ETIOLOGY AND MANAGEMENT OF LEAF SPOT DISEASE IN *MAGNOLIA CHAMPACA* L.

A thesis submitted to

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In partial fulfillment of the requirements for the award of the

Degree of

Master of Science in Botany

Submitted by

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This is to certify that this thesis entitled "**Etiology and Management of Leaf Spot Disease in *Magnolia champaca* L**" submitted to the Assam Science & Technology University, Guwahati, for the award of the degree of **Master of Science in Botany** is a bonafide research work carried out by **Dinku Mahanta, Roll no: 202820047005,** under my guidance and supervision during the period between April 2022 to August 2022 in the **Division of Forest Protection, Rain Forest Research Institute, Jorhat.** I further certify that no part of this thesis has been submitted anywhere else for the award of any Degree, Diploma, Associateship, Fellowship or other similar titles.

Date: 23-03-2022
Place: Silapathar

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(पर्यावरण, वन एवं जलवायुपरिवर्तनमंत्रालय ,भारत सरकार के अधीन एक स्वायत्त परिषद्)
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DECLARATION

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I hereby declare that the work embodied in this thesis entitled **"Etiology and Management of Leaf Spot Disease in *Magnolia champaca* L"** is a research work done by me under the supervision and guidance of **Dr. Jitu Gogoi**, Assistant professor of Botany, Silapathar Science College, Silapathar. I further declare that this work has not been submitted earlier in full or in parts to any other university for the award of any Degree, Diploma, Associateship, Fellowship or any other similar titles.

Date: 23.09.2022

Place: Dhemaji

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Dinku Mahanta

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Dinku Mahanta.

DINKU MAHANTA

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ABRIBIATION

%: Percent

A.I: Active ingredient

BOD: Biological oxygen demand

°C: Degree centigrade

C.D: Critical difference

HEPA: High efficiency particulate absorbing

Hrs: Hour

i.e.: That is

ITS: Internal transcribed spacer

IARI: Indian Agricultural Research Institute

PDA: Potato Dextrose Agar

PDB: Potato Dextrose Broth

ppm: parts per million

R: Replication

RFRI: Rain Forest Research Institute

S.E: Standard error

T: Treatment

WP: Wettable powder

ABSTRACT

Magnolia champaca L. is well-known for its high timber value and medicinal properties. Hence, in India and other places around the world *M. champaca* is being grown in a large scale. But, due to unscientific cultivation practices, new diseases are emerging in the nursery. Leaves of *M. champaca* infected with leaf spot disease were observed in the Silviculture nurseries of Rajavatkhawa, West Bengal. The Causal organism was found to be as *Pestalotiopsis algeriensis* (ID. NO11690.22). During pathogenicity test, similar disease symptoms were noticed on the inoculated leaves of *M. champaca* seedlings and re isolation from the infected leaves revealed *P. algeriensis.* Scanning of literature has revealed *P.algeriensis* as new host record and this is the first record from India.

For the control of the disease, the efficacy of the three fungicides namely Mancozeb 75% WP (Indofil M-45), Carbendazim 50% WP (Bavistin) and Captan 50% WP (Captaf) were evaluated against the pathogen at concentrations of 10, 25, 50 and 100 ppm. The effectiveness of Carbendazim 50% WP (Bavistin) was found to be higher than the Mancozeb 75% WP (Indofil M-45) and Captan 50% WP (Captaf). Hence, the present study revealed that, the use of Carbendazim 50%WP (Bavistin) at a minimal concentration of 25 ppm may be recommended for the management of the leaf spot disease of *M.champaca* caused by *Pestalotiopsis algeriensis* in nursery condition.

INTRODUCTION

CHAPTER I: INTRODUCTION

A plant is define as normal or healthy when its organs or parts are doing their normal function and the processes of growth, development and reproduction are carried out naturally in a balanced way. On the other hand, when any of the plant organs or parts is not doing their normal work and their growth and reproduction are not carried out naturally in a balanced way, then the plant is termed as disease. In nature, a set of environmental factors interrupt the optimum growth and development of a plant. Most plant diseases are caused by some physical, chemical, or biological factors in the environment.

Depending upon the nature of the causative agent, plant diseases are classified as infectious or non-infectious. When a disease is caused by biotic agents (pathogenic organisms), such as viruses, bacteria, fungi, nematodes, and parasitic flowering plants, they are called infectious diseases. These types of diseases can spread from an infected plant to a healthy plant. On the other hand, when a disease is caused by physical or chemical factors i.e. unfavorable environmental conditions, it is called a non-infectious disease as it is not spread from an infected plant to a healthy one. There are some important environmental factors which may affect the development of plant diseases, such as temperature, relative humidity, soil moisture, soil PH, soil type, and soil fertility.

1.1 Nursery diseases and its management:

When the plantlets are grown on nursery beds during their seedling stages, they suffered from various plant disease caused by various agents such as fungi, bacteria, phytoplasma and also due to the environmental factors. Among the diseases leaf spot, damping off, leaf and stem blight, root rot, powdery mildew, canker, wilting, crown gall, leaf blotch and powdery mildew are the common diseases. Among these various causative agents the fungus are greatly responsible as disease causing agent in plants. The symptoms that are developed due to the fungal infection are seen at the site of infection or at that site where the maximum growth of the pathogen takes place. When the infections are take place in the important tissue system of the plant, the symptoms can be seen in the remote places of the plant due to

the malfunctioning of particular tissue. Most commonly fungi cause small irregular to round chlorotic or necrotic lesions on the infected plant part. These areas increase in their size and coalesced to extend into a large area.

Fungal disease can consider as a serious problem in forest regeneration and sometimes fungi may cause heavy mortality in nurseries. The seedling of nurseries particularly susceptible to several diseases because of their tender tissues and often they have difficulty in their establishment. Since, the seedlings grown in the nurseries are use as planting stock, it is necessary to investigate the diseases and apply control measures. Among the nursery diseases leaf spot disease is the most common disease which can characterized by dead tissue of brown to black colour and surrounded by living tissue. The death of the tissue takes place at the site of infection. The death tissue is become separated from the surrounding healthy tissues. The size of the spots is depended on the susceptibility of the host and environmental conditions.

The management of these diseases are carried out to control the diseases in the nursery and to produce healthy seedlings without any infection. The pathogen can be avoided by selection of good geographical area, selection of sterilized green house or rooting medium, selection of proper time of sowing, disease escaping varieties, and selection of healthy plant material for propagation and by modifying the cultural practices. The disease can be also avoided by exclusion of inoculums. The principle of exclusion aims to prevent the entry of the pathogen in a nursery which can be achieved by adaptation of the seed, treatment of the seedlings, quarantine and eradication of vectors. Biological methods are also applied to control the diseases which aim in the eradication of pathogens and protection of plant surfaces through the activity of the other microorganisms. For example *Pseudomonas fluorescence* and *Paecilomyces lilacinus* are used as a combined formulation in seed and nursery bed treatment.

Fungicide plays an important role in the control of the disease through chemical treatment. The inoculums of many infectious diseases are bought by wind from neighbouring area and distant places. Hence the above mentioned methods are generally insufficient to prevent such diseases. Therefore seedlings of the nurseries have to be provided some protective cover to create a toxic barrier between host surface and propagules of the pathogen to protect from such pathogens. Chemicals

sprays of fungicides are recommended to form a protective toxic layer on the host surface so that when the pathogen comes in the contact with the surface it killed or prevent the growth of the pathogen. For example for the control of the blight and leaf spot caused by fungi imperfecti Mancozeb and Chlorothalonil or propiconazole sprayed soon after appearance of initial disease symptoms.

Magnolia champaca Linn. is an evergreen flowering plant that comes under the family *Magnoliaceae*. *Magnolia champaca* seedlings are grown on a large scale in nurseries due to their high timber value, medicinal properties, and economic importance. In seedling stages, due to an imbalance of some important environmental factors such as temperature, light, humidity, water, nutrition and the presence of infectious organisms, seedlings of *Mangnolia champaca* in nurseries have been suffering from foliar and other diseases. This may lead to the production of diseased plantlets. So there is a need for scientific study to control this disease at the nursery level to prevent the further spread of the disease in a new area.

1.2 Objectives:

The present study is carried out with following objectives:

- Collection of Diseased seedlings of *Magnolia champaca* L.
- Isolation of the Pathogen from infected leaf of *Magnolia champaca* L.
- To identify the causal organism.
- To carry out the Pathogenicity test.
- Management of the disease by using Poison Food Technique.

PLANT PROFILE

CHAPTER II: PLANT PROFILE

Magnolia champaca L**.** commonly known as – **English-**Champak, **Hindi-**Champaka, Champaca, Champe-ke-phul, Campa, **Bengali**-Swarnachampa, **Kannada**-Champaka, Kolasampige,**Malayalam**- Campakam, Cempakam, Champa, **Marathi-** Sonchampa, Sona champa, Chamfo, **Sanskrit**-Hempushpa, Hemapuhpika, Campaka, **Tamil-**Amariyam,Sambagam, Shampangi, (Raja *et al.,* 2014).

2.1 Description:

Magnolia champaca Linn is a species of flowering plant which is belongs to the family Magnoliaceae Kumar *et al*., (2011) and consists of 12 genera and 220 species of evergreen trees and shrubs Armiyanti *et al*., (2010). It was previously classified as *Michelia champaca. Michelia* is a historical genus of flowering plants belonging to the *Magnolia* family. Magnolia champaca tree is well known by the names "champaka", "Champak" or "Golden Champa" Savita *et al*., (2019) in Assam it is commonly known as " Tita-Sopa" and in West Bengal this plant is known by „Rani-Sopa". The species include in IUCN red list species as threatened in its Geographical distribution range reported by Negeeb and Sagheer (2021).

2.2 Distribution:

It is indigenous to tropical and subtropical South and Southeast Asian countries such as India, Sri Lanka, China, Indonesia, Malaysia, Myanmar, Nepal, Thailand and Vietnam Rajshree and Ranjana (2016). In the forest of North –Eastern Indian region *M. champaca Linn* grows naturally and also planted in a large scale.

2.3 Taxonomical position:-

Domain: Eukaryota

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Mangnoliophyta

Class: Magnoliopsida- Dicotyledons

Subclass: Magnoliidae

Superorder: Magnolianae

Order: Magnoliales

Family: Magnoliaceae

Genus*: Magnolia*

Species: *champaca L.*

2.4 Habit:

Magnolia champaca L is an evergreen or semi deciduous, and small to medium sized tree up to 50m tall, with straight long cylindrical stem up to 200 cm in diameter and smooth brown bark Savita *et al.*, (2019) showing in Fig: 2.1

2.5 Habitat:

Magnolia champaca Linn grows in areas where temperature is 0-47.5 ℃ and annual precipitation is 2250 to 5000 mm or more reported by Thaware et al., (2010). The plant requires a sunny position in a moist but well drained, deep, fertile, loamy to sandy soil, which prefers a pH in the range between 4.5–5.5, but can tolerate 4-6.

2.6 Morphological Characters of Plant:-

Magnolia champaca Linn is an evergreen or semi- deciduous tall tree with a crown conical to cylindrical that grows up to 30 meters or more showing in Fig: 2.1

ROOT: Tap root and branched.

STEM:

Stem (Fig: 2.1-C) is aerial, erect, branched, cylindrical, woody and solid, bark surface is smooth and gray to grayish white in color, and inner bark is fibrous, yellow to brown in color and about 2 cm thick (Sinha and Varma, 2016).

LEAF:

Leaves are simple, entire, alternate, stipulate, petiolate (petiole is 1.8 to 3.0 cm long), lanceolate, sometimes ovate, reticulate venation, finely acuminate, smooth, leaves are generally 13 to 25 cm long and 5 to 9 cm wide (Fig: 2.1-D) old leaves are yellow in color (Khanal *et al*., 2019).

FLOWER:

The flower is large, axillary, solitary or rarely in pairs, pale yellow when young and becoming orange in matures, each flower is about 2-45 cm long, fragrant, flowering period starts after the age of 4-5 years Oyen *et al*., (1999), tepals 6-21 which is arranged in 3-6 whorls where the outermost tepals are obovate and innermost tepals are narrower, polypetalous, necter is present, stames many, stamens is 0.6-0.8 cm long, gynoecium stipitate spirally arranged, with many ovules (Khanal *et al*., 2019) (Fig: 2.2 E-F).

FRUIT AND SEED:

Fruiting carpels dehiscing along the dorsal suture when free or fused and forming a fleshy or woody syncarp. Aggregate fruits consists of long cluster of 3-20 brown capsules, on a spike about 7.5-15 cm long. Seeds hang from its funicle. Each capsule contains 2-6 reddish seeds. Seeds are dark brown and angular covered with pink fleshy anillus (Zabala, 1990) (Fig: 2.2 G-H)

