}	14.28±10.23^{c}	38.88^{c}	23.33^{de}
*P.paniculata*LeafEthanolic After	22.13±4.76^{d}	60.45±8.89^{e}	56.66^{b}	53.88^{a}

Each value is expressed as mean of triplicates, & column sharing same alphabetical letters are not significantly different ($p \leq 0.05$) using.

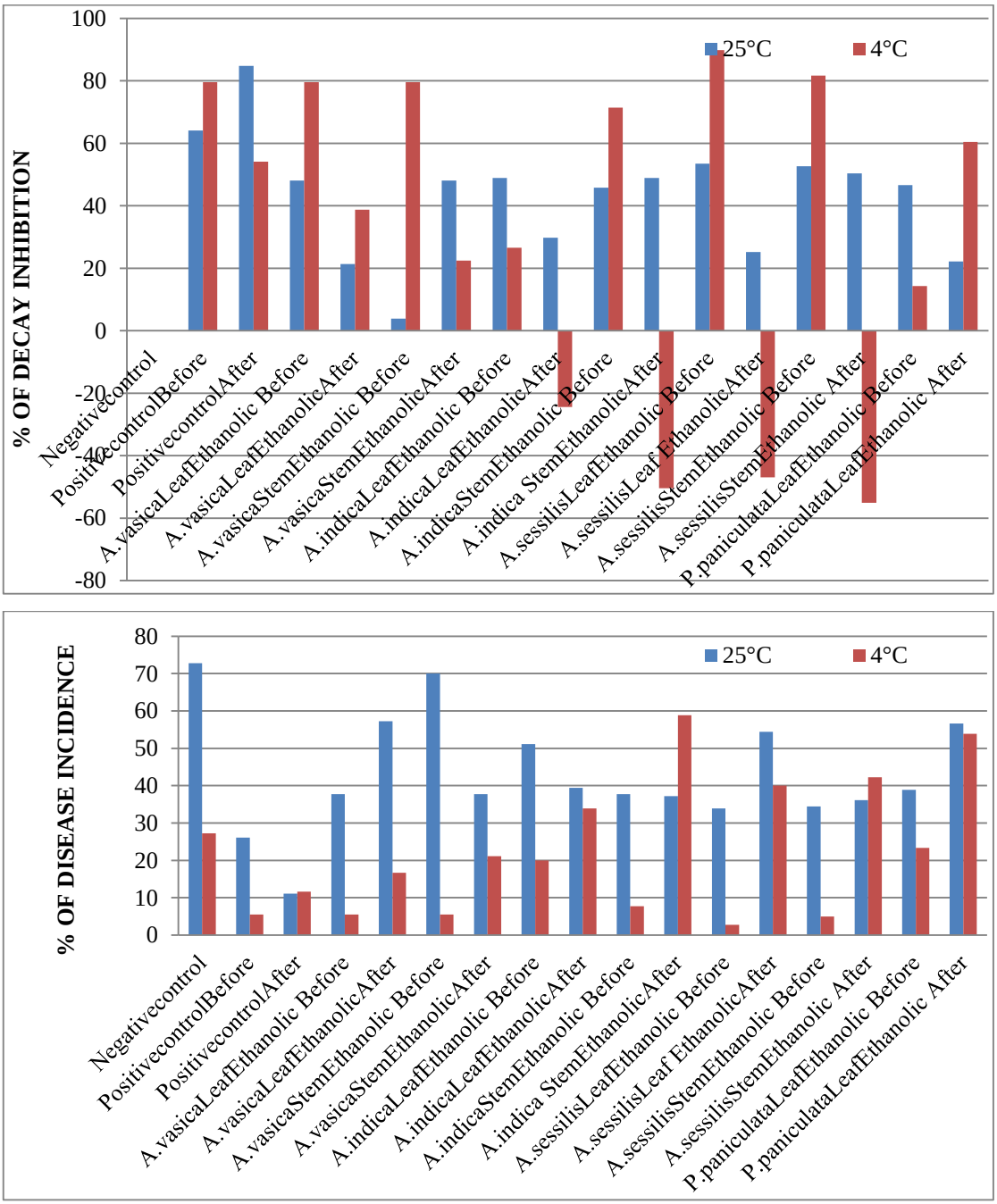


FIG. 5.60 DECAY INHIBITION (A) AND PERCENTAGE DISEASE INCIDENCE (B) IN ETHANOLIC EXTRACT TREATED CHILLI. VARTICAL BARS REPRESENT ERROR BAR OF MEAN.

There was a significantdifference in decay inhibition b/w stemextract treatments after & before chilli inoculation at 4°C. Before applying the extract, the ethanolic stem extract of A.

sessilis showed the maximum decay inhibition (81.63 ± 3.25%). Treatment with ethanolic stem extracts of A. vasica and A. indica had a similar effect. Chilli fruit had a degradation inhibition rate of 79.0 ± 2.04% and 71.42 ± 5.39%. The ethanolic stem extract of A. vasica inhibited deterioration by 22.44±7.35% compared to the control group.

5.4.1. Percentage of Disease Incidence

A substantial variation in percentage illness was seen between 25°C & 4°C (Fig. 5.60 (B) & Table 5.10). Before applying the extract at 25 °C, the minimal disease incidence in chilli was observed to be 33.88% in ethanolic leaf extract of A. sessilis. The remaining ethanolic leaf extracts (A. vasica, P. paniculata, & A. indica) showed 37.77%, 38.88%, and 51.11% decay inhibition, respectively. In the control group, illness incidence was 72.77% at 25 °C.

In contrast, 39.44% disease incidence was observed in A. indica ethanolic leaf extract treatment following chilli inoculation with capsicum capsici at 25 °C. While disease incidence increased in ethanolic leaf extracts of A. sessilis, P. paniculata, and A. vasica, by 54.44%, 56.66%, and 57.22%, respectively.

A similar outcome was observed before adding the stem extract to the chili. A. sessilis had the lowest illness incidence (34.44%), followed by A. indica (37.77%), whereas A. vasica stem extract had a disease incidence of 57.22%.

After applying the ethanolic stem extract of A. sessilis, A. indica and A. vasica were effectively reducing disease incidence in chilli and it was 36.11% (A. sessilis), 37.22% (A. indica) and 37.77% (A. vasica).

Plant extract was found more effective to reduce disease incidence at 4 °C than 25 °C. Before applying the plant extract on chilli. The increasing order of disease incidence were reported in order of 5.55%, 20.00% and 23.33% in ethanolicleaf extract of A.vasica, A. indica and P. paniculata, respectively. While in control disease incidence was observed 27.22%.

Further disease incidence in chilli after capsicum capsici treatment were reported 16.66% in A. vasica leaf extracts followed by 33.88% (A. indica) 40.00% (A. sessilis) and 53.88% (P. paniculata)

Ethanolic extract of stem at 4 °C in A. vasica and A. indica were found effective to reduce disease incidence in chilli. Disease incidence reduced by A. sessilis, A. vasica and A. indica stem extract treatment before inoculation of chilli were 5.00%, 5.55% and 7.77%. But, Ethanolic stem extract of A. indica (58.88%) A. sessilis (42.22%) were not reported effective in reducing disease incidence after inoculation of chilli. Where, as 21.11% disease incidence was found in chilli by the ethanolic stem extract of A. vasica.

5.4.2. Disease Severity

Results of the current investigation revealed that disease severity in the form of lesion diameter of anthracnose on chilli fruit (Table 5.11 and Fig. 5.61). Ethanolic leaf extract of A. sessilis exhibited minimum lesion diameter i.e., 0.15 ± 0.04 cm2 in extract treatment before inoculation chilli at 25 °C. while 0.32 ± 0.04 cm2 lesion diameter was reported after inoculation of chilli. 0.18 ± 0.03 cm2, 0.27 ± 0.046 cm2 and 0.71 ± 0.069 cm2 lesion diameter was reported on chilli via before applying the A. indica, A. vasica and P. paniculata leaf extract treatments. Increase order of lesion diameter 0.18 ± 0.017 cm2, 0.18 ± 0.020 cm2, 0.28 ± 0.02 cm2 and 0.32 ± 0.04 cm2 was found in after applying leaf extract of A. indica, A. vasica, P. paniculata and A. sessilis, respectively. Result showed the less effectiveness of ethanolic A. sessilisleaf extract in afterinoculation in chilli at 25 °C.

Ethanolic stem extract of A. vasica (0.17 ± 0.01 cm2) treatment was found effective in reducing lesion diameter before the inoculation in chilli at 25 °C. Further lesion diameter of A. indica and A. sessilis ethanolic stem extract were reported 0.25 ± 0.05 cm2, 0.43 ± 0.069 cm2, respectively. While the lesion diameter in control was 0.46 ± 0.031 cm2.\

After treatment the stem extract at 25 °C, at par result were found similar the stem extract treatment before inoculation of chilli. lowest lesion diameter was observed in A. vasica (0.16 ± 0.021 cm2) treatment followed by A. indica (0.18 ± 0.017 cm2) and A. sessilis (0.43 ± 0.06 cm2).

Results revealed that A. sessilis leaf extract found more effective to reduce lesion diameter at 4 °C than 25 °C. Before applying the A. sessilis leaf extract, lesion diameter was reported 0.15 ± 0.06 cm2 whereas 0.23 ± 0.04 cm2 was observed after dipped chilli in extract of the inoculation. The lesion diameter was found in the ethanolic stem extract treatment of A. sessilis before (0.25 ± 0.02 cm2) and after (0.39 ± 0.053 cm2) inoculation of chilli and.

Before inoculation of leaf extract in the chilli with C. capsici at 4 °C, increase trend of lesion diameter such as 0.17 ± 0.014 cm2, 0.26 ± 0.049 cm2 and 0.28 ± 0.06 cm2 were found in A. vasica, A. indica and P. paniculata leaf extract treatment, respectively.Whereas in control, lesion diameter was 0.36 ± 0.04 cm2. After treatment of ethanolicleaf extract treatment of A. vasica, A. indica and P. paniculata, lesion diameter was recorded 0.18 ± 0.003 cm2, 0.33 ± 0.035 cm2 and 0.30 ± 0.023 cm2, respectively.

Ethanolic stemextract of A. vasica and A. indica were found less effective to reduce lesion diameter at 4 °C than 25 °C. It was 0.25 ± 0.05 cm2 (before), 0.28±0.014 cm2 (after) and 0.24 ± 0.047 cm2 (before), 0.26 ± 0.049 (after) lesion diameter in A. vasica and A. indica treatment.

5.4.3. Weight Loss

The effectof the extract on weightloss in chilli have been presented in table 5.12 and fig. 5.61 (B). Ethanolic leaf extract treatment of A. sessilis was observed effective to maintain the weight of chilli in before inoculation the chilli at 25 °C. It was found 30.2 ± 1.69% in A. sessilis treatment and control (58.79 ± 0.85%). Decreasing order of effectiveness of extract in respect to weight loss was 26.77 ± 0.04%, 31.11 ± 4.02%, and 36.66 ± 0.50% in before applying ethanolic leaf extract of A. indica, P. paniculata and A. vasica at 25 °C. Whereas decreasing trend of effectiveness in after applying leaf extract was 33.95 ± 0.29%, 45.58 ± 5.29%, 46.25 ± 7.40% and 70.74 ± 3.25% in A. sessilis, A. vasica, A. indica and P. paniculata, respectively.

Before applying ethanolic stem extract, 26.88 ± 1.88% weight loss was reported in A. sessilis followed by A. indica (34.1 ± 3.16%) and A. vasica (48.16 ± 7.26%). In the contrary, A. vasica was found more effective to maintain the weight of chilli (30.67 ± 0.61%) in after

treating with extract. Whereas 33.17 ± 1.22% and 41.05 ± 2.12% weight loss was reported in after applying the A. indica and A. sessilis stem extract on chilli, respectively.