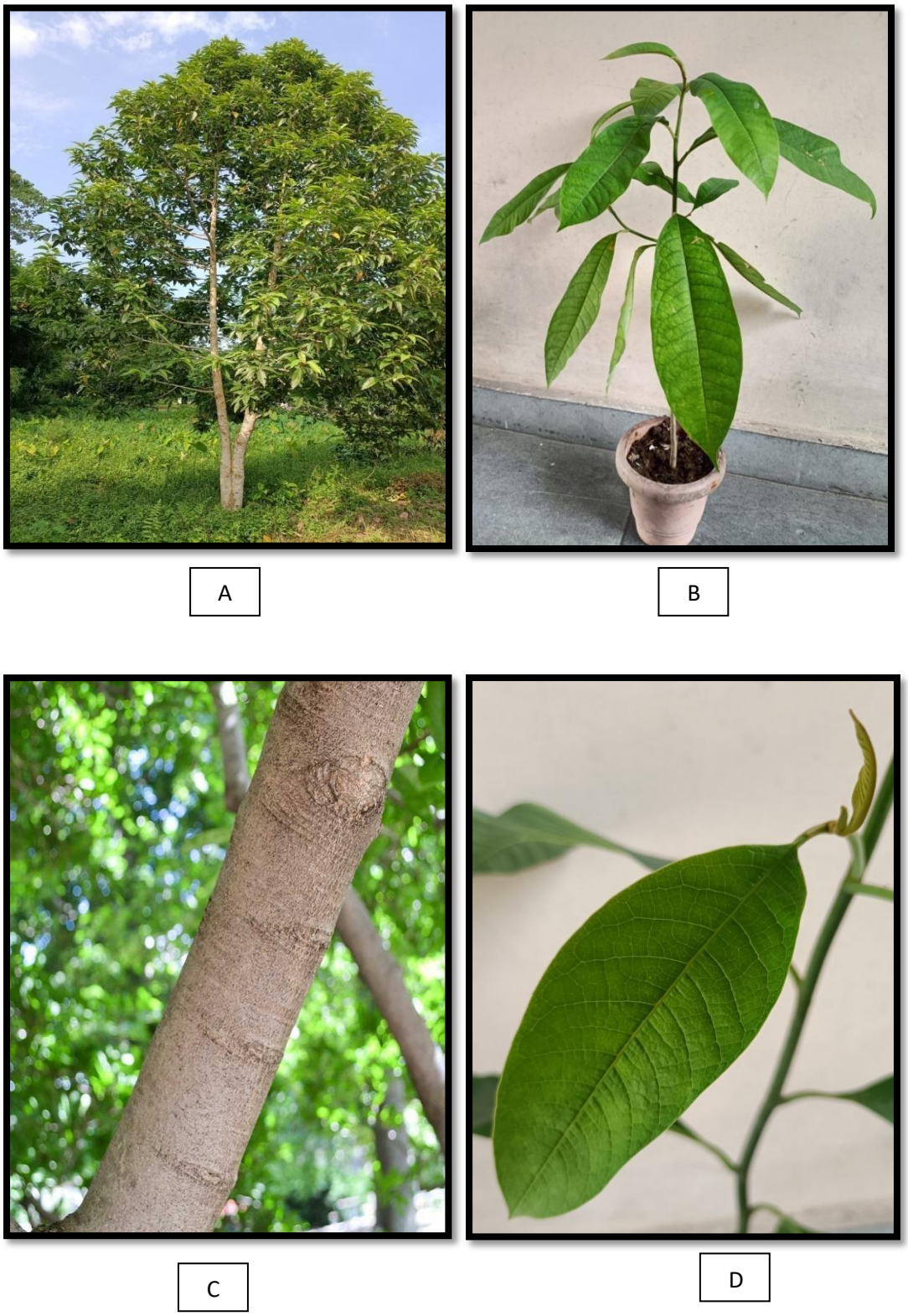

Fig: 2.1: *Magnolia champaca Linn,* A: Mature plant of *Magnolia champaca L.* B: a healthy seedling of *M. champaca L.* C: Stem D: Leaf of *M. champaca*

E

F

G

H

Fig: 2.2 Flower and fruits of *Magnolia champaca,* E&F: Flower, G: Bunch of fruits, H: Mature seeds coming out from fruits

2.7 Uses:

The tree is mostly grown as a timber tree and also well-known for its lovely, fragrant flowers as well as medicinal properties. In India, *Magnolia champaca Linn is* commonly cultivated in the nurseries, gardens, and near the temples as an ornamental plant, especially for its sweet-scented orange or white colored flowers, according to Ruwali *et al*., (2019). Primarily, the flowers are used for worship at temples. The flowers are traditionally used by women for hair decoration. Flowers are also floated in bowls of water to scent the rooms as a natural room freshener.

The tree is also used in reforestation project, and the tree has reclamation properties for soil due to presence of arbuscular mycorrhizae on the roots of the plant Das *et al.,* (2013). The *Magnolia champaca* provides Nitrogen fixation along with increases in soil pH, soil organic carbon and available phosphorous.

However this plant is mostly grown as a timber tree. The wood is soft; the color of the heartwood is light yellow-brown to olive brown. The heartwood of the plant is strong, long-lasting and able to take high polish Hossain *et al*. This is used in furniture making, construction, and cabinetry.

The plant also popular for its commercial importance, each and every part of the plant mostly the flowers has a number of medicinal, cosmetics and economic uses Armiyanti et al., (2010). From the flower of champaka an essential oil could be extracted and used in the production of perfumes and hair oil. Joy by Jean Patou is a one of the top selling perfumes in the world which is produced from the flowers of *M.champaca* reported by Mosquin (2011).

Magnolia champaca Linn is a widely used medicinal plant of the family Magnoliaceae. The plant is traditionally used for the treatment of fever, colic, leprosy, and cure for coughs and rheumatism and for remedies of various disorders Armiyanti *et al.,* (2010). The plant has been using as a medicine for improving immunity and resistance capacity against cold, joint pains, fever etc Ruwali *et al.*, (2019). It also have many pharmacological properties like anti-inflammatory, anticancer, antihelmintic, antipyretic, antihyperglycemic, analgesic, antiulcer, antimicrobial, antifertility , antioxidant, wound healing etc reported by (Raja *et al*., 2014).

REVIEW OF LITERATURE

CHAPTER-III: REVIEW OF LITERATURE

3.1 Different disease incidence reported from *Magnolia champaca L:-*

Mehrotra (1989) reported a new foliar disease in the nurseries of Dehra Dun .During humid months the diseases causing leaf spot and blight in Michelia champaca. The spots were large, irregular and increased in size to produce large infected areas which results premature defoliation in 70-100 percent infected seedlings. From the infected leaves the causal organism was identified as *Rhizoctoina solani*.

Mitra *et al*., (2014) they take *Michelia champaca* as a host and observed the interaction with the fungus *Phomopsis micheliae* and they notice that it is a very common fungal infection in *Magnolia champaca* which possesses some harmful effects on *Magnolia champaca* .The fungus on the infected leaves reduce the stomatal frequency up to 89.8 percent per mm^2 ,*Phomopsis michelia* reduce the number of both chlorophyll a and b mm^2 and also decrease the stomatal diameter up to 75percent as compared to uninfected leaves.

Zhang *et al.* (2018) observed necrotic spots on the mature and young leaves of *Michelia champaca* in Changzhou Country, Sichuan Province, China. Spots are circular to semicircular in shape, water soaked, and later become greyish white in the centre with a dark brown margin bordered by a tan holo. As the disease increased, the spots merged, which caused premature senescence of the leaves. The pathogen was identified as *Colletotrichum fioriniae.*

3.2 *Pestalotiopsis spp.* reported from different Hosts:-

Shino *et al.*, (1999) observed that the fungus *Pestslotiopsis theae* caused a gray blight disease on Tea plants (*Camellia sinensis)* in Korea. At the early stage the symptoms appeared as minute brown color spots, as the disease developed, the spots increased in size and become circular or oval in shape, measuring 10-15mm in diameter. The spots were grayish in the center with a light to dark brown color margin.

Hopkins & Mcquilken (2000) obtained isolates of *Pestalotiopsis* from the foliage, stem-base and roots of hardy ornamentals grown on commercial nurseries in UK. All the isolates (18) were identified as-*Pestalotiopsis sydowiana* based on the result of the pathgenicity test, colony and conidia morphology.

Starosta (2004) carry out an investigation on Ericaceae ornamental plant in the nurseries near to Poznan, Where the Erica spp. lose their ornamental value due to the presence of a pathogen, which causes serious damage on cuttings, stock plant and potted plants of Erica spp. Symptoms appeared as necrotic spot which cause death of foliage. After the isolation of the pathogen from the infected leaf samples the isolate was identified as *Peatalotiopsis sydowiana*.

Elliott (2005) reported that the fungus *Pestalotiopsis spp*. caused a leaf spot disease on the palm tree. The fungus mostly infects the leaf blade, petiole, and rachis. The symptoms appear as minute yellow, brown, or black spots. Under favorable conditions, the spots increase in size and coalesce to form leaf blight and rachis blight, and the spots become grayish with a black outline.

Keith *et al*., (2006) conducted a survey on guava (*Psidium guajaua)* at Waiakea Agricultural Experiment Station; Where the Guava plants are suffered by a scab disease. Disease symptoms appeared as small darkened colored spots at first, but as the disease developed, the spots increased in size and became distinct, circular, dark brown to black colored spots. The isolation of the pathogen was done from the infected leaves and fruits, and as a result, all the isolates were identified as *Pestalotiopsis spp.* on the basis of culture and conidial characteristics.

Rivera & Wright (2007) conducted a survey during September 1998 in azalea (*Rhododebdron spp.)* growing areas in Argentina. They observed brown colored spots on petals and from yellow blight, which causes abscission of petals and based on the morphological characteristics, the pathogen was identified as *Pestalotiopsis guepine.*

Karakaya (2007) observed a new foliar disease on the leaves of Kiwifruit (*Actinidia deliciosa)* Hayward plants in the Artvin-Arhavi region of northeastern Turkey. Symptoms were reported as leaf spot, after the isolation of the pathogen the fungus was identified as- *Pestalotiopsis sp.*

Espinoza *et al*., (2008) observed the presence of Canker and Dieback symptoms on blueberry (*Vaccinium spp)*. After the isolation of pathogens, the isolates were identified as- *Pestalotiopsis clavispora, Pestalotiopsis neglecta* and *Truncatella (Pestalotia) angustata* on the basis of colony characteristics and conidial morphology.

Luan *et al.,* (2008) observed a leaf spot disease on Blueberry (*Vaccinium corymbosum)* in a plant nursery of Dalian,China. From the morphological descriptions, conidial characteristics and sequence data support the identity of the causal organism as *Pestalotiopsis clavispora.*

Valencia *et al.,* (2011) reported a new disease of Avocado in chile. Symptoms appeared as small, irregular, brown colored lesions on the stem and fruit. After the isolation of the pathogen, the isolates were identified as- *Pestalotiopsis clavispora* and *Pestalotiopsis sp* on the basis of the morphological and conidia characteristics.

Gonzalez *et al*., (2012) revealed that *Pestalotiopsis clavispora* causing Dieback disease on Blueberry plants in Uruguary. In the infected plants, the symptoms were characterized by the death of twigs and characterized by the death of twigs and branches frequently in Blue berry plants.

Suwannarach *et al*., (2012) reported that *Pestalotiopsis virgatula* causing a new leaf blight disease on the plantation of *Eucalyptus camaldulensis* at Chiang Mai Province, Thailand in 2011. The symptoms in the initial stage appeared as brown colored, oval or irregular shaped lesions on the leaf margin or leaf tip. As the disease developed the neighbouring spots were coalesced to form the diseased leaf completely blighted and causing premature leaf fall.

Zhang *et al.,* (2012) reported that *Pestalotiopsis microspora* causing a nut black spot disease on *Chinese Hickory(Carya cathayensis)* in China.

Ren *et al*., (2013) observed a twig blight disease on bayberry (*Myrica rubra Sieb & Zucc*) in Zhejiang Province. They collected blighted twig samples from bayberry fields in Xianju, Ruian and Huangyan of Zhejiang Province. From the all isolates, 257(87.9%) fungal isolates were identified as *Pestalotiopsis spp.*

Suwannarach *et al*., (2013) for the first time in the world reported that *Peatalotiopsis theae* caused a leaf spot disease on oil palm (*Elaeis guineensis*) in

Chiang Mai Province Thailand The symptoms were appeared as dark brown to black color spots , surrounded by a yellow to rust brown margin. The spots were rapidly increased in size and the shape of the spots were changing from circular to elliptical lesions.

Ismail *et al*., (2013) revealed that *Pestalotiopsis spp.* causing gray spot disease on the leaves, twigs and panicles of Mango plants in Italy. From the infected plant parts the isolate was identified as *Pestalotiopsis uvicola* and *Pestalotiopsis clavispora* on the basis of the fungal colony and conidial characteristics.

Deng *et al*., (2013) observed a fruit rot disease on the "Cheongsoo" grape in Daejeon, Korea. Isolation of the pathogen was done from the diseased fruits of grape and the causal organism was identified as- *Pestalotipsis sp.*

Maharachchikumbura *et al.,* (2013) isolate a new fungus species from *Syzygium samarangense*, which causes rot disease on the fruits of *S.samarangense* in Thailand. On the basis of the molecular analysis and morphological characteristics, the causal organism is identified as *Pestalotiopsis samarangensis.*

Mahadevakumar and Janardhana (2014) conducted a survey on cowpea fields in Mysore district, Karnataka and reported a new foliar disease on cowpea (*Vigna unguiculata)* symptoms were appeared as leaf spot. The isolated fungus were identified as- *Pestalotiopsis sp.*

Feng *et al*., (2014) they observed leaf blotch symptoms on *Rosa chinensis* in the Tianjin flower Nursery. Symptoms appeared on the basal leaves. After the isolation of the causal organism from the infected leaf of the plant, the pathogen was identified as *Pestalotiopsis sp.* depending on the cultural and conidial characteristics of the fungus.

Sezer and Dolar (2015) carried out a survey on hazelnut growing areas in Ordu, Giresun, and Trabzon provinces in Turkey. The symptoms were appeared as blight. Isolation of the pathogen was done from the infected fruit clusters. Depending upon the cultural morphology, growth rate,conidial morphology and rDNA sequences the fungus was identified as *Pestalotiopsis sp.*

Verma and Verma (2015) isolated *Pestalotiopsis versicolor* as a causal organism from the Pre-harvest fruit rot of *Emblica officinalis* from Jabalpur, Madhya Pradesh, India. They also reported that the fungus is causing sot rot disease for the first time in Aonla.

Argawy (2015) reported that *Pestalotiopsis spp.* causing a scabby canker disease on the plants of Guava in four different regions of El-Behrira Governorate, Egypt. Total forty three *Pestalotiopsis spp.* were isolated from the infected leaves and fruits of guava. By comparing the morphological characters of fungal colony and conidial characters produced on potato dextrose agar medium five isolates were identified as- *Pestalotiopsis psidii, Pestalotiopsis microspora, Pestalotiopsis clavispora, Pestalotiopsis neglecta* and *Pestalotiopsis spp.* In vitro management of the isolates application of chistosan with 25% concentration showing the best result to reduce the growth of *Pestalotiopsis spp.* on 86.53% agar plates.

Dung *et al*., (2016) investigated the pathogen that cause crown rot disease in Strawberries. They collected 150 samples of infected plant parts from three different Strawberry cultivated regions in Dalat. And of all the all isolates, 327 isolates belongs to 6 different fungal species. After fulfilling the pathogenicity test the main fungus was identified as *Pestalotiopsis sp*.

Ara *et al*., (2017) conducted a study to identify the pathogen which was causing the Crown rot disease in Strawberry Plants. For the isolation of the pathogen, they collected 200 samples of infected parts from the 15 Strawberry growing areas in Rajshahi, Bangladesh. On the basis of the morphological and cultural characteristics, the fungus was identified as *Pestalotiopsis sp.* and in vitro management and field condition, the fungicide Bavistin showed 100% growth inhibition of this fungus.

Hemelrijck *et al.,* (2017) conducted a study on Strawberry plant and repeatedly isolated the *Pestalotiopsis spp.* as a pathogen from crown tissues of wilting strawberry plants from different cultivars “Sonata”, “Jive”, “Elsanta”,” Malling Centenary” and “ Portola” in Belgium.