Results indicate that weight loss in chilli was less at 4 °C than 25 °C. Among the various ethanolic leaf extracts of A. sessilis was found more effective to maintain weight of chilli before the inoculation. It was 14.94 ± 0.27% in A. sessilis treatment and 27.29 ± 1.86% in control.

Increase weight loss in before applying the ethanolic extract of leave was 23.56 ± 2.50%, 30.09 ± 1.44% and 32.54 ± 3.35% in A. vasica, A. indica and P. paniculata, respectively at 4 °C. Whereas in after applying of leaf extract of inoculation, increase weight loss was 25.11 ± 2.51%, 28.94 ± 1.19%, 32.68 ± 1.45% and 38.00 ± 5.57% in A. indica, A. vasica, A. sessilis & P.paniculata treatment, respectively.

Minimum weight loss 25.38 ±1.06% was reported in before apply stem extract of A. indica followed by 28.18 ± 1.17% (A. vasica) and 29.30 ± 1.67% (A. sessilis). Whereas after applying stem extract, 24.41 ± 0.84%, 32.39 ± 2.33% and 29.24 ± 0.37% weight loss was observed in A. vasica, A. sessilis, and A. indica, respectively.

5.4.4. Wound in Chilli

Lowest number of wound 50 and 5 were reported in A. sessilis leafextract treatment beforeinoculation of chilli in compare to control 131 and 49 at 25 °C and 4 °C (Table 5.11). Before applying ethanolic leaf extract, increase number of wounds in chilli was 67, 68, and 80 of A. indica, A. vasica & P. paniculata, respectively at 25 °C. While after applying the leaf extract of inoculation, not single leaf extract was found effective to reduce number of wounds in chilli and it was 92, 98, 102, 103 in A. indica, A. sessilis, P. paniculata and A. vasica treatment, respectively.

No variable difference in wound of chilli (65 and 67) was reported in ethanolic stem extract of A. sessilis and A. indica after the inoculation at 25 °C, respectively. Similar results were found in applying the ethanolic stem extract of A. indica (59) and A. sessilis (61) before the inoculation at 25 °C. While ethanolic stem extract of A. vasica was not effective to reduce the number of wounds in chilli and it was 126 wounds after inoculation of chilli.

Increase number of wounds was observed as 10, 36 and 42 in A.vasica, A. indica & P. paniculata leaf extract treatment before the inoculation with capsicum capsici at 4 °C. No ethanolic leaf extract treatments were found effective to maintain minimum number of wounds after the inoculation the chilli at 4 °C. 30, 61, 72, and 97 wounds were observed by A. vasica, A. indica, A. sessilis & P. paniculata, respectively. Application of ethanolic stem extract treatment before the inoculation, A.vasica, A. indica and A. sessilis were reported to reduce number of wounds in chilli. While, it was 9, 10, and 14 wound in A. sessilis, A. vasica and A. indica stem treatment respectively at 4 °C. No ethanolic stem was found effective to reduce anthracnose disease in chilli. The wound was found in A. vasica A. sessilis and A. indica stem treatment treated with capsicum capsici in chilli after the inoculation were 38,76, and 106, respectively.

5.4.5. pH

Table 5.12 showed the effect of extract treatment on chilli pH at 25 °C and 4 °C. Highly acidic pH 4.95 ± 0.014 and 5.12 ± 0.024 was found in A. sessilisleaf extract treatment before the inoculation at 25 and 4 °C. While 6.57 ± 0.006 and 6.46 ± 0.08 pH was found in control chilli at25 and 4 °C. Increasing pH order of chilli was 5.53 ± 0.017, 5.82 ± 0.018, 8.24 ± 0.026 (at 25 °C) 5.64 ± 0.017, 6.37 ± 0.008 and 6.64 ± 0.008 (at 4 °C) in A. vasica, P. paniculata & A. indica leaf extract treatment respectively before the inoculation. Similar results were found in leaf extract treatment after the inoculation the chilli fruit at 25 °C. Increase trend of basicity of pH was found 5.62 ± 0.014, 6.04 ± 0.018, 6.92 ± 0.005, 8.25 ± 0.020 (at 25 °C) 5.14 ± 0.028, 6.25 ± 0.014, 6.45 ± 0.020 and 6.25 ± 0.017 (at 4 °C) after the inoculation of A. sessilis, A. vasica, P. paniculata & A. indica leaf extract, respectively.

The chilli pH was found 5.54 ± 0.020, 5.53 ± 0.018 in A. sessilis & A. vasica stem extract treatment before inoculation at 25 °C, respectively. While basic pH of chilli was found in A. indica (before and after) A. vasica (after) stem extract treatment compare to control at 25 °C. It was 7.27 ± 0.014 (before), 7.22 ± 0.023 (after) and 7.74 ± 0.026 (after) in A. indica and A. vasica stem treatment at 25 °C, respectively. The pH of chilli was found less in stem extract treatment compare to control at 4 °C. It was 5.18 ± 0.008, 5.45 ± 0.017 and 6.25 ± 0.014 in A. vasica, A. indica and A. sessilis respectively before the inoculation. While after the inoculation,

5.34 ± 0.008 pH was found in A. vasica stem extract treatment pH of chilli was found similar to control in A. sessilis and A. indica stem treatment.

TABLE 5.11 LESION DIAMETER, WEIGHT LOSS AND NUMBER OF WOUNDS IN EXTRACT TREATED CHILLI.

TREATMENTS	LESIONDIAMETERAT25±2°C(CM^2)	LESIONDIAMETERAT4°C(CM^2)	WEIGHTLOSSAT25±2°C (GM)	WEIGHTLOSSAT4°C (GM)	TOTALNUMBEROFWOUNDSINCHILLIAT25±2°C	TOTALNUMBEROFWOUNDSINCHILLIAT4°C
Negativecontrol	0.46 ±0.031^{b}	0.36 ±0.04^{ab}	58.79 ±0.85^{b}	27.29 ±1.86^{bc}	131	49
PositivecontrolBefore	0.15 ±0.039^{ef}	0.12 ±0.039^{ef}	44.51 ±1.49^{c-e}	26.21 ±2.84^{bc}	47	10
PositivecontrolAfter	0.19 ±0.042^{def}	0.15 ±0.02^{def}	45.40 ±2.04^{cd}	25.63 ±1.57^{bc}	20	21
A.vasica Leaf EthanolicBefore	0.27 ±0.046^{cde}	0.17 ±0.014^{c-f}	36.66 ±0.50^{d-g}	23.56 ±2.50^{c}	68	10
A.vasica Leaf EthanolicAfter	0.18 ±0.020^{def}	0.18 ±0.003^{c-f}	45.58 ±5.29^{cd}	28.94 ±1.19^{bc}	103	30
A.vasica Stem EthanolicBefore	0.17 ±0.01^{def}	0.25 ±0.05^{b-f}	48.16 ±7.26^{c}	28.18 ±1.17^{bc}	126	10
*A.vasica*Stem EthanolicAfter	0.16 ±0.024^{def}	0.28 ±0.014^{a-d}	30.67 ±0.61^{fg}	24.41 ±0.84^{c}	68	38
A.indica Leaf EthanolicBefore	0.18 ±0.03^{def}	0.26 ±0.049^{b-e}	26.77 ±1.04^{g}	30.09 ±1.44^{bc}	67	36
A.indica Leaf EthanolicAfter	0.18 ±0.017^{def}	0.33 ±0.035^{ab}	46.25 ±7.40^{cd}	25.11 ±2.51^{bc}	92	61
A.indica Stem EthanolicBefore	0.25 ±0.05^{c-f}	0.24 ±0.047^{b-f}	34.13 ±3.16^{e-g}	25.38 ±1.06^{bc}	59	14
A.indica Stem	0.18	0.26	33.17	29.24	67	106

EthanolicAfter	±0.017^{def}	±0.049^{b-e}	±1.22^{fg}	±0.37^{bc}		
*A.sessilis*Leaf EthanolicBefore	0.15 ±0.04^{f}	0.15 ±0.06^{def}	30.20 ±1.69^{fg}	14.94 ±0.27^{d}	50	5
*A.sessilis*Leaf EthanolicAfter	0.32 ±0.04^{c}	0.23 ±0.04^{b-f}	33.95 ±0.29^{e-g}	32.68 ±1.45^{ab}	98	72
*A.sessilis*Stem EthanolicBefore	0.43 ±0.069^{b}	0.25 ±0.02^{b-e}	26.88 ±1.88^{g}	29.30 ±1.67^{bc}	61	9
*A.sessilis*Stem EthanolicAfter	0.43 ±0.06^{b}	0.39 ±0.053^{a}	41.05 ±2.12^{c-f}	32.39 ±2.33^{ab}	65	76
*P.paniculata*Leaf Ethanolic Before	0.71 ±0.069^{a}	0.28 ±0.06^{a-d}	31.11 ±4.02^{fg}	32.54 ±3.35^{ab}	80	42
*P.paniculata*Leaf EthanolicAfter	0.28 ±0.02^{cd}	0.30 ±0.023^{abc}	70.74 ±3.25^{a}	38.00 ±5.57^{a}	102	97

The values are given as the mean of triplicates, and Duncan's multiple range test shows no significant difference ($p < 0.05$) across the columns with the same alphabetical letters.

5.5. Defense Enzymes:

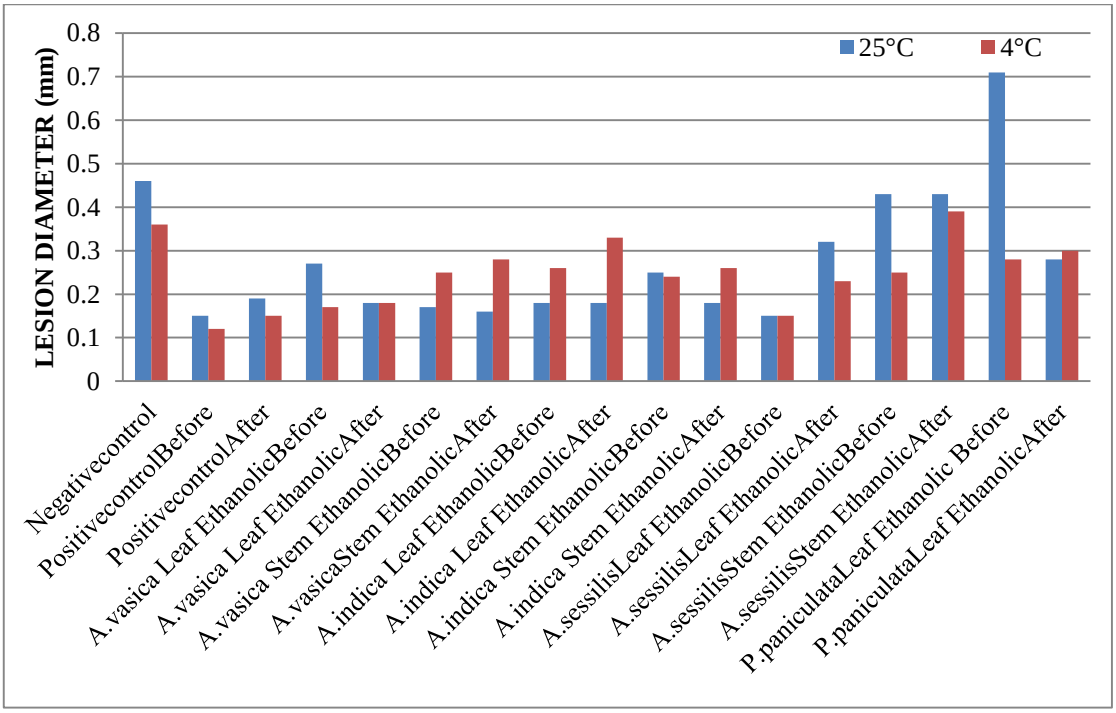
5.5.1. Polyphenol Oxidase (PPO)

The polyphenol oxidase (PPO) production in chilli during the inoculation period has been shown in table 5.14 and fig. 5.62 (A). Peaked PPO was reported 0.154 ± 0.006 unit min-1g-1FW in ethanolic leafextract of A. sessilisbefore the inoculation at 25 °C. PPO, increase 0.018 ± 0.011, 0.026 ± 0.002, and 0.046 ± 0.007 unit min-1g-1FW in before applying leaf extract of P. paniculata, A. indica and A. vasica at 25° C. Whereas increase trend of PPO was 0.014 ± 0.001, 0.032 ± 0.004, 0.036 ± 0.002, and 0.126 ± 0.006 in A. indica, P. paniculata, A. vasica and A. sessilis in leaf extract treatment after inoculation the chilli.