Azlan *et al*., (2018) studied leaf spot and anthracnose disease in nine selected Oil Palm Nurseries in Peninsular Malaysia. The isolation of the pathogen was done from the infected leaf of the oil palm tree. Depending upon the culture and conidial

characteristics, the causal organisms were identified as – *Colletotrichum spp, Curvularia sp.* and *Pestalotiopsis sp.*

Chen *et al*., (2018) reported that *Pestalotiopsis* like species are causing gray blight disease on Tea (*chemellia sinensis)* in China. On the basis of the morphological and phylogenetic analysis the isolates were identified as- *Pseudopestalotiopsis camelliae- sinensis, Neopestalotiopsis clavispora and Pestalotiopsis camelliae.*

Bhagariya and Prajapati (2019) for the first time reported that *Pestalotiopsis clavispora* is causing Crown Rot Disease on Strawberry Field in Hill Millet Research Station, Waghal, Gujarat, India. The infected plants showed drying from the edges of their leaves. At first, on the leaf surface, some dark lesions appeared and spread towards the downward to the crown region.

Mora *et al*., (2019) reported a new leaf spot and anthrancosis disease on the leaves and stems of strawberry plants in Puebla, Maxico. After the isolation of the pathogen on the basis of colony and conidial characteristics, the causal organism was identified as *Pestalotiopsis spp.*

Chelong *et al.,* (2020) reported that *Pestalotiopsis spp.* infecting the leaves of Para Rubber plant in Thailand. The causal organism was identified on the basis of the morphological and microscopic observation.

Bhuiyan *et al*., (2021) for the first time reported that *Pestalotiopsis sp* causing gray leaf spot disease on the leaves of Coconut (*Cocos nucifera* L.) trees in Bangladesh. They also reported that the growth of the pathogen was completely inhibited by Autostin 50 WDG (Carbendazim) at 100ppm.

Zheng *et al*., (2022) recently reported a leaf blight disease on Buddhist Pine (*Podocarpus macrophyllus)* in Anhui Province, China. On the basis of morphological and multilocus phylogenetic analysis the isolated pathogen from the symptomatic leaf was identified as- *Pestalotiopsis lushanensis.*

Jesus *et al.,* (2022) reported the first occurrence of *Pestalotiopsis microspora* as a causal agent of leaf spots disease on desert rose (*Adenium obesum)* in Brazil.

3.3 Management of plant disease caused by *Pestalotiopsis spp.* under *in vitro* condition.

McQuilken and Hopkins (2004) obtained *Pestalotiopsis* isolates from the foliage, stem-base and roots of Ericaceous crops. On the basis of the conidia morphology the isolates were identified as *Pestalotiopsis sydowiana.* They also observed that on five spray programme of alternating prochloraz and Carbendazim disease incidence and foliar browning caused by *P. sydowiana* was less.

Islam *et al*., (2004) reported that *Pestalotiopsis palmarum* causing leaf spot disease on betelnut. In *in vitro* evaluation of fungicides Bavistin at 100,200 and 300 ppm inhibited 100 per cent radial growth of *Pestalotiopsis palmarum.*

Bhanumathi and Rai (2007) reported that under *in vitro* management of *Peatalotiopsis sp* which causing leaf blight disease on *Syzygium cumini.* Among the all fungicides Bavistin (Carbendazim 50% WP) was proven to be most effective fungicide against the *Pestalotiopsis sp* at concentrations of 50 and100, this fungicide showed 100% growth inhibition and nil fungal growth.

Saju *et al.,* (2011) tested four fungicides under *in vitro* condition against *Pestalotiosis sp* which infect large Cardamom (*Amomum subulatum).* All the fungicides were evaluated at the 0.1% concentration and Carbendazim 50 WP was significantly effective at all concentrations tested (0.05%-0.15%).

Esiegbuya *et al.,* (2014) noticed a leaf blight disease on *Vitellaria paradoxa* and from the infected leaf the causal organism was identified as *Pestalotiopsis clavispora.* In *in vitro* evaluation of some selected fungicides Captan at 500 and 250 ppm, Benlate at 500 ppm and Difolatan at 500 ppm completely inhibit the mycelial growth of *P. clavispora.*

Teixeira *et al.,* (2015) carried out a laboratory test in order to select chemicals or biological products with the potential to control the strawberry disease caused by *Pestalotiopsis longisetula.* Result showed that no fungicide was able to inhibit the growth of the pathogen at a 100% rate under *in vitro* condition.

Barman *et al.,* (2015) observed that in the *in vitro* assay of fungicides against *Pestalotiopsis theae.* Among all the treatments Carbendazim50% WP (Bavistin)

showed 100% mycelial growth inhibition against *Pestalotiopsis theae* in Poison food technique.

Hoque *et al.,* (2016) had under taken an *in vitro* experiment to screen ten fungicides and two tannin extracts against the fungal pathogens including *Pestalotiopsis sp.* of Jujube fruit and leaf disease. Out of the ten fungicides studied, complete growth inhibition of *Pestalotiopsis palmarum* was achieved with Bavistin DF.

Ara *et al.,* (2017) described that *Pestalotiopsis sp* causing a crown rot disease on Strawberry plant. They also observed that the per cent redial growth inhibition of *Peatalotopsis sp* was increased with the increasing concentration of fungicide. Among the all tested fungicide Carbendazim 50% WP (Bavistin) at 1000 ppm showed the 100% growth inhibition of the fungus in *in vitro* condition and in field condition.

Moshayedi *et al.,* (2017) used different commercially formulated fungicide at 1000, 2000 and 3000 ppm concentrations to determined the per cent growth inhibition against *Pestalotiopsis sp.* Application of different fungicides on *Pestalotiopsis sp.* Mancozeb 50WP (Bavistin) showed nil growth against *Pestalotiopsis sp.*

Lekete *et al.,* (2018) revealed that *Pestalotiopsis sp.* causing a leaf spot disease on Oil palm (*Elaeis guineensis Jacq).*Under *in vitro* management of fungus no mycelial growth was observed in Carbendazim 500 SC at the concentration of 100 ppm.

Rakesh *et al.,* (2020) reported that *Pestalotiopsis mangiferae* causing grey blight disease in mango. In *in vitro* evaluation of fungicides among the all tested fungicides Carbendazim and Mancozeb showed the 100% growth inhibition against *P. mangiferae.*

Das *et al.,* (2020) performed an *in vitro* experiment by screening different fungicides against *Pestalotiopsis mangiferae,* the causal organism of gray blight of mango by poison food technique. Among all the tested fungicides Carbendazim products i.e Carbendazim 12%+ Mancozeb 63% found best with 100 per cent growth inhibition at each concentration

3.4 Effect of different fungicides in management of foliar disease in plants:-

3.4.1 Indofil M45 (Mancozeb 75% WP):-

Singh and Singh (2005) reported that *Alternaria brassicae, A. brassicicola, Albugo candida* and *Peronospora parasitica* causing *Alternaria* Blight, white rust and Doneny Mildew in Mustard. For the management of these disease they combined the seed treatment with three foliar spary of Indofil M-45 (Mancozeb 75%) at 15 Days of interval. Resulted in lowest *Alternaria blight* and highest seed yield.

Singh *et al.,* (2007) tested twelve different fungicides against the bacterium *Xanthomonas axonopodis* responsible for leaf blight of *Gossypium spp.* 100 per cent growth inhibition of the causal organism was observed in the broth medium containing 0.02% Indofil M-45.

Baiswar *et al.,* (2011) reported that *Phakopsora pachyrhizi* causing Rust disease on Soyabean. They also reported that Indofil M-45 is not effective in reducing the yield loss of Soyabean caused by *P. pachyrhizi*.

Hajano *et al.,* (2012) tested five different fungicides including Mancozeb for the management of Rice Blast disease caused by *Magnaporthe oryzae.* Among the all tested fungicides Mancozeb was most effective against *M.oryzae.*

Chethana *et al.,* (2012) tested different fungicides including Mancozeb, against *Alternaria porri* responsible for the purple blotch disease of onion. Among the all tested fungicides, Mancozeb and Probineb showed 100 per cent inhibition of the mycelial growth.

Ajay *et al.,* (2013) applied eleven fungicides against leaf blotch disease under field condition and revealed that 0.2 per cent of Indofil M-45 (Mancozeb 75% WP) can completely inhibit the growth of the causal organism.

Sharma and Saikia (2013) evaluated different fungicides for the management of leaf blight disease on potato. Indofil M-45 (Mancozeb 75% WP) when applied after the appearance of late blight, gave 29.99% and 45.70% severity of the disease, respectively with 61.24 and 40.94 % control of the disease.

Rajbongshi *et al.,* (2014) tested commonly used five fungicides including Indofil M45, Bavistin and Captan on the mycoflora of tea agro-ecosystem. Result was fungicides affect the soil mycoflora both quantitatively and qualitatively.

Neha *et al.,* (2017) evaluated six fungicides including Indofil M-45 by poisioned food technique against *Rizoctonia solani* responsible for Sheath blight disease of Rice. Where 59.76 per cent growth inhibition of the pathogen was recorded for Indofil M-45.

Mohammdi (2019) reported that *Ascochyta rabiei* causing Ascochyta blight in Chickpea. Under *in vitro* evaluation of fungicides Indofil M-45 (Mancozeb 75% WP) was recorded for 44.18 % fungal growth inhibition.

3.4.2 Bavistin (Carbendazim 50% WP):

Nikam *et al.,* (2007) studied the management technique for Wilt disease on Chickpea caused by *Fusarium oxysporium* and reported that chemical seed treatment with Thirum (0.15%) + Carbendazim (0.1%) is proved to be most effective against the *F. oxysporium.*

Bhanumathi and Rai (2007) reported that *Collectotrichum dematium* and *Fusarium solani* causing leaf blight disease on *Azadirachta indica.* For the management of these pathogens under *in vitro* condition they tested 7 fungicides at 50,100 & 150 ppm concentrations. Among all the tested fungicides Highest 88.54-86.32 % mycelial inhibition was noticed in all the three concentration of Bavistin.

Jahan *et al.,* (2015) investigate the efficacy of Biofungicide and Bavistin against anthracnose of Soybean. The highest per cent (76.25%) reduction of anthracnose infected plant plot over control was observed in Bavistin treated plot.

Khalequzzaman *et al.,* (2016) tested five different fungicides for the management of the Wilt disease of Cumin. Among the all tested fungicides lowest disease incidence of Wilt was observed in Bavistin treated plot.

Barupal and Sharma (2017) revealed that *Curvularia lunata* responsible for the leaf spot disease in Maize. For the control of the disease they evaluated the antifungal activity of *Lawsonia inermis* by Poison Food Technique where Bavistin was used as standard check. After 7 days of inoculation period the mycelial growth

was recorded for 52.67 mm on Bavistin treated media which is accounted for 36.28 per cent mycelial growth inhibition.

Shrestha *et al.*, (2017) tested different fungicides for the control of brown leaf spot disease caused by *Bipolaris oryzae* in *Sabha Mansuli* Variety of Rice. Among the all fungicides highest disease progress value was shown by Bavistin at 2 gm/lit which means the efficacy of Bavistin is low against the causal organism.

Kumar *et al.,* (2020) carried out an experiment for the management of *Alternaria* Leaf spot of Broad Bean (*Vicia faba L.)* by foliar spray and Seed treatment with Bavistin (Carbendazim 50% WP). Bavistin is recorded for the reduction in mycelial growth of *A. alternate.*

Kumar and Reddy (2021) evaluated the antifungal activity of Bavistin on (*Sclerotium) rolfsii Sacc* by using Poison Food Technique at specific concentration. They revealed that Bavistin was very toxic at 40 ppm and the growth was completely inhibited.

3.4.3 Captaf (Captan 50%WP):

Khan *et al.,* (2007) evaluated systematic and non-systematic fungicides for the management of *Alternaria* Leaf blight of rapeseed (mustard) caused by *Alternaria brassicaceae.* The combination of Bavistin and Captaf were found to be most effective in the reduction of disease severity.

Das *et al.,* (2014) tested efficacy of systematic and non-systematic fungicides including Captan 50% WP *in vitro* condition at different concentrations against *Sclerotium rolfsii Sacc* by using poison food technique. They revealed that Captan showed higher inhibitory effect on the growth of the causal organism as compaired to the other non-systematic fungicides.

Rani *et al.*, (2017) tested Captan 50% WP against early blight of Tomato caused by *Alternaria solani.* In their study Captaf was most effective fungicides against *A. solani* and exhibiting 55.19 mm mycelial growth which was accounted for 47.96% of per cent growth inhibition.

Altaf *et al.,* (2022) evaluated Captan 50 WP at 0.05, 0.1 and 0.2 per cent concentration against the green mold disease of *Agaricus bisporus.* In their study Captan reported with inhibition percentage of 9.10%.

MATERIALS AND METHODS

CHAPTER IV: MATERIAS AND METHODOLOGY

Field analysis and laboratory experiments were carried out from March 2022 to August 2022 to study the etiology and management of leaf spot disease in *M. champaca L.* The laboratory experiments were carried out in the Forest Pathology Department of Rain Forest Research Institute, Jorhat (RFRI).

4.1 Study area:

The study conducted at Rain Forest Research Institute, Jorhat, Assam, India**.** Study was conducted during April to August 2022. The study site is characterized by a climate with most rainfall occurring during the summer months (May to July) with relatively little or scanty rainfall during the winter months (November to February). The study area map has been presented in Fig. 4.1.

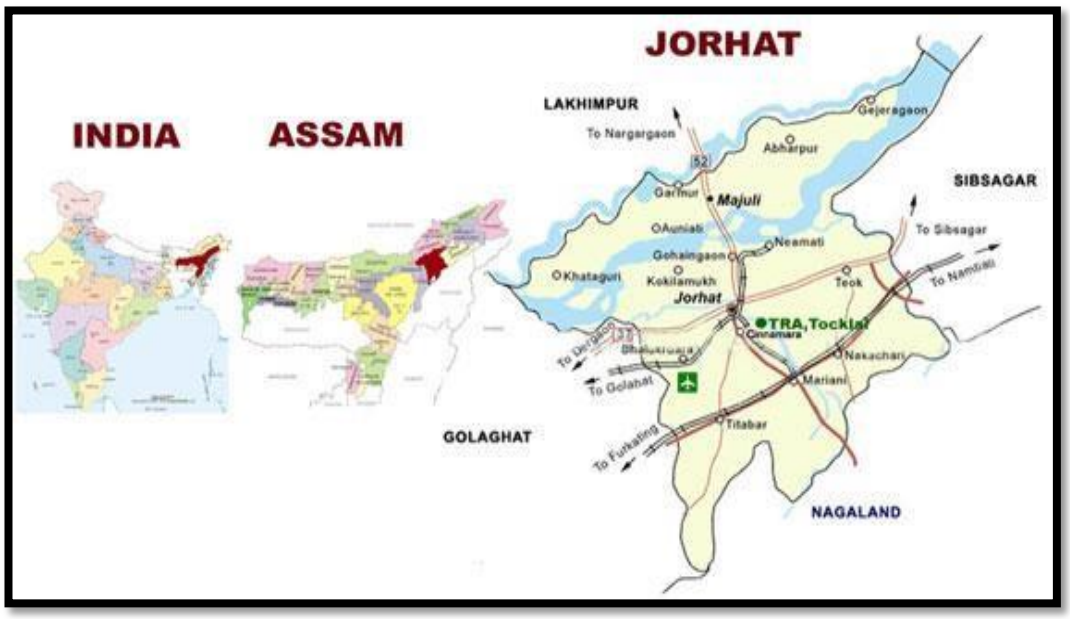

Fig: 4.1 - Study Area

- During the course work the following equipments are used for different purpose.

4.2 Autoclave:

An autoclave is an electrical device which is used to kill microorganisms such as bacteria, viruses, and spores present on the surface of equipment and research sample put inside of the vessel using steam under pressure. By using an autoclave (Vertical autoclave, Size – 14x22, Load – 3KW), we can sterilize the materials that contain water and that cannot be sterilized by the dry heat sterilizing method. They are used to decontaminate specific biological waste and in sterilization of culture media, instruments, lab wares and other laboratory materials etc. There are various types of autoclave but their working principle is same. An autoclave is the most commonly used steam sterilizer equipment that is used to sterilize objects under regulated pressure, so it provides a physical method for sterilization so that they come under moist heat sterilization. Autoclave makes use of high temperature (121℃), and high – pressure steam (15Psi) for 30 minutes to ensure decontamination Tsephel *et al., 2018*. The steam is allowed to from inside the cylinder by heating water when the steam comes in contact with the surface of the equipments it kills the microbes by dehydrating the cells and cause coagulation of proteins.

Precautions:-

- Wear proper gloves, glasses, shoes, and a lab coat before operating the autoclave.
- Do not autoclave reactive, flammable, toxic, corrosive or radioactive materials.
- Before operating an autoclave the water level should be checked.

4.3 Hot Air Oven:

A hot air oven is a type of electrical device which use dry heat to sterilize. Dry heat sterilization is used on equipment that cannot be wet, and on material that will not melt, catch fire, or change form when exposed to high temperatures. Items that are sterilized in a hot air oven include - Glassware (Petri dishes, flasks, pipettes, and test tubes) Powders, Materials that contain oils, Metal equipment (Scalpels, Scissors, and blades) etc.

Materials to be sterilized are placed inside the oven in such a way that heat gets uniformly circulated between the objects. The heat is absorbed by the outside surface of the item, then the heat, passes towards the center of the item, layer by layer. Dry heat sterilization is used to destroy spores as well as vegetative from of microorganisms that are present on the outer surface of the objects. The mechanisms by which microorganisms are destroyed by using dry heat are through coagulation of protein of cell, oxidizing the molecules and by destroying the essential cell constituents and the organism dies. The most common time and temperature relationship for sterilization with hot air sterilizers" are 170℃ (340℉) for 30 minutes, 160℃ (320℉) for 60 minutes, and 150℃ (300℉) for 150 minutes or longer depending upon the volume.

Precautions:-

- Glass apparatus must be wrapped with the news paper or brown paper.
- Do not keep the material at the bottom where it receives relatively more heat which may cause cracking of material.
- Keep space in between material for proper circulation of hot air. Avoid over loading.

4.4 Laminar Air Flow Chamber

A laminar air flow is electrical device which is mostly used in microbiology laboratories. A laminar air flow is an enclosed working station that is applied to provide a contamination free work environment through filters to capture all the particles entering the cabinet. To form a contamination free working environment a special type of filter is used known as high – efficiency particulate air filter or HEPA filter, which can remove the airborne impurity particles that are up to 0.3 micrometers in size. The working principle of laminar air flow cabinet is based on the laminar flow of air through the cabinet. The cabinet works by application of an inward movement of the air over one of HEPA filters in order to create a dust free environment. The air passes through a filtration system, and is then throw out across the surface of the working area. At first the air passes through the pre- filter or filter pad that allows a smooth flow of air in to the cabinet. After that the blower or fan directs the air

towards the HEPA filters and the HEPA filters catch the all bacteria fungi, spores, and other particulate materials and give particulate free air then the air passes through the perforation which is present on the bottom rear end of the cabinet, but mostly the air coming out through the working station towards the face of the operator.

Precautions:

- The operator must wear safety goggles, long gloves, and a laboratory coat while operating the device.
- All the components and devices present inside the cabinet must be sterilized before and after use.
- The laminar air flow cabinet needs to be thoroughly irradiated with UV light before and after use.
- Any sort of ongoing process should be immediately terminated while the UV light is still on.

4.5 BOD Incubator:

The BOD incubator (Serial NO. CLW.267, Model – 112-171) is also known as the Biological Oxygen Demand Incubator. This enclosed device is mostly used in microbiological laboratories for cell culture, tissue culture growth, and storage of bacterial and fungal cultures by providing an optimal condition of temperature, humidity and other environmental conditions which is required for the growth of the organisms. Temperature greatly influences the microbial growth. That‟s why the BOD incubator is generally designed in such a way that it can allow the desired microorganisms to grow a particular temperature normally at 20°C. BOD incubator is additionally recognized as a low temperature incubator because it provides a temperature limit between 5°C to 60°C or including cooling and heating capacities under one unit. In BOD incubator oxygen depletion is occurs when microbes are started to consume oxygen, Microbes use oxygen as electron receptor, when the microbes intake organic material it provides them energy to survive and multiply.

Precautions:-

- The fluctuations in temperature of the cabinet by repeatedly opening the door should be avoided because Microorganisms are more susceptible to temperature.
- Inside the incubator the culture plates should be placed upside down with the lid at the bottom to prevent the condensation of water on the media.
- The inside of the incubator should be cleaned regularly.
- While running the incubator for a long period of time, sterile water should be placed underneath the shelves to prevent the culture media from drying out.

4.6 Distillation Unit:

The distillation unit contains the liquid which is intended to be purified. When the liquid is heated into vapor it is separated from impurities or other liquids with different vaporization temperatures. The vapor then moves into another portion of the apparatus where it can be cooled and condensed back into a liquid. The purified from of the original liquid is called the distillate. Distillation is used for many commercial processes, such as the production of gasoline, distilled water, Xylene, alcohol, paraffin, kerosene and many other liquids. Gas may be liquefied and separate. For example: nitrogen, oxygen, and argon are distilled from air.

4.7 Analytical Balance:

An Analytical balance (Model: ALC – 210.4, Max. – 210g) is highly used lab instrument which is designed to measure the small masses of solids or liquid in the sub – milligram range. Their readability has a range between 0.1 mg to 0.01 mg. Analytical balance has a measuring plate that is present in a transparent box with a glass door and this box give protection so the dust particles and wind will not affect the operation of the balance. This transparent box is also known as a windshield.

Precautions:-

- Keep the balance calibrated.
- Place the balance in an area with controlled humidity and temperature.
- Always were gloves and use a pair of clean forceps while placing the samples.

4.8 Refrigerator:

Refrigerator works on the principle of second law of Thermodynamics. Where, the refrigerator that is in liquid state that passes through the expansion value and turns in to cool gas due to the sudden drop in pressure. As the cool refrigerant gas flows through the chiller cabinet, it absorbs the heat from the substances kept inside the fridge. In Microbiological laboratories Refrigerator is used to maintenance of cultures in pure form for further studies is carried out at low temperature(0-5°C) because at law temperature , all the life processes slow down and culture can be maintained without losing their identity for a longer period

Precautions:-

- Avoid overcrowding and always keep labels while keeping things inside the refrigerator.
- Check the door seal as loose seals allows cool air to seep out causing wastage of energy.

4.9 Heating Mantle:

A Heating Mantle (Size 1000ml, Watt- 300) is laboratory equipment used to heat different samples, liquids, fluids and chemicals by using electricity. Therefore the solutions that has to be heated , is put into round bottom flask such as conical flask, beaker, vessels and keep inside the Heating Mantle.

Precautions:-

- Heating Mantles should never be plugged directly into an outlet.
- Use heat resistant gloves while heating the solution.
- The container should be removed from the heating mantle after use.
- Heat sensitive elements should be avoided.

4.10 Microscope:

The Zeiss Scope A1 microscope (Serial No. 3329000167) was used in the observation of the slides. The Zeiss Axioscope A1 can be used with reflected and transmitted light techniques such as Brightfield, darkfield, polarization, DIC, circular DIC, fluorescence, and phase contrast. The Axio Scope A1 model of Zeiss microscopes can be used with most cameras traditionally used for photomicroscopy as well as all Zeiss Axio Cam cameras and interfaces with the Zeiss Axio Vision Imaging software.

4.11 Growth Media:

Two different kinds of growth media were used in the present investigation for the isolation and growth of the fungus. They are Potato Dextrose Agar (PDA) media and Potato Dextrose Broth (PDB).

4.11.1 Potato Dextrose Agar (PDA):

It is used as a growth medium for the cultivation of fungus. It is recommended as a general purpose medium for the cultivation of the Yeast and Moulds from the pharmaceutical products.

Table-4.1: Composition of Potato Dextrose Agar (PDA):-

SL NO	Ingredients	Gms/ltr
1.	Infusion from Potatoes	200.00
2.	Dextrose(glucose)	20.00
3.	Agar	15.00
4.	Distilled Water	1000ml
5.	P^H after Sterilization	5.6±0.2

4.11.2 Potato Dextrose Broth (PDB):

It is recommended for cultivation and enumeration of Yeast and Moulds. Potato Dextrose Broth is also used for stimulating sporulation, for maintaining stock cultures of certain dermatophytes and for differentiation of typical varieties of dermatophytes on the basis of pigment production. Potato infusion and dextrose promote the fungal growth. The growth of the bacteria in the medium inhibit by adjusting P^H of the medium to 3.5 by adding tartaric acid.

Table-4.2 Composition of Potato Dextrose Broth (PDB):-

SL NO	Ingredients	Quantity (gm/ltr)
1.	Dextrose	20.000
2.	Potato infusion from 200 gm	4.000
3.	pH	5.1±0.2 at 25°C
4.	Distilled water	1000

4.12 Fungicides used for poison food method

Following fungicides were taken to perform the in vitro management technique for the leaf spot disease of *Magnolia champaca*.

4.12.1 Indofil M-45:

Indofil M-45 is an effective protectant contact fungicide which can control disease caused fungus of different classes. The active ingredient of Indofil M-45 is Mancozeb75% WP. Mancozeb is a protective fungicide which reacts with, and inactivates sulfhydryl (SH) groups of amino acids and enzymes of fungal cells, resulting in disruption of lipid metabolism, respiration and production of ATP.

Table-4.3 Composition of Indofil M-45

Sl. No	Ingredients	Amount
1	Mancozeb (a.i)	75%WP
2	Other	25%

4.12.2 Bavistin:

Carbendazim 50% WP is a broad spectrum systematic fungicide containing 50% carbendazim by w/w which is common name for methyl to benzimidazole carbanet carbendazim 50% WP (MBC) is effective against wide range of pathogenic fungi and is highly specific in its control of important plant pathogens on variety of corps, ornamental plants and plantation crops. It is also use as a seed dresser. Carbendazim 50% WP can be applied as a foliar spray before the onset of the disease.

Table-4.4 Composition of Bavistin

Sl. NO	Ingredients	Amount
1	**Carbendazim (a.i)**	**50% WP**
2	**Other**	**50%**

4.12.3 Captan:

Captan is an excellent fungicide for the control of leaf spots, blights, fruit rots and fruit vegetables, flowers and grasses. Captan has also been reported to repel “seedpulling” birds Captan is an organic effective fungicide of Micronized particle size and other balance adjuvant. It contains 50% Captan as active ingredient in WP. It is used for the control of Scab, Brown rot, Downy mildew and early blight on Apple, Cherry, Potato and Tomato.

Table-4.5 Composition of Captan:-

SL. NO.	Contents	Amount
1	Captan Content(Active ingredient)	50.000 % w/w
2	Magnesium carbonate	1.000 % w/w
3	Wetting and Suspending agent – Sodium salt of Alkyl aryl sulphonate sodium lignosulphonate	10.0 w/w

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4.13 Collection of Disease Samples:

Infected Seedlings of *Magnolia champaca Linn* from a forest nursery RajabhatKhawa, West Bengal, India (Latitude: 26.618523^0, longitude: 89.531869^0) were observed and the infected leaves were collected and placed separately into brown paper bags. Photographs were taken and the symptoms are recorded. Infected leaves of *Magnolia champaca Linn* were brought to laboratory of Rain Forest Research institute of Jorhat, for further investigation.

4.14 Sterilization

4.14.1 Cleaning of glass wares:

Before using the glass wares such as: Petri dishes ,test tubes, beakers, measuring cylinders, conical flasks, and funnels, they were kept in the cleaning solution of potassium dichromate and, following that, the glass wares were washed with the detergent (Labolene) followed by tap water. After that, all the glass wares were rinsed with distilled water, and then the glass wares were kept in the sunlight to air dry and then finally cleaned with ethanol.

4.14.2 Flame Sterilization: - Metal objects such as forceps, needles, inoculation loop, and scissors were sterilized by exposing on to flame.

4.14.3 Sterilization in Autoclave: - After cleaning the Petri dishes were covered with news paper and brown paper and placed in autoclave bag for sterilize in the autoclave. The mouth conical flasks and test tubes containing growth media were sealed with cotton plug and covered with brown paper and all the materials were carefully placed under the autoclave and sterilized at 121℃ at the pressure of 15 psi for 30 minutes.

4.14.4 Sterilization in Hot Air Oven: - The glass wares were kept inside the hot air oven and sterilized at 160℃ for an hour.

4.15 Preparation of Culture Medium:

4.15.1 Potato Dextrose Agar Medium:-

One liter (1000ml) distilled water was taken in a 2000 ml volume of conical flask and 39.0 gm of Potato Dextrose Agar(PDA) suspended was added in the 1000 ml of distilled water, the mouth of the conical flask was sealed with cotton plug and covered with brown paper . After that the solution was heated in the heating mantle at 70-80℃ for 5-10 minutes to boil when the media is dissolve completely the Potato Dextrose Agar(PDA) solution was sterilized by Autoclave at 121℃, 15psi for 30 minutes then it was allowed to cool down up to a normal temperature.