Among the stem extract at 25° C, highest PPO was observed 0.076 ± 0.005 unit min-1g-1FW in before applying A. sessilis on chilli fruit. Similar result was found in after applying the stem extract on chilli. Maximum PPO 0.050 ± 0.006 unit min-1g-1FW was also observed in A. sessilis treatment but ethanolic stem extract A. indica was reported less effective to induce

the PPO production (0.014 ± 0.001 unit min-1g-1FW) in chilli compare to control (0.028 ± 0.010 unit min-1g-1FW) after inoculation of chilli.

Result indicate that increased trend of PPO, 0.014 ± 0.004, 0.053 ± 0.007, 0.085 ± 0.001 and 0.107 ± 0.016 unit min-1g-1FW was found in before applying the leaf extract of P. paniculata, A. indica, A. vasica and A. sessilis respectively at 4 °C. Whereas after applying the leaf extract of inoculation, A. vasica was found effective to induce production of PPO (0.070 ± 0.001 unit min-1g-1FW) in chilli followed by 0.075 ± 0.002, 0.036 ± 0.009, and 0.030 ± 0.008 unit min-1g-1FW in A. indica, A. sessilis and P. paniculata, respectively.



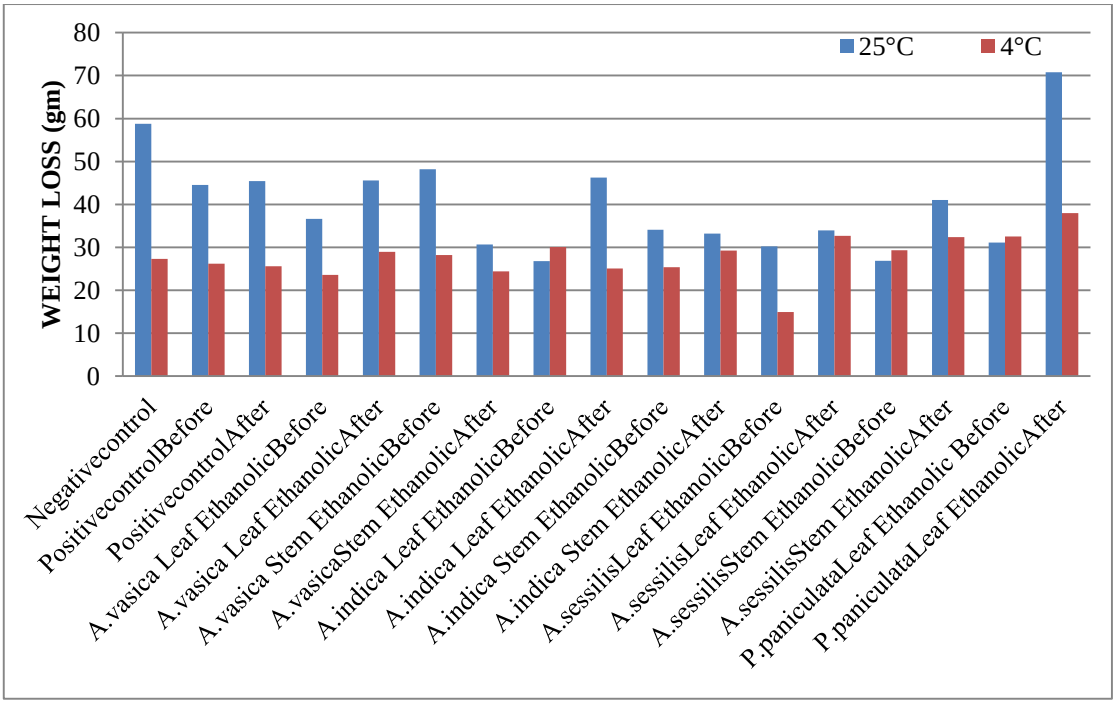


FIG. 5.61 LESION DIAMETER AT WOUNDED AREA (A) AND WEIGHT LOSS (B) IN ETHANOLIC EXTRACT TREATED CHILLI. VARTICAL BARS REPRESENT ERROR BAR OF MEAN

FIG. 5.62 EFFECT OF PLANT EXTRACT ON CHILLI FRUITS AGAINST ANTHRACNOSE DISEASE AT 25 °C. WHERE, - CON = NEGATIVE CONTROL, + CON BE = POSITIVE CONTROL BEFORE, + CON AF = POSITIVE CONTROL AFTER, P L E BE = P. PANICULATA LEAF ETHANOLIC BEFORE.

FIG. 5.63 EFFECT OF PLANT EXTRACTS ON CHILLI FRUITS AGAINST ANTHRACNOSE DISEASE AT 25 °C. WHERE, P L E AF = *P. PANICULATA* LEAF ETHANOLIC AFTER, AD L E BE = *A. VASICA* LEAF ETHANOLIC BEFORE, AD L E AF = *A. VASICA* LEAF ETHANOLIC AFTER, AD S E BE = *A. VASICA* STEM ETHANOLIC BEFORE.

FIG. 5.64 EFFECT OF PLANT EXTRACTS ON CHILLI FRUITS AGAINST ANTHRACNOSE DISEASE AT 25 °C. WHERE, AD S E AF = A. VASICA STEM ETHANOLIC AFTER, AC L E BE = A. INDICA LEAF ETHANOLIC BEFORE, AC L E AF = A. INDICA LEAF ETHANOLIC AFTER, AC S E BE = A. INDICA STEM ETHANOLIC BEFORE.

FIG. 5.65 EFFECT OF PLANT EXTRACTS ON CHILLI FRUITS AGAINST ANTHRACNOSE DISEASE AT 25 °C. WHERE, AC S E AF = A. INDICA STEM ETHANOLIC AFTER, ALT L E BE = A. SESSILIS LEAF ETHANOLIC BEFORE, ALT L E AF = A. SESSILIS LEAF ETHANOLIC AFTER, ALT S E BE= A. SESSILIS STEM ETHANOLIC BEFORE.

FIG. 5.66 EFFECT OF PLANTS EXTRACT ON CHILLI FRUITS AGAINST ANTHRACNOSE DISEASE AT 25 °C AND 4 °C. WHERE, ALT S E AF = A. SESSILIS STEM ETHANOLIC AFTER, NEG CON = NEGATIVE CONTROL, +VE CON BE = POSITIVE CONTROL BEFORE, +VE CON AF = POSITIVE CONTROL AFTER.

FIG. 5.67 EFFECT OF PLANTS EXTRACTS ON CHILLI FRUITS AGAINST ANTHRACNOSE DISEASE AT 4°C. WHERE, AD L E BE = A. VASICA LEAF ETHANOLIC BEFORE, AD L E AF = A. VASICA LEAF ETHANOLIC AFTER, AD S E BE = A. VASICA STEM ETHANOLIC BEFORE, AD S E AF = A. VASICA STEM ETHANOLIC AFTER.

FIG. 5.68 EFFECT OF PLANT EXTRACTS ON CHILLI FRUITS AGAINST ANTHRACNOSE DISEASE AT 4 °C.WHERE, AC L E BE = A. INDICA LEAF ETHANOLIC BEFORE, AC L E AF = A. INDICA LEAF ETHANOLIC AFTER, AC S E BE = A. INDICA STEM ETHANOLIC BEFORE, AC S E AF = A. INDICA STEM ETHANOLIC AFTER

FIG. 5.69 EFFECT OF PLANTS EXTRACTS ON CHILLI FRUITS AGAINST ANTHRACNOSE DISEASE AT 4 °C.WHERE, ALT L E BE = A. SESSILIS LEAF ETHANOLIC BEFORE, ALT L E AF = A. SESSILE LEAF ETHANOLIC AFTER, ALT S E BE = A. SESSILIS STEM ETHANOLIC BEFORE, ALT S E AF= A. SESSILIS STEM ETHANOLIC AFTER,

FIG. 5.70 EFFECT OF PLANTS EXTRACTS ON CHILLI FRUITS AGAINST ANTHRACNOSE DISEASE AT 4 °C. WHERE, PP L E BE = P. PANICULATA LEAF ETHANOLIC BEFORE, PP L E AF = P. PANICULATA LEAF ETHANOLIC AFTER

.

Stem extract was less effective to induce production of PPO in chilli at 4 °C compare to 25 °C. Maximum 0.044 ± 0.010 unit min-1g-1 FW PPO production in chilli was found in an

A. sessilis stem extract before the inoculation. No variable difference was observed in induction of PPO production in chilli by ethanolic stem extract of A vasica (0.014 ± 0.004 unit min-1g-1 FW) after the inoculation at 4 °C. Significant variation was not found in ethanolic stem extract after the inoculation of A. indica, A.vasica and A.sessilis to induce the production of PPO after\ inoculation the chilli as 0.023 ± 0.005, 0.030 ± 0.006 and 0.038 ± 0.007unit min-1g-1FW in A. vasica, A. indica and A. sessilis, respectively.

5.5.2. Peroxidase Enzyme (POD)

Effect of extract to induce production of peroxidase enzyme in chilli is shown in table 5.14 and fig. 5.71 (B). Peaked peroxidase production in chilli (0.090 ± 0.002 unit min-1g-1 FW) was observed ethanolic leafextract of A. sessilis treatment before the inoculation at 25 °C, whereas A. indica, A. vasica & P. paniculatawere found less effective to induce production of peroxidase in chilli at 25 °C. It was 0.033 ± 0.003, 0.035 ± 0.003 and 0.016 ± 0.08 unit min-1g-1 FW in A. vasica, A. indica, and P. paniculata, respectively. After applying these extract of inoculation, A. indica and P. paniculata were not able to produce significant peroxidase enzyme in chilli at 25° C. It was produced 0.021 ± 0.006 unit min-1g-1 FW (A. indica) and 0.020 ± 0.002 unit min- 1g-1 FW (P. paniculata) Peroxidase enzyme. While A. vasica and A. sessiliss were activate the production of peroxidase in chilli on after applying the extract. 0.85±0.006 unit min-1g-1 FW was produce in chilli by leaf extract of A. sessilis followed by 0.050 ± 0.006 unit min-1g-1 FW in A. vasica.

`The stem extract treatment at 25 °C was not effective to produce of peroxidase before the inoculation of chilli. The peroxidase enzyme production was 0.019 ± 0.008, 0.035 ± 0.003 and 0.030 ± 0.003 unit min-1 g-1 FW found in A. indica, A. sessilisand A. vasica in treated chilli respectively. While increase trend of peroxidase enzyme in chilli was 0.025 ± 0.00, 0.035 ± 0.006 and 0.037 ± 0.008 by the ethanolic stem extract A. sessilis, A. indica, and A. vasica treatment after the inoculation respectively

.