4.15.2 Potato Dextrose Broth Medium:-

One liter (1000ml) distilled water was taken in a 2000ml volume of conical flask and 24gm of PDB Powder was added in the 1000ml of distilled water. The mouth of the conical flask was sealed with cotton plug and covered with the brown paper. The solution was heated in the heating mantle at 70-80℃ for 5-10 minutes to dissolved the media completely. Then the media was sterilized by Autoclave at 121℃, 15psi for 30 minutes. Then it allowed cooling at room temperature.

4.16 Isolation of the Pathogen:

The infected leaves of *Magnolia champaca L.* were collected and brought to the laboratory in brown paper bag. The isolation of the pathogen was done from the transition zone of healthy and diseased areas of the sample. For the isolation of the pathogen the samples were cut into small pieces of about 2-5mm from the adjoining point of diseased and healthy areas. The cut pieces were transferred in to sterile Petri dishes. Prepared media was heated and a trace amount of streptomycin and lactic acid were added in the media to inhibit the growth of the bacterial population and to make the media acidic, respectively, and then mixed the media gently and poured in to sterile Petri dishes or test tubes in the Laminar Air Flow and kept them for 30 minutes to solidify. The cut pieces were surface sterilized in 4.0 percent sodium hypochlorite (NaOCl) for 2-3 minutes, and then the cut pieces were transferred into Petri dishes containing sterile distilled water and washed the cut pieces properly. The surface sterilized cut pieces were transferred in the blotting paper to remove the

excess amount of moisture. Then the treated cut pieces were transferred in the sterilized Potato Dextrose Agar (PDA) media with the help of a sterilized needle or forceps. All the Petri dishes were sealed with paraflim to avoid the father air born contamination. To maintain an aseptic environment above mention all the steps were carryout under the Laminar Air Flow. The Petri dishes were incubated at 24±1℃ and the growth of the fungus was examined periodically for up to 10-15 days (Borah *et al*., 2019).

- **Pure culture**:

After 15 days of incubation (24±1℃) period one colony of fungi was developed from the cut pieces of the infected leaves. The fresh growth of the fungi was transferred into the freshly prepared Potato Dextrose Agar (PDA) medium for sub – culturing and to obtain the pure culture of the pathogen. At the same time, microscopic slides were prepared from the isolated fungi. Prepared slides were observed under a compound microscope. From the microscopic observation all the morphological features were recorded and micro photographs were taken. For confirmed identification, the fungal cultures were referred to Indian Agricultural Research Institute (IARI), New Delhi.

4.17 Preparation of Slide:

A clean microscopic slide was taken and a drop of lacto phenol was placed on the center of the slide by a dropper. The fungal mycelium was added on the lacto phenol drop by using a sterilized inoculating needle and gently spread the fungal mycelium on the slide by using the inoculating needle. After that a clean cover slip was placed over the fungal specimen. Then the edges of the cover slip were sealed by nail polish to make it semi permanent and observed under compound microscope.

4.18 Pathogenicity Test:

For the Pathogenicity Test the seedlings of *Magnolia champaca* were grown in the nursery of Rain Forest Research Institute, Jorhat. The seedlings were raised in earthen pots and filled with sterile soil under glass house condition. Five healthy seedlings of *M. champaca* were taken to carry out the pathogenicity test, four seedlings for treatment and one seedling for control. The pathogenicity test was performed with the help of pinprick method and the pathogenicity test was confirmed through Koch‟s postulation. The inoculation sites of the leaf were wounded by using a sterilized needle. With the help of a sterile cork borer five mm diameter plugs of mycelia agar inoculums were taken from the 15 days old fungal culture and carefully inoculated on the inoculation site of the leaves Borah *et al*., (2019). At the same time the control plant was inoculated only with the sterilized agar bits. Both the inoculated plants as well as the controlled plants were covered with moistened polythene bags and kept in natural condition for 6-7 days. The inoculated plants were observed regularly for symptoms development. After the appearance of similar disease symptoms on the inoculated leaves of *M. chamapaca* seedlings, the causal organism was again re- isolated from the artificially infected leaves of *M. champaca* on Potato Dextrose Agar (PDA) medium. The newly isolated fungus was compared with the original culture for conformation. As compared to the treatment plants the control plant remains healthy.

4.19 Poisoned Food Technique:

The poisoned food technique was performed to evaluate the inhibition of the mycelial growth *in vitro* condition. For the management of the disease two contact fungicides Mancozeb 75% WP (Indofil M-45) and Captan 50% WP (Captaf) and one systematic fungicide Carbendazim 50% WP (Bavistin) was procured. The fungicides were tested at different concentration such as 10 ppm, 25 ppm, 50 ppm and 100 ppm.

4.19.1 Preparation of Fungicide amended media:

10 ml Potato dextrose Agar media was prepared for each concentration of the treatments. For the preparation of the stalk solution the 1000 ml autoclaved distilled water has been taken in a conical flask. Then a required amount of fungicide was suspended on to it. The stalk solution was prepared for every concentration of treatments separately. About 9 ml sterile water taken in a test tube and 1ml stalk solution was added into it, then the solution was poured in the PDA media to make it fungicide amended media.

The requisite amount fungicide need to prepare the stalk solution was determined by following method.

Example: For the preparation of 10 ppm concentration if fungicide amended PDA medium the concentration of the stalk solution would be –

$$S1 = \frac{S2 - V2}{V1} \times 100$$

Where, S1 – Concentration of the stalk solution, S2 – 20, (2x value of the required of concentration), V1 –1ml i.e. amount of stalk solution that need to poured in PDA medium, V2 -10 ml(9ml sterile water + 1ml stalk solution).

The amount of fungicide need to be suspend on the 1000 ml of water to make required concentration of stalk solution is calculated as given bellow-

Example – for 200 ppm concentration of stalk solution-

To get 50 ppm Carbendazim we need = 100mg of Bavistin

To get 1 ppm Carbendazim we need = $\frac{100\ mg}{50}$ of Bavistin

For 200 ppm Carbendazim we need = $\frac{100\ mg}{50\ ppm} \times 200\ ppm$

= 400mg

Therefore, the amount of fungicide or Indofil M-45 that needs to be transfer to the 1000ml sterile water to get 200 ppm concentration of stalk solution is 400 mg.

In the same way, for each replication of a treatment the requisite amount of fungicide for the stalk solution was calculated by the above method. Details of the treatments for poison food technique are presented in table 5.19.1

Table- 4.6: Details of the treatments for poisoned food technique

Treatments	Concentrations/ppm
T1 - Indofil M45 (Mancozeb 75%WP)	10 ppm, 25 ppm, 50 ppm, 100 ppm (Indofil M-45 + Fungus)
T2 – Bavistin (Carbendazim 50%WP)	10 ppm, 25 ppm, 50 ppm, 100 ppm (Bavistin + Fungus)
T3 - Captaf (Captan 50% WP)	10 ppm, 25 ppm, 50 ppm, 100 ppm (Captan + Fungus)
T4 - Control	Control (Fungus)

No. of treatment – 4

No. of replication - 3

4.19.2 Plating and Incubation:

The fungicide amended PDA media were sterilized by autoclaving and the media without fungicide were prepared for control. A trace amount of streptomycin and lactic acid were added to fungicide amended PDA media to avoid the bacterial growth. About 20 ml PDA media were poured in the 9cm diameter of each Petri plate and allowed to solidify. About 5mm diameter of mycelial agar bit from the periphery of the ten days old culture was inoculated on the PDA medium with the help of sterilized cork borer in the centre of the Petri plates and incubated at room temperature 25±2℃.

Three replications were maintained for each concentration of a treatment. After 10 days of incubation period the average diameter of the mycelial growth of three replication of each concentration were taken and per cent growth inhibition was calculated by using following formula (Gautam *et al.*, 2017).

Per cent (%) growth inhibition = $\frac{C-T}{C} \times 100$

Where,

C = Growth of the control plate

T = Growth of fungicide treated plate

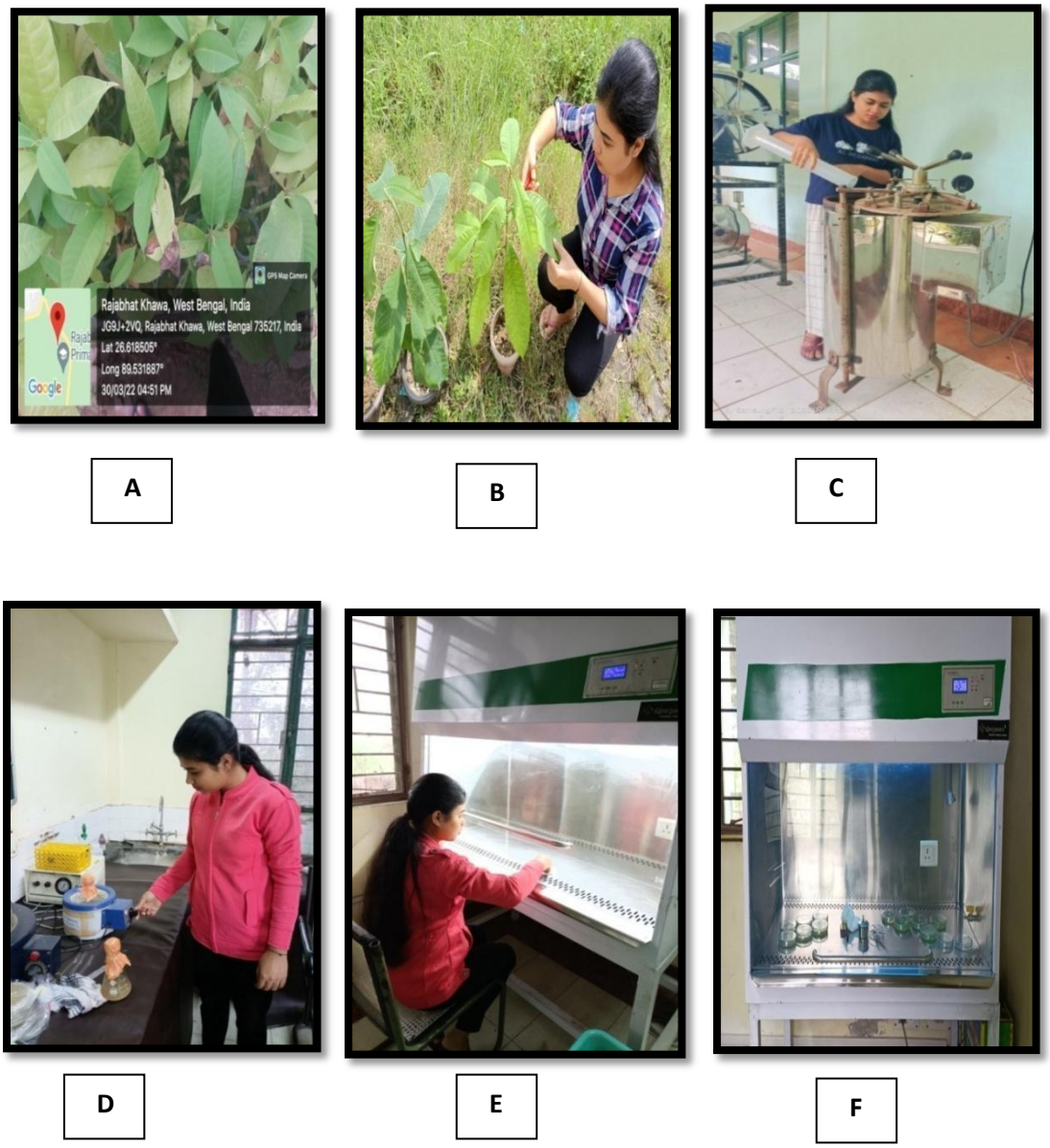
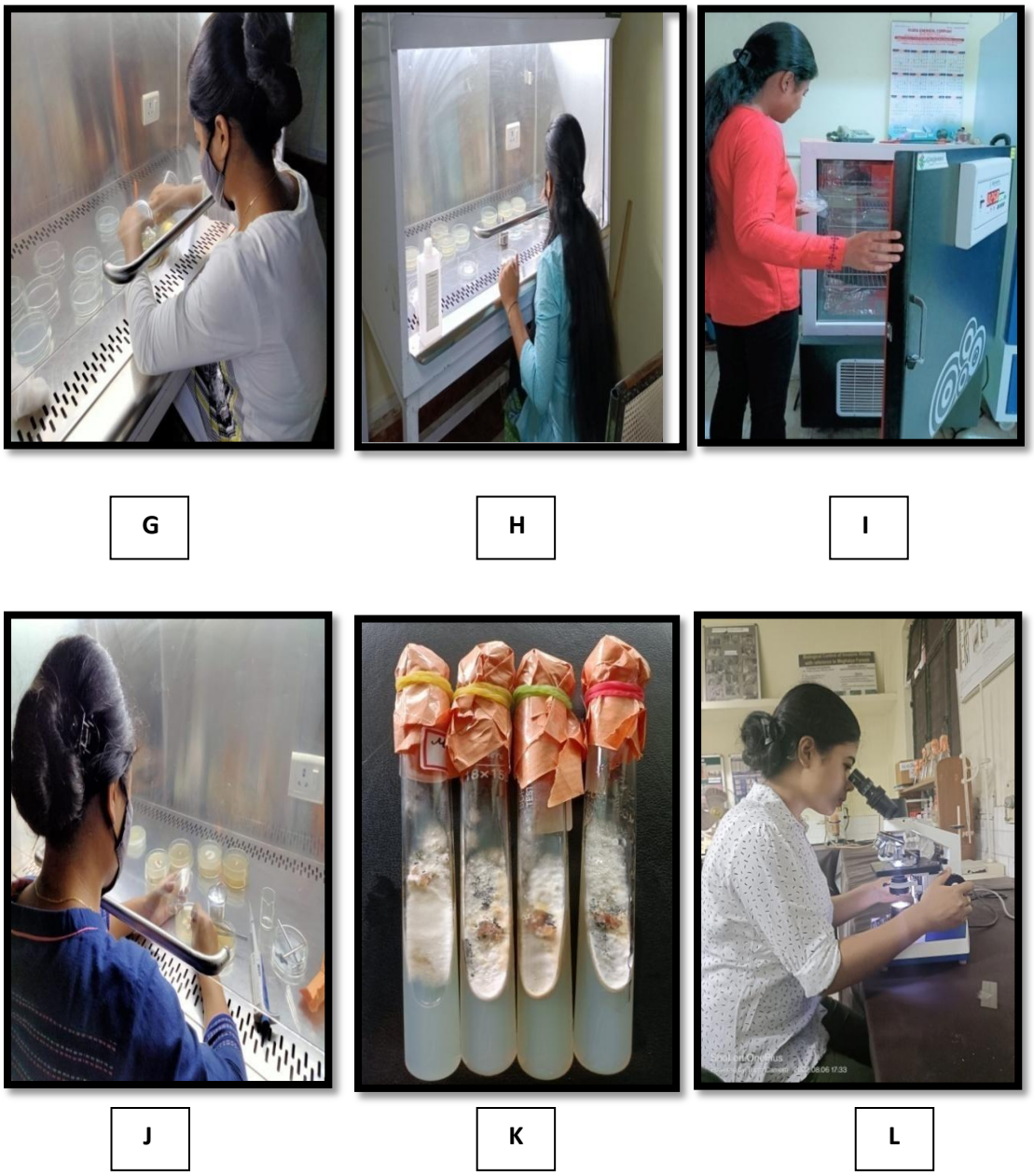