Similar result was found before applying the leaf extract at 4 °C, highest 0.061 ± 0.006 unit min-1g-1 FW peroxidase was reported in chilli by A. sessilis. Whereas the remaining leaf extracts were expressed peroxidase production in chilli were 0.010 ± 0.001, 0.012 ± 0.001 and 0.019 ± 0.002 unit min-1g-1 FW by A. indica, P. paniculata and A. vasica, respectively. In after application of leaf extract, increase trend of peroxidase production in chilli was found 0.009 ±

0.002, 0.009 ± 0.003, 0.016 ± 0.003 and 0.051 ± 0.001 unit min-1g-1 FW by P. paniculata, A. indica, A. vasica and A. sessilis, respectively.

Stem extract was not effective in activation of peroxidase enzyme production in chilli before the inoculation at 4 °C as 0.013 ± 0.002, 0.027 ± 0.01 and 0.029 ± 0.003 unit min-1g-1 FW by the A. vasica, A. sessilis and A. indica, respectively. While after applying the stem extract on chilli, 0.008 ± 0.001, 0.014 ± 0.008 and 0.021 ± 0.001unit min-1g-1 FW peroxidase was produce by A. vasica, A. sessilis and A. indica, respectively.

TABLE 5.13 PH AND TITRATABLE ACIDITY IN EXTRACT TREATED CHILLI.

TREATMENTS	P^H AT 25 °C	P^H AT 4 °C	TITRATABLE ACIDITY % AT 25°C	TITRATABLE ACIDITY % AT 4°C
Negativecontrol	6.58 ±0.07^{e}	6.47 ±0.09^{b}	0.094 ±0.006^{f}	0.076 ±0.002^{def}
PositivecontrolBefore	5.07 ±0.02^{j}	5.18 ±0.02^{h}	0.21 ±0.02^{c}	0.097 ±0.003^{cd}
PositivecontrolAfter	6.00 ±0.06^{f}	5.63 ±0.06^{e}	0.05 ±0.009^{g}	0.092 ±0.005^{cde}
*A.vasica*LeafEthanolic Before	5.54 ±0.017^{i}	5.65 ±0.017^{e}	0.24 ±0.008^{ab}	0.098 ±0.001^{cd}
*A.vasica*LeafEthanolic After	6.05 ±0.018^{f}	6.26 ±0.014^{d}	0.23 ±0.005^{b}	0.086 ±0.002^{cde}
*A.vasica*StemEthanolic Before	5.54 ±0.019^{i}	5.19 ±0.009^{h}	0.08 ±0.005^{f}	0.12 ±0.003^{def}
*A.vasica*StemEthanolic After	7.75 ±.026^{b}	5.35 ±0.008^{g}	0.15 ±0.006^{e}	0.06 ±0.006^{fg}
*A.indica*LeafEthanolic Before	8.25 ±0.027^{a}	6.47 ±0.009^{b}	0.14 ±0.008^{e}	0.11 ±0.004^{bc}
*A.indica*LeafEthanolic After	8.26 ±0.021^{a}	6.53 ±0.018^{a}	0.010 ±0.005^{f}	0.093 ±0.002^{cde}
*A.indica*StemEthanolic Before	7.28 ±0.015^{c}	5.46 ±0.018^{f}	0.23 ±0.008^{ab}	0.12 ±0.003^{bc}
*A.indica*StemEthanolic	7.23	6.47	0.19	0.076

After	±0.024^{c}	±0.009^{b}	±0.008^{d}	±0.003^{def}
*A.sessilis*LeafEthanolic Before	4.96 ±0.015^{j}	5.13 ±0.025^{h}	0.34 ±0.02^{a}	0.18 ±0.004^{a}
*A.sessilis*LeafEthanolic After	5.63 ±0.015^{hi}	5.15 ±0.026^{h}	0.15 ±0.009^{e}	0.097 ±0.002^{cd}
*A.sessilis*StemEthanolic Before	5.55 ±0.021^{i}	6.26 ±0.015^{d}	0.18 ±0.005^{d}	0.13 ±0.008b
*A.sessilis*StemEthanolic After	5.71 ±0.15^{gh}	6.47 ±0.009^{b}	0.19 ±0.008^{d}	0.050 ±0.008^{g}
*P.paniculata*Leaf EthanolicBefore	5.83 ±0.019^{g}	6.38 ±0.008^{c}	0.14 ±0.005^{e}	0.092 ±0.005^{cde}
*P.paniculata*Leaf EthanolicAfter	6.93 ±0.006^{d}	6.46 ±0.021^{b}	0.15 ±0.005^{e}	0.070 ±0.003^{efg}

Each value is expressed as mean of triplicates, & column sharing same alphabetical letters arenot significantlydifferent ($p \leq 0.05$).

TABLE 5.14 POLYPHENOL OXIDASE AND PEROXIDASE ENZYME IN EXTRACT TREATED CHILLI.

TREATMENTS	**PPO AT 25 °C**	**PPO AT 4°C**	**POD AT 25°C**	**POD AT 4°C**
Negativecontrol	0.028 ±0.010ef	0.014 ±0.004^{h}	0.016 ±0.008^{c}	0.008 ±0.001^{e}
PositivecontrolBefore	0.092 ±0.002^{c}	0.035 ±0.006^{e-g}	0.033 ±0.002^{bc}	0.044 ±0.006^{bc}
PositivecontrolAfter	0.032 ±0.001^{f}	0.042 ±0.001^{e-g}	0.029 ±0.005^{bc}	0.029 ±0.003^{cd}
*A.vasica*LeafEthanolic Before	0.046 ±0.007^{de}	0.085 ±0.001^{ab}	0.033 ±0.003^{bc}	0.019 ±0.002^{de}
*A.vasica*LeafEthanolicAfter	0.036 ±0.002^{ef}	0.070 ±0.001^{b-d}	0.050 ±0.006^{b}	0.016 ±0.003^{de}
*A.vasica*StemEthanolic Before	0.012 ±0.004^{g}	0.014 ±0.004^{h}	0.030 ±0.003^{bc}	0.013 ±0.002^{e}
A.vasica StemEthanolicAfter	0.030 ±0.005^{g}	0.023 ±0.005^{gh}	0.037 ±0.008^{bc}	0.008 ±0.001^{e}
*A.indica*LeafEthanolic Before	0.026 ±0.002^{f}	0.053 ±0.007^{d-f}	0.035 ±0.003^{bc}	0.010 ±0.001^{e}
A.indica Leaf EthanolicAfter	0.014 ±0.001^{g}	0.075 ±0.002^{bc}	0.021 ±0.006^{c}	0.009 ±0.003^{e}

*A.indica*StemEthanolic Before	0.039 ±0.002^{ef}	0.016 ±0.004^{gh}	0.019 ±0.008^{c}	0.029 ±0.003^{cd}
*A.indica*StemEthanolic After	0.014 ±0.001^{g}	0.030 ±0.006^{f-h}	0.035 ±0.006^{bc}	0.021 ±0.001^{de}
*A.sessilis*LeafEthanolic Before	0.154 ±0.006^{a}	0.107 ±0.016^{a}	0.090 ±0.002^{a}	0.061 ±0.006^{a}
*A.sessilis*Leaf Ethanolic After	0.126 ±0.006^{b}	0.036 ±0.009^{e-g}	0.085 ±0.006^{a}	0.051 ±0.001^{ab}
*A.sessilis*StemEthanolic Before	0.076 ±0.005^{c}	0.044 ±0.010^{e-g}	0.035 ±0.003^{bc}	0.027 ±0.015^{cd}
*A.sessilis*StemEthanolic After	0.050 ±0.006^{de}	0.038 ±0.007^{e-g}	0.025 ±0.006^{bc}	0.014 ±0.008^{e}
*P.paniculata*LeafEthanolic Before	0.018 ±0.011^{d}	0.014 ±0.004^{gh}	0.016 ±0.008^{c}	0.012 ±0.001^{e}
*P.paniculata*LeafEthanolic After	0.032 ±0.004^{f}	0.030 ±0.008^{f-h}	0.020 ±0.002^{c}	0.009 ±0.002^{e}

Each value is expressed as the mean of triplicates, and the column sharing the same alphabetical letters are not significantly different ($p \leq 0.05$).

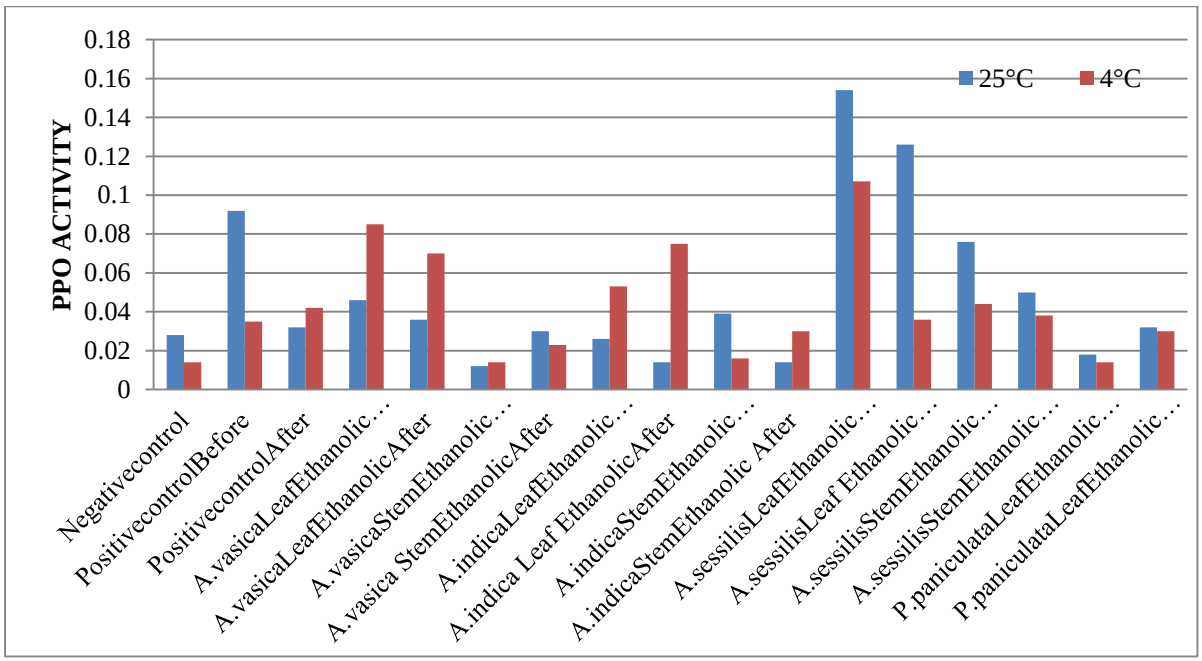
5.5.3. Catalase Enzyme (CAT)

The resultsare summarized in table 5.15 & fig. 5.72 (A) indicates that the effectivity of plant extracts in catalase production on before the pathogen inoculation. The highest catalase was observed 95.04 ± 1.83-unit min-1g-1 FW treated with the ethanolic leaf extract of A. sessilis followed by 56.18 ± 2.21-unit min-1g-1 FW (A. vasica), 43.67 ± 2.50 unit min-1g-1 FW (A. indica) and 25.01 ± 1.07 unit min-1g-1 FW (P. paniculata) before the capsicum capsici inoculation at 25 °C. The leaf extracts treated after capsicum capsici inoculation, A. sessilis, A. vasica, and P. paniculata was found less effective to activate the CAT production in chilli. It was 41.36 ± 1.34, 36.74 ± 1.83, and 29.05 ± 1.07-unit min-1g-1 FW in A. sessilis, A. vasica and P. paniculata, respectively. Whereas CAT was less produced (16.73 ± 0.57 unit min-1g-1 FW) in chilli when leaf extract of A. indica compare to negative control (21.35 ± 2.64 unit min-1g-1 FW).