Fig: 4.2: Steps carried out during the project period:- A: Study area, B: Sample collection, C: Sterilization in autoclave, D: Heating of PDA media, E-F: Sterilization of laminar air flow



G H I

J K L

Fig: 4.2 Steps carried out during the project period :- G: Pouring of PDA in Petri plates, H: Isolation of Pathogen, I: Incubation, J: Subculture of the pathogen, K: Slant culture, L: Observation in microscope

M N O

P Q R

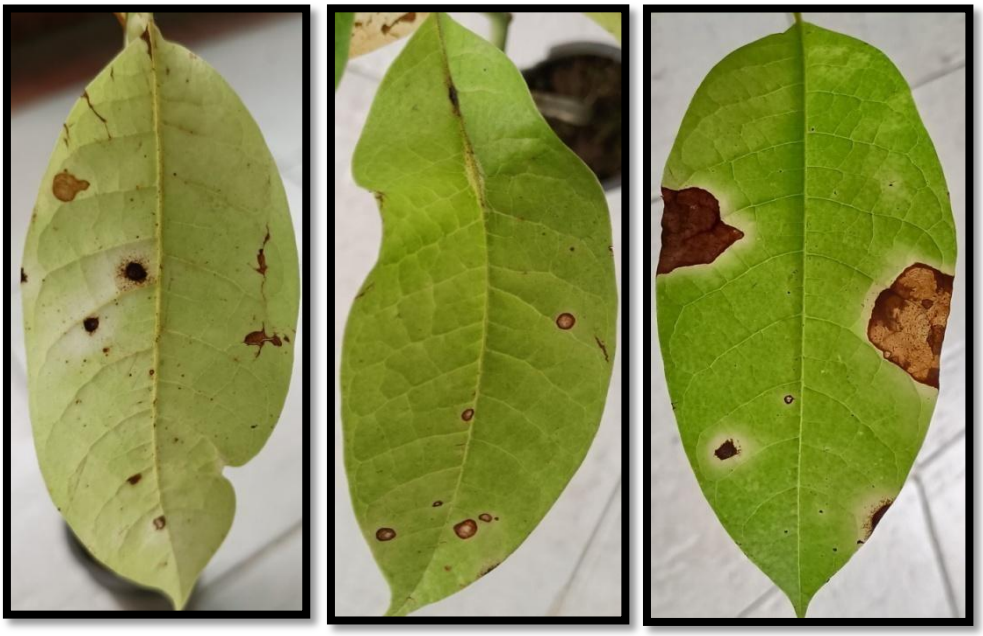
Fig 4.2 Steps carried out during the project period: M: Inoculation of the pathogen, N: Re-isolation of the pathogen, O: Preparation of stalk solution, P: Stalk solution, Q: Double stranded PDA media, R: Fungicide solution

RESULTS

CHAPTER V: RESULTS

5.1 Symptomology:

Initially symptoms appear on the seedling‟s leaves of *Magnolia champaca Linn* as tiny grayish brown dots which later increase in size and become large, semicircular brown to black colored spots. The spots are light brown in color in the middle with a black color margin surrounded by living or healthy tissues. Spots are varying in size and shape and the margins of the spots also vary in size and pattern. In advance stages of infection the tissues around the spots turn yellowish in color. Sometimes the neighboring spots coalesced to from large infected area. The spots are clearly noticeable on the both dorsal and ventral surface of the lamina as well as on the tip. (Fig 5.1)



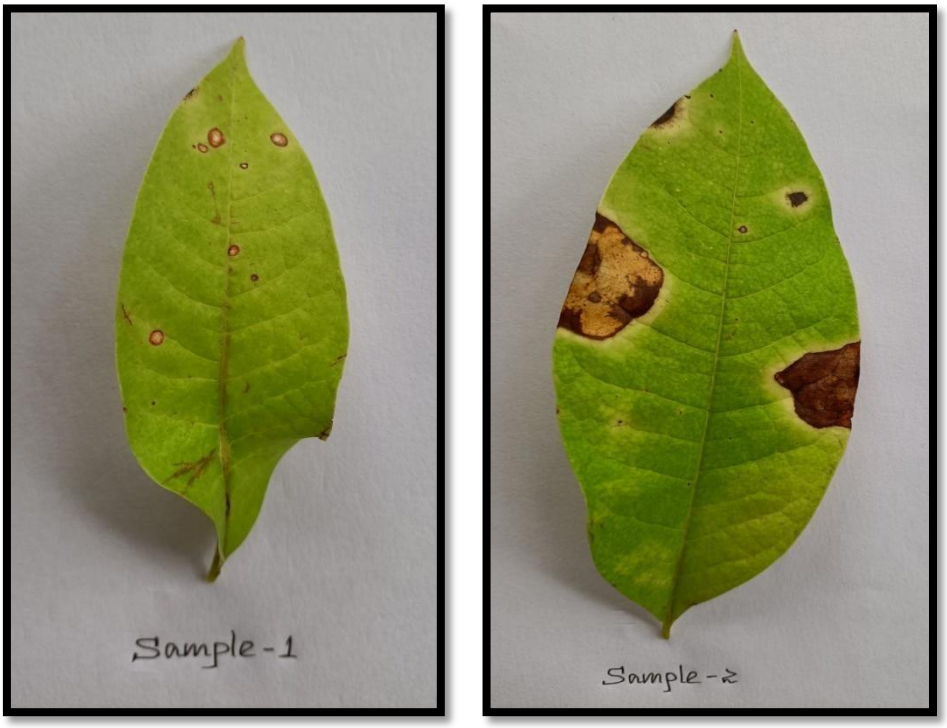
A B C

D

Fig 5.1: Symptoms, A-C: Symptoms of leaf spot disease on *Magnolia champaca, D:* Infected seedlings

5.2 Identification of the Pathogen:

The isolation was done from the infected leaf of *Magnolia champaca Linn*. The isolated fungus was identified on the basis of the fungal culture and microscopic observation. On potato dextrose agar (PDA) growth media the fungus produce smooth, wooly-cottony, white mycelial growth. After 14 days, black colored acervuli were formed all over the mycelial mat. On microscopic observation the conidia was five celled, straight or slightly curved (fusiform), four septate, the middle cells were colored (the upper most two cells were slightly darker then the lower one), slightly constrictions at the septa, septa darker than the rest of the cells and the apical and basal cells were hyaline, most the apical cells have two hyaline appendages and rarely three. On the basis of the above mentioned characters and confirmed identification result given by Indian Agricultural Research Institute (IARI) the isolated fungus was identified as *Pestalotiopsis algeriensis* (I.D No. 11690.22). (Fig 5.2 – 5.4)



A B

C D

Fig 5.2: Isolation and identification of the Pathogen, A&B. Infected leaf samples used for the isolation of the pathogen C&D.Isolation of the pathogen on PDA media C- (Front side), D-(Back side)

Fig 5.3 : Colony Produced by isolated pathogen, .E&F: 7 Days old pure culture of isolated fungus on PDA medium (E: Front side, F: Back side) G-H:- 14 Days old pure culture of isolated fungus on PDA medium (G: Front side, H: Back side)

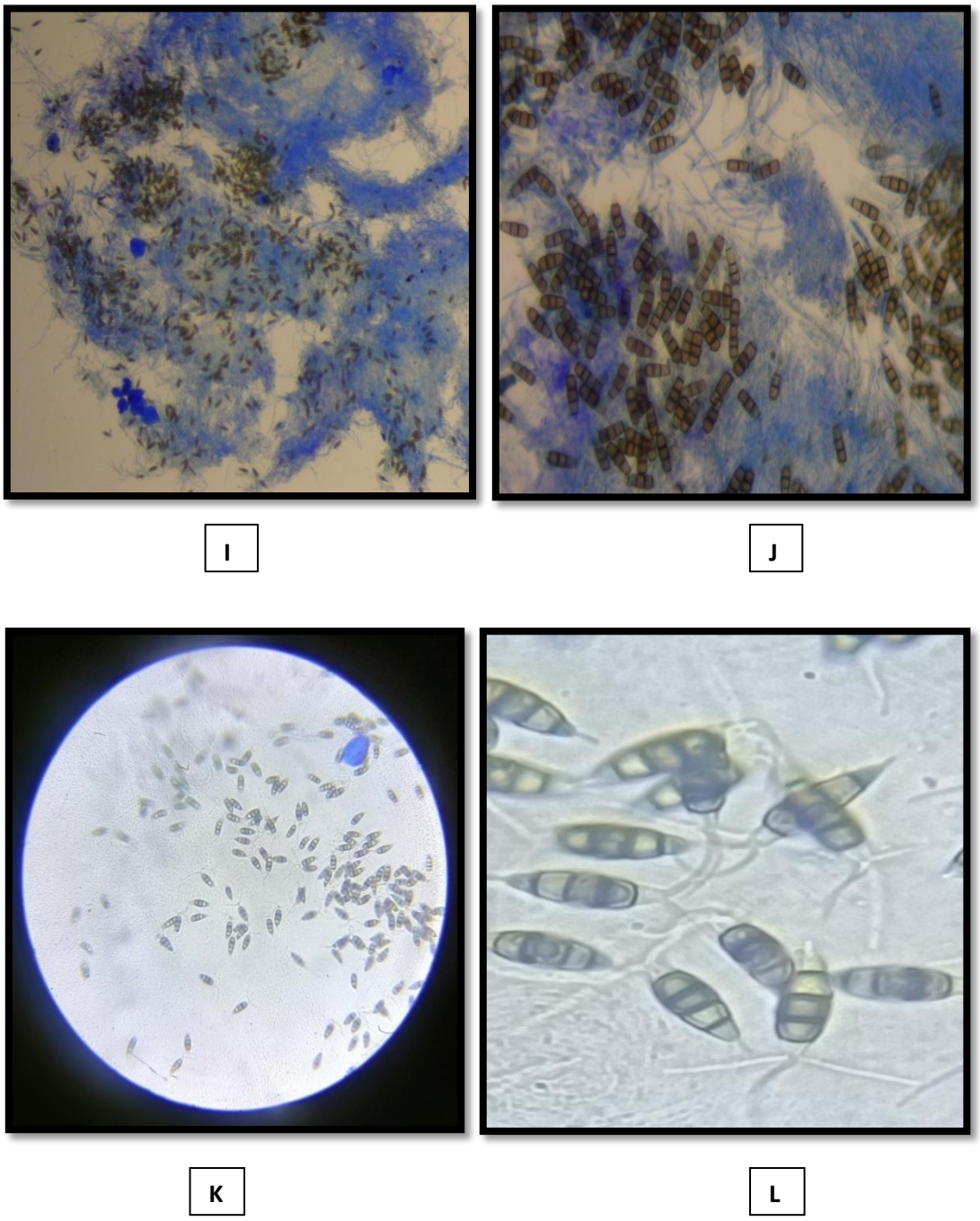


Fig 5.4:- I-L: Conidia of the isolated pathogen

5.3 Pathogenicity Test:

Result of Pathogenicity test revealed that the isolated fungus forming the similar leaf spot symptoms on the artificially inoculated leaves of *Magnolia champaca* seedlings after 3 Days of inoculation. The leaf spots were varying from circular to semi- circular in shape. The spots were light brown in color in the center, with a black color margin and the sounding tissue become yellowish.

The pathogen was re-isolated from the artificially infected leaves of *Magnolia champaca* on Potato Dextrose Agar (PDA) growth media. The fungus produces white cottony mycelial growth after 2 Days of inoculation under 25±℃. The morphology of the colony and characters of the conidia were compared with the isolate of the naturally infected leaves and all the characters resembles with each other. Hence the pathogen isolated from the artificially inoculated leaves was identified as *Pestalotiopsis algeriensis* (ID NO 11690.22) (Fig: 5.5 – 5.9).

A

B

C

Fig 5.5: Pathogenicity Test. A-B. Inoculation of mycelial bit on the healthy leaves of *Magnolia champaca* C. Inoculated and control seedlings of *M. champaca*

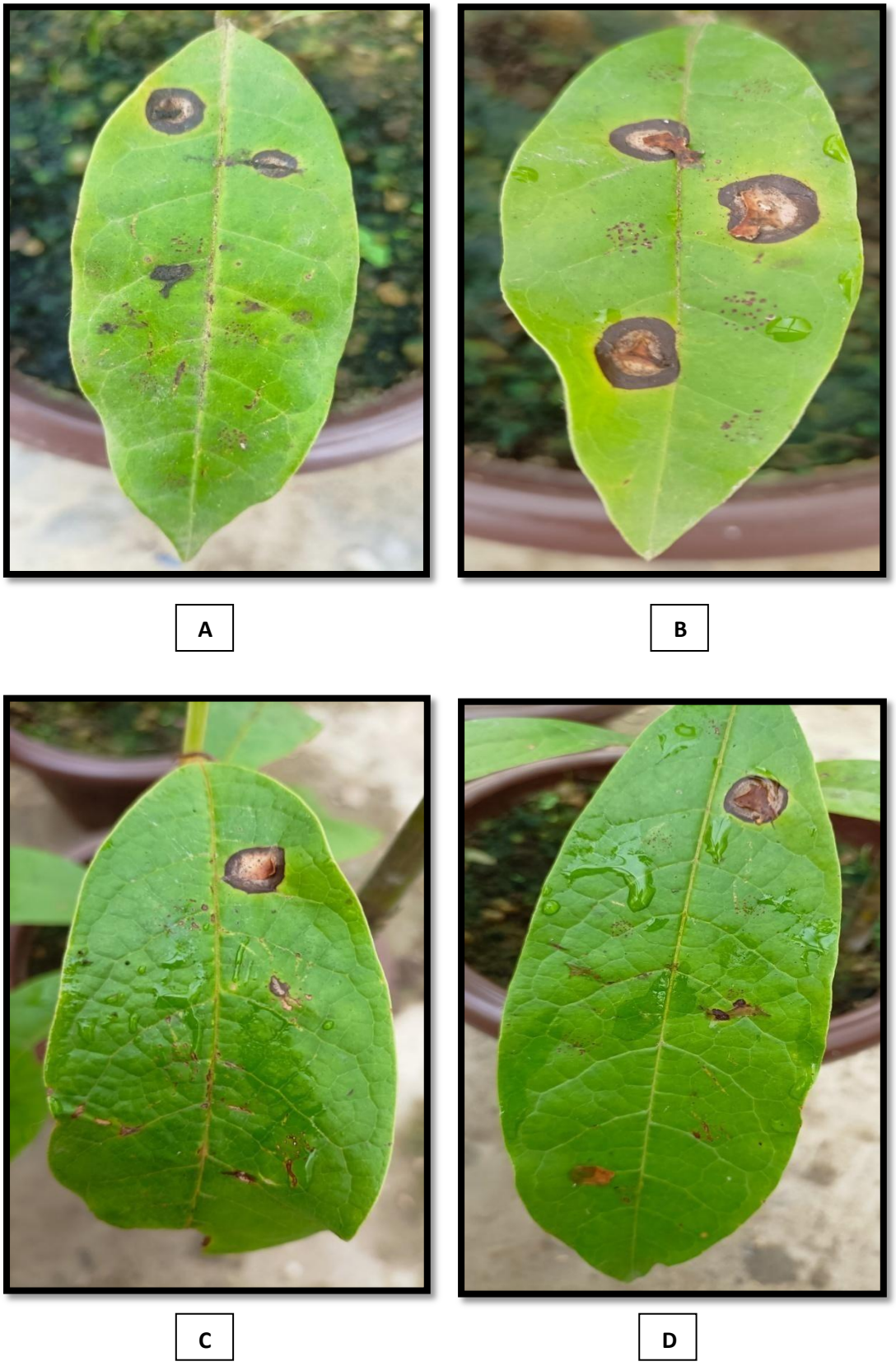


Fig 5.6: A-D. Symptoms appeared on the inoculated leaves of *Magnolia champaca*

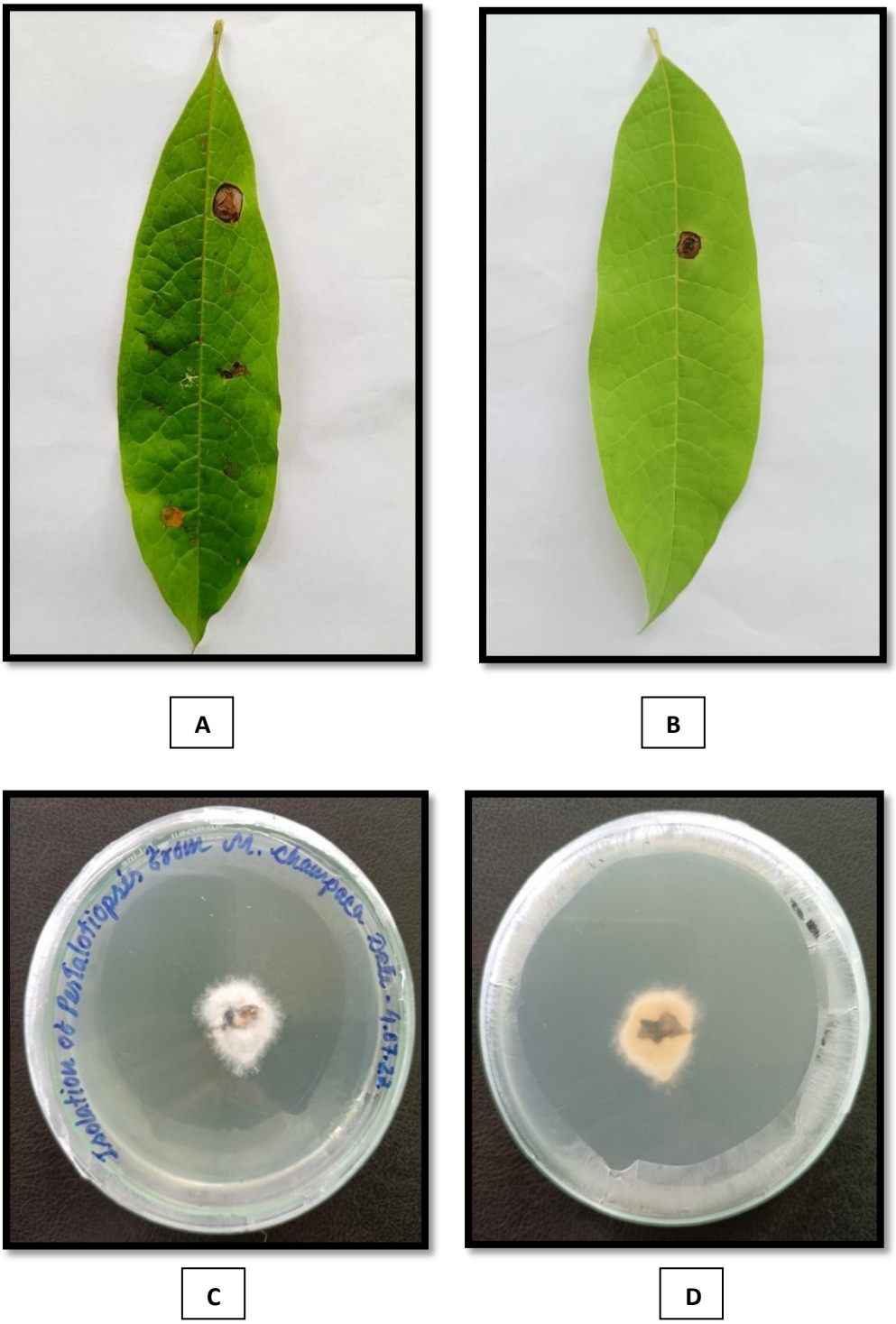


Fig: 5.7: Isolation of the Pathogen from artificially inoculated leaves, A&B: Infected leaf used for the re-isolation of the pathogen, C&D: Isolated pathogen on PDA. C: Front side, D: Back side

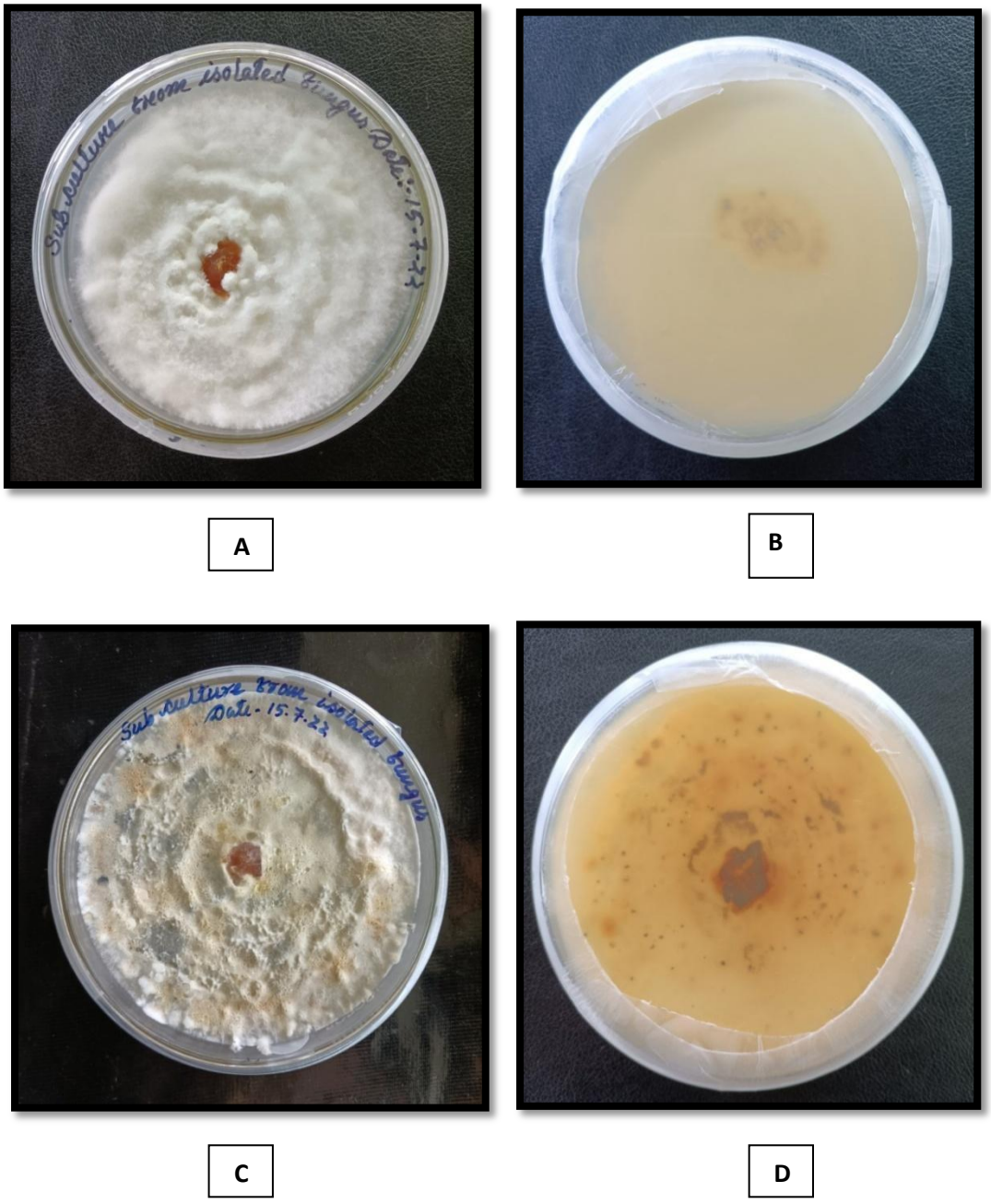
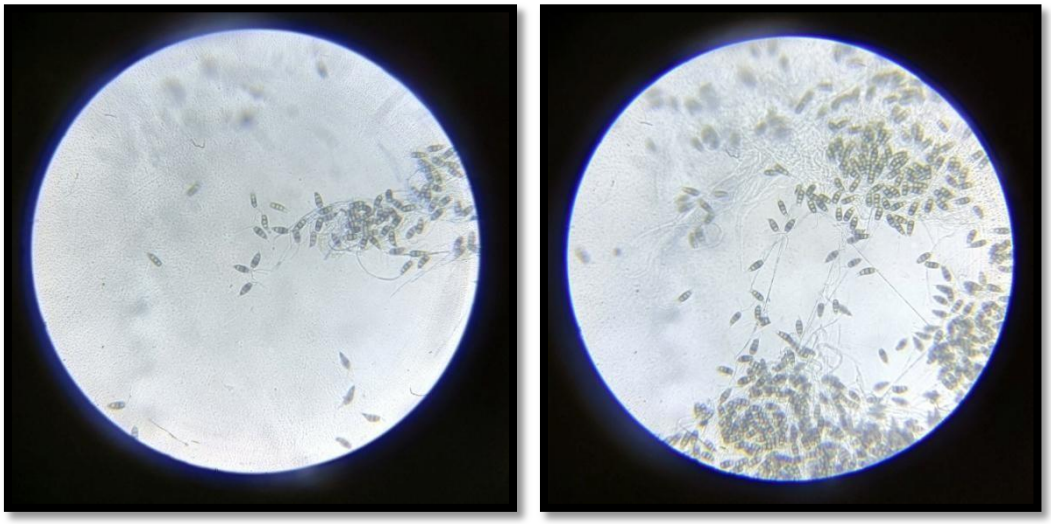
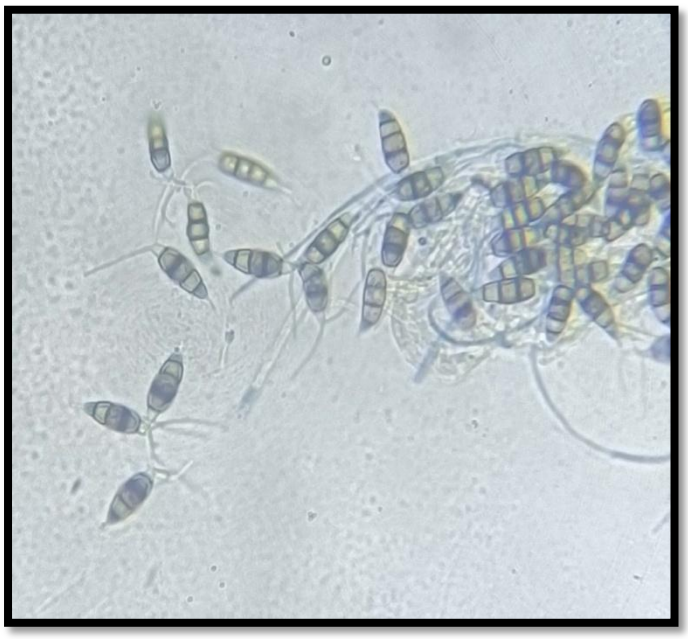


Fig 5.8: Pure culture of the re-isolated fungus, A&B: 14 days old Pure culture of the re-isolated fungus. A: Front side. B: Back side. C&D: 25 days old pure culture of re-isolated fungus on PDA media C: Front side, D: Back side



A B



C

Fig : 5.9: Conidia of the re – isolated pathogen

5.4 Poisoned food Technique:

The present study was carried out to find out the efficacy of three commercially available fungicides, Mancozeb 75% WP, Carbendazim 50% WP and Captan 50% WP to evaluate the inhibitory effect against the *Pestalotiopsis algeriensis* responsible for leaf spot disease in *Magnolia champaca*. After 10 days of incubation period the average diameter of the mycelial growth on PDA medium was taken and per cent growth inhibition was calculated. The diameter of the colony growth on the presence and absence of fungicide was measured from the edge of the colony. The photographs of the culture plates with and without fungicide were documented.

The radial colony growth of the pathogen in every 24 hrs of interval in the Mancozeb 75% WP (Indofil M-45), Carbendazim 50% WP (Bavistin) and Captan 50% WP (Captaf) incorporated PDA medium is presented on the Table 5.1, Table 5.2 and Table 5.3 respectively.

In **treatment-1**i.e. **Indofil M-45** (Mancozeb 75% WP) , the minimum growth was recorded in 50 ppm with 27 mm followed by 50 ppm with 38.37 mm mycelial growth. The maximum mycelial growth was observed in 10 ppm concentration with diameter of 43.67mm. However nil growth was recorded in the concentration of 100 ppm. The maximum mycelial growth inhibition was obtained in 50 ppm concentration which is accounted for 64.47% reduction of mycelial growth over control (Fig: 5.10).