While drawing the result of ethanolic stem extract in activation of CAT production with A. sessilis extract treated before inoculation. It was 87.34 ± 0.50 unit min-1g-1 FW. While 46.75 ± 3.5 and 36.55±3.23 unit min-1g-1 FW was reported in A. vasica, and A. indica. In the contrary, A. sessilis was observed less effective to activate the production of CAT (27.12 ± 2.90 unit min-1g-1 FW) in chilli after the inoculation. Whereas 34.82 ± 2.34 and 40.40 ± 2.96

unit min-1g-1 FW CAT production in chilli was reported treated with stemextract of A. vasica & A. indica after the inoculation.

In the before inoculation of chilli at 4 °C, similar trend of activation of CAT production was found in chilli. It was 36.74 ± 2.83, 58.87 ± 2.40, 64.26 ± 1.50, and 70.41 ± 2.60 unit min-1g-1 FW in an increase trend of activation of CAT production in chilli by ethanolic leaf extract of the P. paniculata, A. indica, A.vasica and A. sessilis, respectively. Similar result was also observed in leaf extracts treatment after inoculation in the chilli. Leaf extract of A. sessilis & A. vasica were found more effective in CAT production in chilli. It was 68.68 ± 1.20 and 35.20 ± 1.52 unit min-1g-1FW



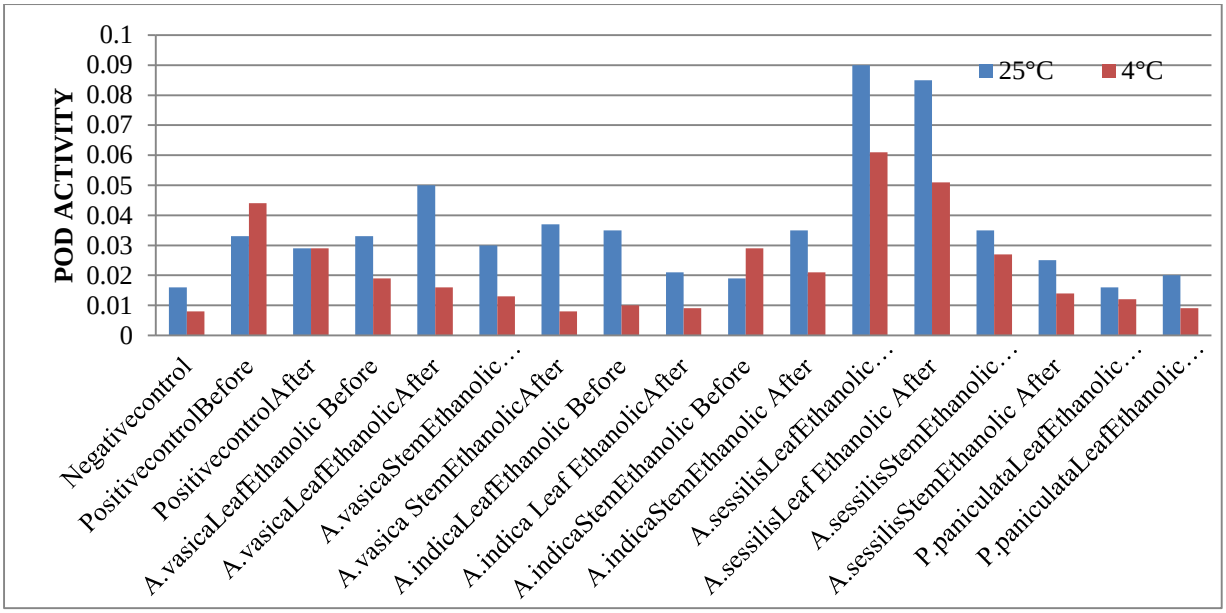


FIG. 5.71 POLYPHENOL OXIDASE (A) AND PEROXIDASE (B) ENZYME ACTIVITY IN ETHANOLIC EXTRACT TREATED CHILLI. VARTICAL BARS REPRESENT ERROR BAR OF MEAN.

While less CAT production 19.43 ± 0.69, 22.12 ± 1.12 unit min-1g-1 FW was reported in A. indica and P. paniculata leaf extract treatment respectively after the inoculation with capsicum capsici in chilli.The stem extract of A. sessilis before the inoculation was found less activated to produce CAT at 4° C. which was 19.43 ± 0.69 unit min-1g- 1 FW in treated than the control (21.35 ± 0.66 unit min-1g-1 FW). The stem extract of A. indica A. vasica and A. sessilis increased almost double fold activity of CAT enzyme when after inoculated in chilli, 43.67 ± 2.14, 35.97 ± 2.58 and 20.58 ± 1.34 unit min- 1g-1 FW,respectively.

TABLE 5.15 CATALASE AND PHENOLIC ACID IN EXTRACT TREATED CHILLI.

TREATMENTS	CAT AT 25˚C	CAT AT 4˚C	PHENOLIC AT 25˚C	PHENOLIC AT 4˚C
Negativecontrol	21.35±2.64^{kl}	21.35±0.66^{f}	2.16±0.12^{c}	2.42±0.01^{c}
Positivecontrol Before	74.07±0.96^{c}	65.47±2.26^{ab}	3.25±0.13^{b}	3.84±0.02^{ab}
Positivecontrol After	33.09±1.01^{g-i}	38.28±0.69^{de}	3.1±0.05^{b}	3.52±0.01^{ab}
A.vasica Leaf EthanolicBefore	56.18±2.21^{d}	64.26±1.50^{bc}	4.65±0.02^{a}	4.60±0.01^{a}
A.vasica Leaf EthanolicAfter	36.74±1.83^{fg}	35.20±1.52^{e}	2.65±0.02^{bc}	4.06±0.03^{a}
A.vasica Stem EthanolicBefore	46.75±3.85^{e}	63.10±2.03^{bc}	3.45±0.02^{b}	2.85±0.02^{bc}

*A.vasica*Stem EthanolicAfter	34.82±2.34^{f-h}	35.97±2.58^{e}	2.03±0.03^{c}	2.65±0.02^{bc}
A.indica Leaf EthanolicBefore	43.67±2.50^{e}	58.87±2.40^{c}	2.76±0.03^{bc}	2.74±0.02^{bc}
A.indica Leaf EthanolicAfter	16.73±0.57^{l}	19.43±0.69^{f}	3.1±0.15^{b}	2.27±0.03^{c}
A.indica Stem EthanolicBefore	36.55±3.23^{fg}	20.09±1.34^{f}	2.68±0.06^{bc}	2.06±0.03^{c}
A.indica Stem EthanolicAfter	40.40±2.96^{ef}	43.67±2.14^{d}	2.25±0.04^{c}	2.02±0.04^{c}
A.sessilis LeafEthanolicBefore	95.04±1.83^{a}	70.41±2.60^{a}	5.05±0.02^{a}	4.3±0.05^{a}
*A.sessilis*Leaf EthanolicAfter	41.36±1.34^{ef}	68.68±1.20^{ab}	2.85±0.02^{bc}	4.23±0.02^{a}
A.sessilis StemEthanolicBefore	87.34±0.50^{b}	19.43±0.69^{f}	3.05±0.02^{b}	4.26±0.08^{a}
*A.sessilis*Stem EthanolicAfter	27.12±2.90^{i-k}	20.58±1.34^{f}	2.66±0.03^{bc}	2.42±0.01^{c}
*P.paniculata*Leaf Ethanolic Before	25.01±1.07^{jk}	36.74±2.83^{e}	2.48±0.04^{c}	2.25±0.05^{c}
*P.paniculata*Leaf Ethanolic After	29.05±1.07^{h-j}	22.12±1.12^{f}	2.38±0.4^{c}	2.21±0.07^{c}

Each value is expressed as mean of triplicates, & column sharing same alphabetical letters are notsignificantly different ($p \leq 0.05$).

5.6. Discussion

Pesticides and fungicides are being used in crops with appropriate quality and quantity. But pesticides have been extensively used. Due to which, microbes became resistant that become a major public health concern globally in recent years (Khanam et al., 2015).

The commonly used pesticides on plants enters the biological systems through their mode of action and produce free radicals which damage exogenous cell components. They produce some toxic andadverse effects on liver, kidney. (Ibtissem et al., 2017).

Medicinal plants are rich source of potent natural antimicrobial products, they can be used against phytopathogenic fungi (Khanam et al., 2015).

Recently, there is a significant increase in usage of herbal productsin both developed and undeveloped countries. Many plant species are traditionally utilized for treatment

ofdifferent plant diseases. Natural goods are safer to use than manufactured ones. Due to food and environmental safety concerns, biological control agents have arisen as an alternative to synthetic fungicides. Many plant extracts have been tested in vitro and in vivo against a variety of post-harvest fungus, including Colletotrichum gloeosporioides, capsicum musae, capsicum linelemuthainum, and capsicum kahawae. Plant extracts, essential oils, and purified substances have been shown to have potent antifungal properties (Kekuda et al., 2014).

In the present study, aqueous, methanolic and ethanolic extracts (leaf & stem) of A. indica, A.vasica, A.sessilis, capsicum hirsutus, M. parvifolia, P. paniculata and T. bellirica were evaluated for their antifungal potential. Among these extracts, ethanolic extracts of A. sessilis showed promising effects against capsicum capsici.

Plants contain bioactive chemicals in low concentrations. An extraction process can recover and isolate phytochemicals from plant material with a high yield and few modifications. Solvent extractability is primarily determined by the compound's solubility, the mass of the product transference kinetics, and the solute/matrix strength communication, with matching constraints on heat and mass diffusion rate extraction (Dhanani et al., 2017).

In addition to this, important factors of crude extraction are considered as pressure, temperature & dynamic time. Extract solubility is very sensitive to temperature and pressures in the critical range. This might be due to flavonoidyield changedsignificantly with temperature over range of 40–60 °C. Vapourpressure of extractable compounds also increase with increased temperature. Thus, compounds are extracted in the supercritical fluid phase with increased tendency (Bimakr et al., 2011).

In present study, extraction was carriedout in aqueous, hexane, 70% ethanol and 70% methanol. Plant extract was extracted in aqueous (100 °C), ethanol (70 °C) and methanol (60 °C). Highest crude extract was reported in aqueousextract of A. indica (leaf) & A. vasica (stem) followed by methanolic, ethanolic and hexane extract. According to the literature, extract yield is obtained in a high percentage from the methanolic solvent.

Lai et al., (2009) found that methanolic plant material extract yield was higher than ethanolic and aqueous extracts which is similar to capsicum hirsutus leaf and stem extract yield. Wang et al., (2017) reportedthat extract yield is increasedwith increasing the ethanolic

concentration upto 70% but gradually decrease from 70 % to 100%. In present study, higher crude extract was reported from capsicum hirsutus by using methanolic solvent followed by ethanol, aqueous and hexane. Our present results supported the Asekunowo et al., (2017) who reportedthat high extract yield of Acalypha sp. obtained in aqueous than the methanolic and ethanolic. Because of the existence of numerous compounds with varying chemical properties, the efficiency of extraction and biological activities are heavily reliant on the properties of the extraction solubility in a certain solvent. Present investigations are further confirmed by Dhanani et al., (2017) who stated that different flavonoid compounds were extracted by using different solvents.

However, the flavonoids yield increases with the increase in extractiontime of 1, 1.5, 2 & 2.5 h. But dropped as the extraction time increased in 70% ethanol concentration (Wang et al., 2017). Our result agree with the finding of Brahmam and Sunita (2018) who reported high extract yield of methanolic extract of C. hirsutus than chloroform and ethyl acetate. But in M. parvifolia, T. bellirica and A. sessilis, leaf and stem extract yield were high in aqueous followed by the ethanolic and methanolic solvent that support the result of (Fatima et al., 2015) who observed that extract yields decrease drastically due to different availability ofextractable components from varied chemical compositionof plant metabolites as polarity of extractionsolvent changes from highlypolar water tonon-polar n-hexane.

Dhanani et al., (2017) supported our finding and reported that extract yield was obtained 3.74 times lower than in ethanol (3.17%,) compared to (11.85%) in water. Moreover, Bimakr et al., (2011) also reported that the extraction yield is increased with temperature range. 267.3 mg/g extraction yield was observed in methanolic extraction while 257.6 mg/g in 70% ethanol extraction. In our finding, decreased order of crude yield in leaf extract of A. indica was aqueous, ethanolic, methanolic, and hexane.

The currentstudy evaluated application of ethanolic, methanolic and aqueous extracts of leaves & stems of A. indica, A. vasic,. A. sessilis, capsicum hirsutus, M. parvifolia and T. bellirica as an antifungal against capsicum capsici. Ethanolic leaf & stem extract of A. indica, A.vasica and A. sessilis showed good inhibitory effect than capsicum hirsutus, M. parvifolia and T. bellirica. These resultsare in agreement with findings of Sakthi et al., (2011) who observed that ethanolic leaf extracts strongly inhibited the colony growth of Candida albicans, capsicum glabrata, and Aspergillus flavus than ethyl acetate leaf extract. Rony et al., (2021)

whoreported that 20% extract concentration of A. indica inhibit the radial growth 100% and 79.16% in Colletotrichum dematium and Colletotrichum gloeosporioides. Akarsh et al., (2016) found thatmethanolic leaf extract of A. vasica inhibited 50% radial growth in capsicum capsici and F. oxysporum f.sp. zingiberi. However, ethanolic extract of A. vasica was not found to shown any antifungal activity against Sclerotium rolfsii (Bapat et al., 2016). Methanolic leaf and stem extract of all the studied plants were not reported in significant growth inhibition of fungus. Radwan et al., (2014) supported our study which stated that isolated compounds from methanolic extractof Myristica fragrans fruit were also not expressed significant growth inhibition of Colletotrichum sp.

Giwa (2010) reported that the ethanolic leaf extract of P. paniculata showed radial growth inhibition of Aspergillus niger, A. clavatus and Rhizopus stolonifer at mg/ml concentration. In addition to this, presenceof steroids, alkaloids, phenols, flavonoids, saponins & tannins was reported in ethanolic extract of P. paniculta with the inhibition of Escherichia coli,Bacillus cereus & Staphylococcus aureus (Jarakiraman, 2012). Moreover, leafextract of P. paniculata wasreported to reduced 90% radial growth of capsicum capsici than control (Akarsh, 2016).

Ishnava et al. (2012)who studied that the effect of aqueousextract of A. vasica, which showed inhibition againstAlternaria sp., Aspergillus parasi,Aspergillus nidulans, Trichodermaharzianum & Aspergillusflavus. Anarse, (2019) also reported thataqueous extract of A. indica & A. vasica inhibited growthof Fusarium sp. Kumari (2016) supported our resultthat aqueous leaf extract of A. sessilis is unable to inhibit the growth of fungus. Devi et al., (2013) reportedthat aqueousextract of A. indica and A. sessilis leaf extract inhibit the growth of sunflower leaf blight causing pathogen Alternaria helianthin but in our finding, it was not observed similar against the capsicum capsici. Very few literatures have been reported on T. bellirica against phytopathogen. Hexane leaf extract of T. bellirica showed good inhibitory effect against bacteria than fungi (Chanda, 2013).

Shukla et al., (2012) supports the present finding that methanolic aqueous extract of T. belliricasignificantly inhibitedgrowth of fungus. Moreover, Rastogi et al., (2018) reported high concentration of ellagic acid in ethanolic leaf extract of T. bellirica followed by fruit and stem. But flavonoid content was reported in higher concentration in methanolic leaf and stem extract than the phenolic compound.

Chandel et al., (2019) observed the high flavonoid in T. bellirica leaf extract over the phenolic content. Which supports our result that ethanolic extract of T. bellirica expressed antifungal activity might because of ellagic acid and methanolic due to flavonoid compound activity.

The result of capsicum hirsutus antifungal activity is supported with the finding of Meena et al., (2015) who evaluated that methanolic leaf and stem extractof capsicum hirsutus have highest antimicrobial activity. Wayal & Gurav (2019) reported that carbohydrates, steroids,cardiac glycosides, phenolics like tannins & flavonoids are present in methanolic extract of capsicum hirsutus. While only phenolic compounds & carbohydratesare found in aqueous extract. Moreover, Meena et al., (2015) reported that methanol is good solventsystem for extraction of total phenolic compounds. Therefore, methanolic leaf extract possesses high flavonoid, glycosides and lignins that might be playing a role in antimicrobial activity (Kumidini and Ranganyakulu, 2018).

According to literature, methanolic leaf extract of M. parvifolia was recorded against human pathogenicbacteria such asEscherichia coli, Pseudomonasaeruginosa & Bacillussubtilis but no result has been reported of ethanolic, methanolic and aqueous leaf and stem extract against the plant pathogens. Panda et al., (2016) studied antibacterial activity & found that methanolic leaf extract inhibit the bacterial growth of B. cereus, S. aureus, S. flexneri and V. cholera but aqueous extract expresses the antibacterial activity against only V. cholera. Padmavathi (2021) have reported least activity of aqueous and great activity of ethanolic leaf extract M. parvifolia against the Candida albicans, Microsporum gypseum, and Aspergillus niger Vasmatkar et al., (2014) studied phytochemicals of M. parvifolia and observed the alkaloids are the prominent constituents of methanolic extract. In additional to this Badgujar and Surana (2010) also reported methanol and ethanol extract are rich in alkaloids. M. parvifolia might be less effective due to rich alkaloid against capsicum capsici constituents.

Devi (2013) reported that aqueous extract of A. sessilis inhibited the mycelium growth and sporegermination of Alternaria helianthi. But in our study, aqueous extract against capsicum capsici was not inhibited growth. Oon et al., (2021) studied antifungal activity ofhexane, chloroform, ethyl acetate, ethanol, methanol, distilled water of A. sessilis against the yeasts & 2 species of filamentous fungi. Leaf extract in hexane, chloroform, and ethyl acetate

have good antifungal activity to the all fungus except Aspergillusfumigatus than ethanol, methanol, & water extract of the plants had stronger. Moreover, Kumar (2014) investigated ethanol extract of A. sessilis against gram-positive, gram-negative bacteria and fungi. In addition to this, Sivakumar and Sunmathi (2016) was also found that ethanol extract of A. sessilis inhibit growth of bacteria & fungus. Similar to our finding, ethanolic extract of A. sessilis significantly inhibited the Penicillium notatum, Aspergillus niger, Candida albicans. Ethanol leaf extract of A. sessilis has higher phenolic compounds such as ferulic acid, rutin, quercetin and apigenin (Hazli, 2018). Pernin (2019) reported that ferulic acid inhibits Listeria monocytogenes. Moreover, Apigenin causes cell shrinkage and changes the cell membrane potential via membrane dysfunction and increases cell permeability. Thus, apigenin induces inhibition of fungus growth (Lee, 2018). Nair (2018) evaluated that pomegranate peel extract hasrutin which inhibits th Colletotrichum gloeosporioides.

Very few literatures are provided on the relationship between appressoria formation of capsicum capsici and plant extract. However, less research work on Colletotrichum sp., and it's appressorium formation have been observed. In present study, 90.38 ± 1.22% conidia germination inhibition was observed in ethanolic leaf extract of A. vasica treatment. Bussaman et al., (2012) alsoreported similar result that methanolic leaf extractof Piper sarmentosum inhibit 100% conidia germination in vitro in capsicum gloeosporioides. This is in accordance with findings of Rahman et al., (2011) who reportedthat ethanolic leaf extract of Azadiracta indica, Ocimum sanctum and Curcuma longa inhibit the conidia germination in capsicum capsici. Moreover, methanolic extracts of Zingiber officinale and Polyalthia longifolia were found to show good inhibitory effect on the spore germination in capsicum musae. Rex et al., (2019) also have reported aqueous extract of Zingiber officinale, Allium cepa, Murrya koenigii and Azadiracta indica inhibited the 22.45, 30.61, 38.77 and 46.93% conidia germination in Alternaria solani. Saini et al., (2021) reported that 30% aqueous extracts of Cinnamomumzeylanicum, Alliumsativum, Syzygium aromaticum & Phyllanthus emblicacompletely inhibit conidial germination in capsicum karstii. But S. aromaticum was found to be shrinking the cell components of fungus. Addition to this, the interaction of ulvan (algal polysaccharide) and Colletotrichum gloeosporioides was studied and observed that ulvan inhibit the appressorium formation without interfering conidial germination and stimulating germ tube formation (Araujo, 2014). Bhutia et al. (2016) found that 0.3% Zingiber officinale rhizome and 0.5% Polyanthia longifolia leaf extract inhibited Colletotrichum musae conidia formation by 68%.

In our study, delay in the appressorium formation with germ tube was observed in methanolic leaf extract of A. sessilis than control while complete inhibition was found in ethanolic leaf extract. There is also strong evidence that plant extract completely inhibits the appressoria formation (Alvindia and Mangoba, 2020). Singh et al., (2016) observed that conidia germination inhibits due to the incorporation of phytochemicals into the cell membrane which make the unstable cell membrane of the cell. In addition to this, Bordohet al., (2020) reported that methanolic extract of ginger (10.0 g L^{-1}) inhibited 88.46 % conidia germination in capsicum gloeosporioides. Moreover, ginger crude extract delayed germ tube formation of conidia into hyphae as well as distorted and swollen it. Boyette and Hoagland (2013) found that refine corn oil was not effective against Colletotrichum truncatum. However, Ethanol extract of Primula root and Hedera helix were observed to highly effected against conidia germination and appressoria formation of Phyllostica ampelicida. Moreover, H. helix & Primula root was possessed a high amount of saponin that was main factor of black rotcontrol (Koch, 2013).

Buyu, (2018) studied that mitogen activated protein kinase kinase (MPAKK) STE11 family gene is found to regulate the formation of appressorium. In addition to this, Takano et al. (2001) studied conidia treated with benomyl and observed that cell cycle regulates the appressorium formation in Colletotrichum sp. and found that cell polarity of conidia determinants with interaction of microtubules. MPAKK interferes appressoria development and also effects conidia germination. In our study, methanolic extract of A. vasica leaf and stem treatment expressed similar result. In addition to this, PMK1gene is responsible for formation of appressoria and infectious hyphae growth. But mutant PMK1 gene failed to show appressoria formation with swollen bodies of conidia (Xu and Hamer, 1996). Present findings also support that conidia germinate and form only germ tube might be mutated PMK1gene due to phytochemicals of plant extracts.

Organic substances are subject to deterioration due to oxidation, heat, or UV irradiation. An essential aspect of this research was determining the stability of the chemical while kept as a plant extract under varied conditions (Han et al., 2004). Oyourou et al. (2013) heated a methanol extract from the leaves of Lippia. javanica at 56 °C and found that antifungal activity of extract remains only 13.7% to reduce from 86.6%. Our present study displayed that, ethanolic and methanolic plants extracts efficacy are not much significantly affected on heating

at 50 °C. Rex et al. (2019) also found similar result of antifungal activity of plant extract on heating 60 °C. Unstable antifungal activity was found by most of plant extract at 100 °C. But in some cases, increase in the antifungal activity was found in ethanolic extract of A. sessilis (stem), P. paniculata (leaf) and methanolic stem extracts of A. indica on heating at 100 °C. Thery et al., (2020) supports our result by observing the retained antifungal activity of broccoli napin against Fusarium culmoruma. Majumder et al., (1998) also reported that antifungal activity ofplant extracts are increased due to an increase in release of active compounds and free radical on heating. In addition to this Wang et al., (2005) isolated lysozyme from Phaseolus mungo seeds and studied the antifungal effect on Fusarium oxysporum,Fusarium solani,Pythium aphanidermatum,Sclerotium rolfsii, & Botrytis cinere. The antifungal activity of lysozyme was found stable below 60 °C. But lysozyme activity was rapidly loss when lysozyme heated above 80 °C. Ghosh (2006) reported that 70 % antifungal activity of extract decrease at 70 °C and also found that enzyme nature present in the extract is affected with temperature range. Enzyme activity remain stable from 5 to 40 °C but maximum antifungal activity is found at 36 °C.

The Trypsin digestion effected antifungalactivity of plant extracts.Antifungal activity ofethanolic and methanolic (leaf & stem) extract of A. indica, A.vasica and A. sessilis were found to reduce in trypsin digestion except the methanolic extract of A. vasica (leaf), P. paniculata (Leaf and stem). However, the antifungal capacity of an ethanolic leaf extract of M. parvifolia was shown to increase after trypsin digestion. This demonstrates that the active antifungal components comprised proteinaceous molecules and had high heat stability. Rizzello et al. (2017) also found that protease pretreatment increases the antifungal activity of the water-soluble extract by releasing oligopeptide sequences.

During postharvest storage, disease occurrence on fruit might occur due to the cellular membrane systems damage, which is responsible for damage of structural integrity ofcellular membrane & resulted in enzymaticbrowning as well asloss of resistance topathogen which acceleratеddisease development ofharvested fruit (Wang et al., 2018). Abiotic or biological elicitors are responsible for inducing th accumulation of defense-related enzymes in fruits to develop resistanceagainst the postharvest diseases. Pan et al., (2020), reported that Zingiber officinale and Clerodendrum tinfortunatum were found to reduce the anthracnose lesion diameter on chilli fruit when compared to untreated fruit (Choudhury et al.,2017). Moreover, Ali et al., (2014) reported thatextract treatment is more effective to reduce disease incidences

before the inoculation as compared to after the dipping in the extract treatment. In addition to this, propolis extract and cinnamon oil also reported to reduce disease incidence & disease severity in chilli caused by capsicum capsici.

A hollow, porouswall structure and anon-uniform shape is found in chilli surface whichmakes it prone to waterloss. Thereby water loss depends up on stomata shape during the transpiration process (Nair et al., 2018)., that Aloe vera coatings and Fagonia cretica gel was reported to play as a water barrier b/w fruit surface & environment during whole storagperiod (Khaliq et al., 2019). In addition to this Aloe vera coatings and Fagonia cretica gel was found to reduce physicochemical changes & slowing downweight loss which acts as a preservant and maintains the shelf life of fresh fruits. Similarly, Hassan and Fetouh (2019) have observed that Moringa leaf extract coating maintains the weight and quality of gladiolus cut flower via reduces the transpiration rate. Moreover, weight loss in plum fruit was reported on application of chitosan through reduce the respiration rate (Bal, 2013). Thus, the slow down the weight loss in chilli is indicated that extract treated fruits can be stored.

TA (titrable acidity) is indicated of acidity of fleshy fruit and determine the quality parameter. Fruit acids are used as a substrate and it reduce due to the rate increases respiration during the ripening stage. In present study, extract treated fruit increased TA compare to untreated fruit. A. sessilis leaf extract treatment expressed highest TA in post-inoculated fruit followedby A. vasica leaf extract compare to control at 25 °C. Similar finding also reported by Pobiega et al. (2020) whoobserved that TA was increased in blue berry fruit through pullulan coatingswith propolis extract treatment comparison to uncoated fruits and reduce degree offruitmaturity by promoted to decrease respiration rate.

The defense-relatedenzymes activity defines host resistance againstplant pathogens by its accumulation on physiologicalconditions & pathogen type of the plant species (Gholamnezhad, 2019). The function of defense enzymes is corrupt the fungal cell wall (Long et al., 2018). Gholamnezhad (2019) reported that PAL enzyme activity was increased in aqueous neem extract against Botrytis cinerea. PAL accumulate in the leaves increase level of salicylic acid (a signaling molecule) that contribute to disease resistance. In phenyl propanoid biosynthesis pathway, it is the firstenzyme that involve for plant defense network via synthesis of phytoalexins or phenols (Saxena et al.,2016). Edirisinghe etal., (2014) reported that chitosan increases accumulation ofPPO, POD & total phenolics infruits. Moreover, suggested that

quinines are obtain from PPO oxidization that restrict pathogenic growth & causing lignification in plant cells. POD responsible for the cell wall reinforcement process, such as suberisation and lignification of host plant cells, oxidation of phenols, producing structural barriers against the pathogen. Similar finding was also observed by Padilhaet al., (2019) who reported polyphenol oxidase (PPO) and POD activities increased in C.annuum that induced resistance mechanism against capsicum capsici. In addition, Hassan and Fetouh (2019) also supported that moringa leaf extract increased the POD & CAT accumulation in gladiolus cut spikes after harvesting. Moreover, under physiological conditions, SOD, CAT and POD are scavenge of relative oxygen species (ROS) and minimize oxidative damage. SOD plays important role to catalyze free radical $O_2 -$ into H_2O_2 and it decomposes H2O2 into H2O and O2 by the CAT or POD to protect cellular damage in the plants (Deng et al., 2015). Hayat et al., (2018) also found an increase amount of enzyme in extract treated chilli that indicated the plants have an excess of H2O2 burst. In this situation, plant establish a defense response signaling that alert the plants in stress conditions. Long et al., (2018) found that phenolic compounds are accumulated in high level and diminished or reduced the development of pathogens at site of the pathogen invasion (Maqbool et al., 2013).

Phenolic and flavonoid disrupts permeability barrier ofmembrane structure & play an important rolein antifungal activity (Singburaudom, 2015). Da et al., (2019) have reported that phenolic impairs the biosynthesis of ergosterin, disrupting membrane integrity, cell damage, induction of apoptotic DNA fragmentation, inappropriate ROS regulation hydrocarbons. Oxygenated components of terpenes can penetrate the fungi cell membrane and blocking synthesis of cell wall, cytomembrane, cytoplasm & organelles (Kong et al. 2019). Mohamed et al., (2017) studiedantifungal activity of Horwood dicksoniae, Citrullus colocynthis, Gypsophila capillaris, Pulicaria incisa and Rhanterium epapposum extract against Fusarium oxysporum. Pulicaria incise was found to have antifungal activity at 0.0092 g L-1 IC50 concentration while capsicum colocynthis was found to be less effective in inhibiting the growth. Moreover, phenolic compounds were reported in both plant extracts in higher concentration than flavonoid compounds. Isolated phenolic compounds were observed similar but flavonoid (Quercetin and Rutin) was only reported in P. incisa. Addition to this, Sati et al., (2019) who reported that phenolic compound, ferulic acid, phydroxybezoic acid and caffeic acid werefound tobe active against F. oxysporum than Quercetin. Moreover, P. incise causes plasmalemma distortation, autophagosome formation in cell and structural disorganization in cytoplasma.

Wang et al., (2019) have reported that crude extract of Gingobioloba has kaempferol in higher concentration than quercetin but quercetin and rutin inhibited fungus effectively than quercetin, kaempferol and isorhamnetin. This result indicated that compound activity is expressed by action mechanism not concentration. Moreover, similarity in structure of carvacrol and thymol are found very closely of phenolic difference of OH group is found in position at benzene ring. But antifungal property of compound was reported different. Thymol expressed higher antifungal activity than carvacrol. Furthermore, Shi et al. (2019) modified the β-pinene with amide moieties and acylthiourea moieties. Ethyl group on metha position was changed in pinene that improve antifungal activity againstColletotrichum gloeosporioides, Fusariumproliferatum, Alternaria kikuchiana & Phomopsis sp. Similar finding was found in Brachylaena elliptica and Brachylaenailici folia that contained 11.5 ± 5.05 and 8.86 ± 2.25 g/Kg flavonoid content, but similar antimicrobial activity was recorded in both plants extract (Sagbo et al., 2017).

Resveratrol is a form of stilbene phytoalexin generated by biotic elicitation. According to Flamini et al. (2018) and Nasir et al. (2021), resveratrol is a significant metabolite in Curculigo latifolia rhizome and leaf extracts. Furthermore, Curculigo latifolia leaf extracts have been shown to inhibit the development of Staphylococcus aureus and Salmonella choleraesuis. Two types of stilbene plant-based antioxidants can be induced by biotic elicitation: inducible viniferins and a substance called oligomers are produced as part of a plant's active defense system, and metabolized viniferins were generated by the action of enzymes released by pathogens in an effort to eliminate toxic compounds. According to Mayo-Prieto et al. (2019), Trichoderma spp. induces the expression of genes for secondary metabolites in Phaseolus vulgaris L. in response to the plant's defense activities against fungus. Moreover, 36 compounds were found different in Trichoderma treated plants in comparison to control plants. Caffeoylquinic acids also reported in A. sessilis leaf extract. Caffeoylquinic acidsis a polyphenol compound that formedby estherication & condensation ofquinic acid & a multimoleculecaffeic acid (Wang et al., 2009). It is widely existing in plant kingdom, especiallyin asteraceae, umbelliferae & caprifoliaceae. Ge et al., (2018) was found the presence of caffeoylquinicacids in flowerbuds extract of Lonicera japonicaThunb. Which showed antiviral activity against the hepatitis B virus. Luvangetin were found to be active against the phytopathogenic fungi Pyriculariaoryzae and Zanthoxylum avicennae (Xiong et al., 2019).

Santhakumari et al., (2018) also isolated phenolic compound 2,6-Di-tert-butyl-4-methylphenol from Chroococcus turgidus that have in-vivo antibiofilm potential against Vibrio.spp. A Polyketides (6,8a-Seco-6,8a-deoxy5-oxoavermectin "2a" aglycone) was also reported in our A. sessilis leaf extract. Risdian et al., (2019) has observed that Polyketides are found plants, have antibacterial,antifungal, anticancer, antiviral,immune-suppressing,anti-cholesterol, & anti-inflammatoryactivities. Thodi et al., (2021) observed rutaretin1' -(6''-sinapoylglucoside) in the leaf extract of Pittosporum dasycaulon that found potential inhibitor of COVID-19 3CLpro virus.

The phytocompound was analysed after TLC, column chromatography, FT-IR and NMR. The FT-IR results showed that carboxyl group is main functional group of the components. 2-D, H1 and C13 NMR results showed the partial characterization of steroid group present in compound. Salvador et al., (2004 & 2009) & Sundar et al., (2019) reportedthat Alternanthera maritima, Alternanthera tenella and Alternanthera sessilis are partial characterization of steroid group though FTIR and NMR, respectively.

CHAPTER 6
CONCLUSIONS

Chilli is a ubiquitous spice, which is cultivated in every state of India, and quality of chilli varies fromstate to state. Global consumption of chilli is approximately 6.2 million tons which makes about 90 percent of the total production of India. Presently, India is one of core suppliers of red chilli in international market (25%) followed by China (24%) and has become the world's largestproducer and exporter ofchili to USA, Canada, UK, Vietnam, Germany, East, and South Asia, and many other countries around the world. Chilli has been accepted as the prime constituent of various cuisines in tropical and subtropical countries.

Chillicrop is susceptible to different pests & pathogens during pre- & postharvest; mycotoxins being prime hindrances in chilli cultivation. Worldwide, capsicumis susceptible to various pests, weeds, fungal, bacterial, & viralpathogens; and amongst fungal disease anthracnose, dieback, and fruit-rot of chilies are prime causes of major loss during production, transport, and storage. The species ofgenus Colletotrichum belonging toascomycetes group causes anthracnose disease affecting economically with reduction in yield by 50% of chilli production.

Disease management through crudeextracts of medicinalplants has been reviewed in the past few yearsfor their efficient antifungal & antimicrobial properties. plants are considered environment friendly, safe, and clean alternative bioagents for control of fungi & mycotoxins in agricultural production. Essential oils, spices, herbs, & crude extracts of plants are promising source of bio fungicides to prevent mycotoxicosis and related fungal infections.

The current studyaimed to decipher application ofplant extracts of seven medicinalplants Mitragyna parvifolia, Cocculus hirsutus, Alternanthera sessilis, Peristrophe paniculata, Acalypha indica, Adhatoda vasica, and Terminalia bellirica and assess their biological activity and phyto-chemical constituents in the prevention of fungal infection of Colletotrichum capcici without anyadverse effect on nutritional valueof chilli.

- Capsicum capsici was collected from infected chilli pods for research purposes from the local market of Gwalior, Madhya Pradesh. The identification of the fungus was confirmed by National Fungal Culture Collection of India, ARI, Pune, Maharashtra.

- Leaves and stems of A. indica, A. vasica,A. sessilis, capsicum hirsutus, M. parvifolia, P. paniculata and T. bellirica were collected from Jiwaji University, Gwalior.
- Plants extract were prepared in 70% methanol, 70 percent ethanol, hexane, and distilledwater in soxhlet apparatus. The obtained extract was concentrated in rotaevaporator & dried in lyophilizer at -55 °C temperature and 1.0 torr pressure (BioEra-55°C model clout) for 2 hours. To determine the yield percentage of crude extract wasweighed after drying & stored in sterilized vials at 4 °C for further use.
- The heatstability test was determinedby heating the extractsat 50 and 100°C for 5 min.Extracts were treatedwith trypsin to proteolyze the extracts.
- After the treatment, food poison techniquewas used to evaluate antifungal activity of the methanol, ethanol, hexane, and aqueous extracts of selected medicinal plant leaves and stems. The volume of 500 μL of five different concentrations (1–5 mg/ml) of plant extracts dissolvedin 0.5% DMSO was taken. Radial growth was measured from the center.
- A modifiedmethod by DeCorato et al.,(2017) wasused to observe conidial germination.
- The time-kill assaywas used to estimate effect of extract on fungal colony growth within the time period.
- The crude powder of sample wassubjected to silicagel column chromatography. The collected eluted were spotted on the dried TLC plate.
- In-vivo screening ofextracts for antifungal activity wascarried out using the similar ripping stage of chilies at 25°C and 4°C.
- After the in-vivo antifungal evaluation of chilli fruits, fresh weight loss (FWL, %), diameter ofanthracnose lesion (DL), number olesions (NL), titratable acidity (TA), and pH were analyzed.
- Plant defense enzyme assays likephenylalanine ammonia-lyase(PAL), peroxidase (POD),polyphenol oxidase (PPO), superoxidedismutase (SOD), and catalase (CAT) were carried out on chilli treated with extract, fungicide treated and non-treated control chilli.
- For bio-autography, TLC plates with extract spots weresprayed with a concentrated suspensioncontaining 1.0 × 106 cells/mL ofactively growing conidiaand observed at 530 nm.
- The phytochemistry of crude extract ofA. sessilis and M. parvifolia were analysed by HR-LCMS using Agilent system (6550A Funnel Q-TOF).

- NMR was performed to identified the isolated phytochemical.
- The highest moisture content was recorded in A. indica leaf and stem while lowest moisture content was observed in A. vasica leaf.
- Percentage yield of selected plant extracts in aqueous, ethanol, methanol and hexane showed variation due to presence of diverse chemical compounds.
- The ethanolic leaf and stem extractof A. indica, A. vasica and A. sessilis expressed highest antifungal activity in in-vitro study against the capsicum capsici followed by methanol, aqueous and hexane extract.
- The ethanolic leaves extract of A. indica, A. vasica, A. sessilis & P. peniculata, showed minimuminhibitory concentration(MIC) at 5 mg/ml whereas the ethanol stem extractof A. indica expressed at 2 mg/ml concentration against capsicum capsici.
- Highest conidialgermination percentage inhibitionwas reported atMIC of the ethanolic leafextract of A. vasica and A. indica followed by A. sessilis.
- Appressoria was not formed from conidia in the ethanol leaf and stem extract of A. vasica but minimum number of germ tube was formed to germinate the conidia. While bulb-like structure was found in methanolic leaf extractof A. vasica.
- The extracts of A.indica and A.vasica showed reduced while Asessilis ethanolic (leaf) & methanolic (leaf and stem) extracts and P. peniculata leaf extract showed increased antifungal activity at both 50 °C and 100 °C.
- The antifungal activity of ethanolic & methanolic (leaf & stem) extract of A. indica, A. sessilis, A. vasica, P. peniculata and T. bellirica were more effective with trypsin treatment and reduced the growth activity of capsicum capsici than nontrypsin treated extract. On the contrary, ethanolic leaf extractof M. parvifolia were found to increase growth of capsicum capsici compare to non-trypsin treated extract.
- For the 1X MIC (5 mg/ml) concentration of A. indica, A.vasica and A. sessilis leaf & stem extracts, CFU value tended to stable for 6 h treatment with extract. But in 2X MIC and 4X MIC, CFU value continuously decline by the end of experiment (48 h) in A. indica, A. vasica (leaf) and A. indica, A. vasica (stem) and A. sessilis extract treatment.
- On the basis of conidia germination inhibition ethanolic extract of four plants were selected for the in-vivo antifungal study in chilli. Based on MIC, A. sessilis, A. indica, A. vasica and P. peniculata were selected to study decay inhibition, disease severity, disease incidence and defense enzymes.

- Before the inoculation of chilli at 25 °C, maximum percentage of decay inhibition was reported in leafextract of A. sessilis at 4 °C & 25 °C respectively, while ethanolic stem extract of A. vasica was reported less effective in decay inhibition at 25 °C .
- A significant difference was reported in percentage disease incidence at 25 °Cand 4 °C. Before application of the extract, minimum disease incidence of ethanolic leaf extract of A. sessilis on chili was reported at 25 °C and 4 °C respectively.
- Lowest number of wound was reported in A. sessilis leaf extract treatment before inoculation of chilli compare to control at 25°C and 4°C.
- Highly acidic pH was found in A. sessilis leafextract treatment before the inoculation at25 °C and 4 °C while lower acidic pH was found in control chilli at 25 °C and 4 °C. Increased trend of basicity of pH was found in A. sessilis, A. vasica, P. paniculata & A. indica at both temperatures.
- Maximum titratable acidity was found in A. sessilis leafextract treated chilli before the inoculation at25 and 4°C. Increased trend of titratable acidity was observedin leaf extract treatment of A.indica, P. peniculata and A. vasica before the inoculation at 25 °C, while P. peniculata, A. vasica and A. indica at 4 °C.
- Maximum PPO was reported in ethanolic leave extract of A. sessilisbefore the inoculation at 25 & 4 °C. Stem extract was less effective to induce production of PPO in chilli at 4 °C compare to 25 °C.Maximum PPO production in chilli was found in an A. sessilis stem extract before the inoculation.
- Maximum peroxidase production in chilli was observed in ethanolic leaf extract ofA. sessilis treatment before the inoculation at 25 °Cand 4 °C.
- The maximum catalase was observed in ethanolic leaf extract ofA. sessilis before the C. capsici inoculation at 25 °C & 4 °C. An increased trend of activation of CAT production in chilli by ethanolic leaf extract of the P. peniculata, A. indica, A.vasica and A. sessilis before the inoculation at 25 and 4 °C.
- Theleaf extract of A.sessilis was found effective to induce production of phenolic compound in chilli before the inoculation compare to negative and positive control in chilli at 25 °C and 4 °C.
- Extract treatments were found effective in production of SOD in A. sessilis at both temperatures. In before leaf extract treatment, highest SOD was found in A. sessilis at 25 °C and 4 °C. The decrease trend of SOD production was reported in A. sessilis, A.

vasica,P. peniculata & A. indica at 4 °C. While A. vasica, A. sessilis, A. indica and P. peniculata at 25 °C.

- Maximum PAL enzyme production was observed in A. sessilis ethanolic leaf extract treatmentbefore the inoculation of chilli compare to negativecontrol at 25 °C and 4 °C.
- The ethanol leafextract of A. sessilis was eluted using different ratio of solvents, hexane: chloroform: ethyl acetate: methanol: ethanol in silica gel column. Twenty-one major fractions were obtained from the ethanol leaf extract of A. sessilis. Rf value of antifungal compound was 0.51, 0.64, 0.80, in CHCl3: EtoAc(30:70), 0.84 in 100 % EtoAc, 0.67, 0.70 in EtoAc: MeoH (30:70), 0.76 in Hex: CHCl3 (70:30) and 0.83 in MeOH:EtOH (50:50). Percentage of radial growth inhibition in capsicum capsici by the fraction of these Rf values were recorded via food poison technique.CHCl3: EtoAc (70:30) showed complete radial growth inhibition of capsicum capsici.
- Seven phytochemicals viz. Resveratrol, Caffeoylquinic, 2,6-Di-tert-butyl-4-methylphenol, 6,8a-Seco-6,8a-deoxy five-oxoavermectin "2a" aglycone, Luvangetin, manumycin, rutaretin1’ -6’’-sinapoylglucoside were found different in A. sessilis than in M. parvifolia by HR-LCMS technique.
- This study was mainly focused on antifungal activity ofselected seven plant species viz. A. indica, A. vasica, A. sessilis, C.hirsutus, M. parvifolia, P. paniculata, and T. bellirica. The present study is concluded as follows:
- Our investigations on the phytochemicalanalysis of selected plant extracts have revealed presence of organic compounds & their other constituents. These compounds are valuable sources of biologically active molecules including antifungal compounds. These compounds are found to beeffective against capsicum capsici. Hence, plant extracts can be used for controlling the pre- & postharvest pathogens of different horticulture crops.
- Inthis study, aqueous, ethanolic, and methanolic and hexane solvent were selected for the plant extractions. Ethanolic extracts were found tobe more effectiveagainst the capsicum capsici than aqueous, hexane and methanol extract.
- The ethanol extracts of A.indica, A. vasica and A.sessilis werefound effective in reducing growth of capsicum capsici than methanol, aqueous & hexane extracts.
- The ethanol & methanol extract (Leaves and stem) were reported to be heat sensitive & heating affected the antifungalactivity of extracts.

- The antifungal capacity of plant extracts was altered by trypsin digestion. This demonstrates that the active antifungal components comprised proteinaceous molecules and had high heat stability.
- In-vivo & in-vitro studies on efficiency of crude plantextracts, fractions and purified secondary metabolites were found to show significant growth inhibition against capsicum capsici.
- In an in-vivo trial, A. sessilis leaf extract reduced the spread of anthracnose in chili the most of any extract tested.
- A. sessilis decreased disease incidence and severity while improving decay inhibition in chilli fruits. Furthermore, A. sessilis leaf extract increased the shelf life of chilli fruit by up to 30 days at 4 °C without compromising food quality.
- Antifungalactivity of A. sessilis was found moreeffective in before inoculation of chilli than after inoculation at 25 °C and 4 °C.
- A. sessilis leaf extract boosted the defensive enzymes (PPO, POD, CAT, PAL, and SOD) in chilli. So, our study concluded that defense-related enzymes are the key protection systems, and that plant extract-induced defensive mechanisms will assist small producers in storing chilli fruits for an extended period of time without deterioration. To meet the consumer's need for agricultural goods free of hazardous toxic chemicals, farmers can employ natural products that are both environmentally and consumer-friendly. However, additional research is needed to discover the key bio-compounds in A. sessilis extract that are important for disease management.

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