In **treatment-2** i.e. **Bavistin** (Carbendazim 50% WP)**,** the minimum mycelium growth was observed in 25 ppm with diameter of 22.67 mm and maximum mycelial growth was observed in 10 ppm concentration with the diameter of 39.67 mm. In 50 ppm and 100 ppm concentration nil mycelial growth was recorded. Maximum inhibition of mycelial growth was observed in 25 ppm concentration which is accounted for 73.32% reduction of mycelial growth over control (Fig: 5.11).

Similarly in **treatment 3** i.e. **Captaf** (Captan 50% WP), the minimum mycelial growth was observed in 100 ppm concentration with the diameter of 59.67mm and maximum mycelial growth was observed in 50 ppm conc. which was measured for 73.67mm. The maximum mycelial growth inhibition was obtained in 100 ppm with 29.8% reduction of mycelial growth (Fig: 5.12).

The present investigation was undertaken with a view to compare the effect of three fungicides and it is evident from the table-5.4 that all the three treatments or fungicides were significantly effective over control reducing the growth of the mycelium. Maximum growth of the mycelial colony of the pathogen was recorded in the control plates followed by the 10 ppm concentration of all three treatments. In contrast, maximum per cent growth inhibition was observed in the concentration of 25 ppm of treatment 2 i.e. – Bavistin (Carbendazim 50% WP) followed by 50 ppm of Indofil M-45 (Mancozeb 75% WP) which was accounted for 73.32% and 64.47% respectively. Therefore among the three fungicides, Bavistin (Carbendazim 50% WP) was found to be most effective for control the disease followed by Indofil M-45 (Mancozeb 75% WP).

5.4.1 Indofil M-45 (Mancozeb 75% WP)

Table- 5.1: The inhibitory effect of Mancozeb 75% WP (Indofil M-45) over mycelial growth of *Pestalotiopsis algeriensis.*

Fungicide: Indofil M-45 A.I – Mancozeb 75%													
Concentration (ppm) →	**10 ppm**			**25 ppm**			**50 ppm**			**100 ppm**			**Control**
Intervals ↓	Radial growth (mm)			Radial growth (mm)			Radial growth (mm)			Radial growth (mm)			Radial growth (mm)
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	
24 Hrs	5	6	5	9	5	5	5	5	5	-	-	-	17
48 Hrs	10	8	11	12	13	10	9	5	5	-	-	-	24
72 Hrs	24	19	27	16	15	13	12	9	11	-	-	-	35
96 Hrs	31	25	32	21	20	23	18	12	14	-	-	-	49
120 Hrs	36	30	35	23	26	26	23	15	19	-	-	-	55
144 Hrs	38	32	38	26	30	29	25	16	20	-	-	-	59
168 Hrs	40	34	42	27	31	31	29	18	22	-	-	-	64
192 Hrs	45	36	46	29	35	33	31	21	25	-	-	-	70
216 Hrs	45	36	47	31	36	37	31	22	26	-	-	-	72
240 Hrs	46	37	48	35	40	40	32	23	26	-	-	-	76

A.I- active ingredient in the fungicide

ppm – parts per million,

R – Replication,

Hrs- Hours

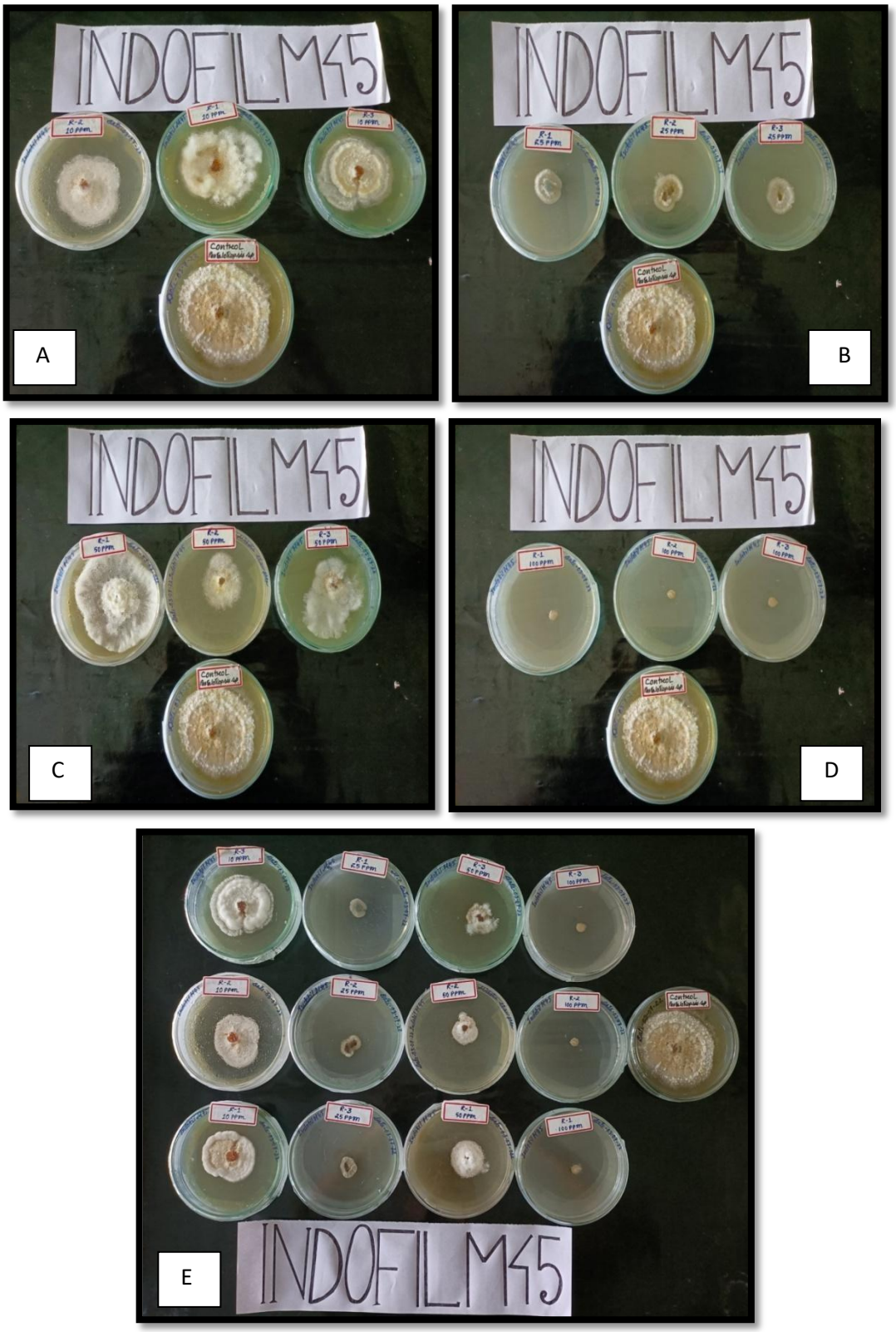


Fig 5.10: Mycelial growth of the pathogen on different concentration of the Fungicide (Indofil –M45) amended PDA media, A: 10 ppm, B: 25 ppm, C: 50 ppm, D: 100 ppm, E: Complete set of Indofil M-45 for poison food

5.4.2 Bavistin (Carbendazim 50% WP)

Table: 5.2– The inhibitory effect of Carbendazim50% WP (Bavistin) over mycelial growth of *Pestalotiopsis algeriensis*

Fungicide: Bavistin A.I – Carbendazim 50%													
Concentration (ppm) →	**10 ppm**			**25 ppm**			**50 ppm**			**100 ppm**			**Control**
Intervals ↓	Radial growth (mm)			Radial growth (mm)			Radial growth (mm)			Radial growth (mm)			Radial growth (mm)
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	
24 Hours	5	5	5	5	5	5							8
48 Hours	12	10	13	6	7	6							10
72 Hours	19	16	18	9	10	8							23
96 Hours	21	20	23	11	12	10							39
120 Hours	24	26	27	17	15	13							53
144 Hours	26	30	29	20	19	17							60
168 Hours	27	31	31	21	20	18							65
192 Hours	30	37	35	22	21	18							69
216 Hours	32	40	38	24	22	19							74
240 Hours	35	44	40	25	23	20							85

A.I- active ingredient in the fungicide

ppm – parts per million

R – Replication

Hrs: Hours

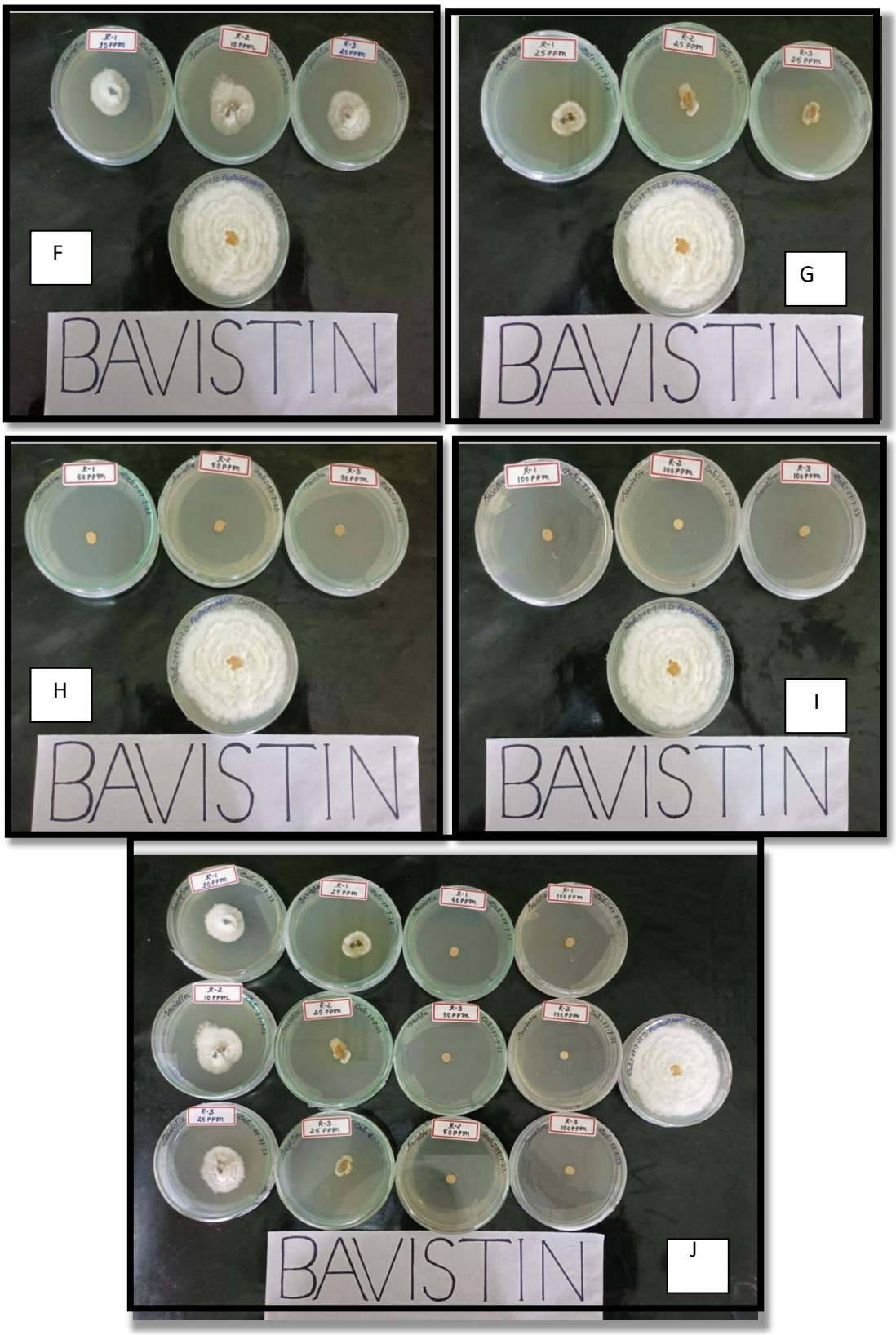


Fig 5.11: Mycelial growth of the pathogen on different concentration of the Fungicide (Bavistin) amended PDA media, F: 10 ppm, G: 25 ppm, H: 50 ppm, I: 100 ppm, J: Complete set of Bavistin for poison food technique

5.4.3 Captaf (Captan 50% WP)

Table 5.3: The inhibitory effect of Captan 50% WP (Captaf) over mycelial growth of *Pestalotiopsis algeriensis*

Fungicide: Captaf A.I – Captan 50%													
Conc. (ppm) →	**10 ppm**			**25 ppm**			**50 ppm**			**100 ppm**			**Control**
Intervals ↓	Radial growth (mm)			Radial growth (mm)			Radial growth (mm)			Radial growth (mm)			Radial growth (mm)
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	
24 Hours	8	6	5	9	5	6	5	5	5	5	5	5	10
48 Hours	12	10	9	13	12	9	13	14	11	6	7	6	15
72 Hours	19	23	15	20	21	21	19	20	17	11	9	8	20
96 Hours	27	30	29	24	27	31	24	24	24	17	17	13	29
120 Hours	29	39	37	32	36	39	29	33	29	19	18	18	52
144 Hours	39	50	42	40	45	44	43	43	39	25	19	22	56
168 Hours	49	58	50	47	56	54	52	51	49	32	29	28	64
192 Hours	55	66	54	53	63	60	60	62	56	44	38	31	72
216 Hours	63	73	60	66	71	68	68	70	65	56	41	36	80
240 Hours	69	83	66	71	77	70	74	75	72	62	52	42	85

A.I- active ingredient of the fungicide

ppm – parts per million, R – Replication, Hrs: Hours

Fig 5.12: Mycelial growth of the pathogen on different concentration of the Fungicide (Captan) amended PDA media, K: 10 ppm, L: 25 ppm, M: 50 ppm, N: 100 ppm, O: Complete set of Captan for poison food technique

Table-5.4: Average Mycelial growth and per cent growth inhibition

Treatment No.	Treatment	Average Diameter of the mycelial growth (mm)				Percent growth inhibition over the control (mm)			
		Concentration of the fungicide (ppm)				Concentration of the fungicide (ppm)			
		10 ppm	25 ppm	50 ppm	100 ppm	10 ppm	25 ppm	50 ppm	100 ppm
T1	Indofil M-45	43.67	38.37	27	-	42.53	49.51	64.47	-
T2	Bavistin	39.67	22.67	-	-	53.32	73.32	-	-
T3	Captaf	72.67	72.67	73.67	59.67	14.50	14.50	14.11	29.8
T4	Control	85				-			
T5	SE CD	2.488.76	1.154.07	1.033.65	1.926.78				

T- Treatment

ppm- parts per million

mm- milimeter

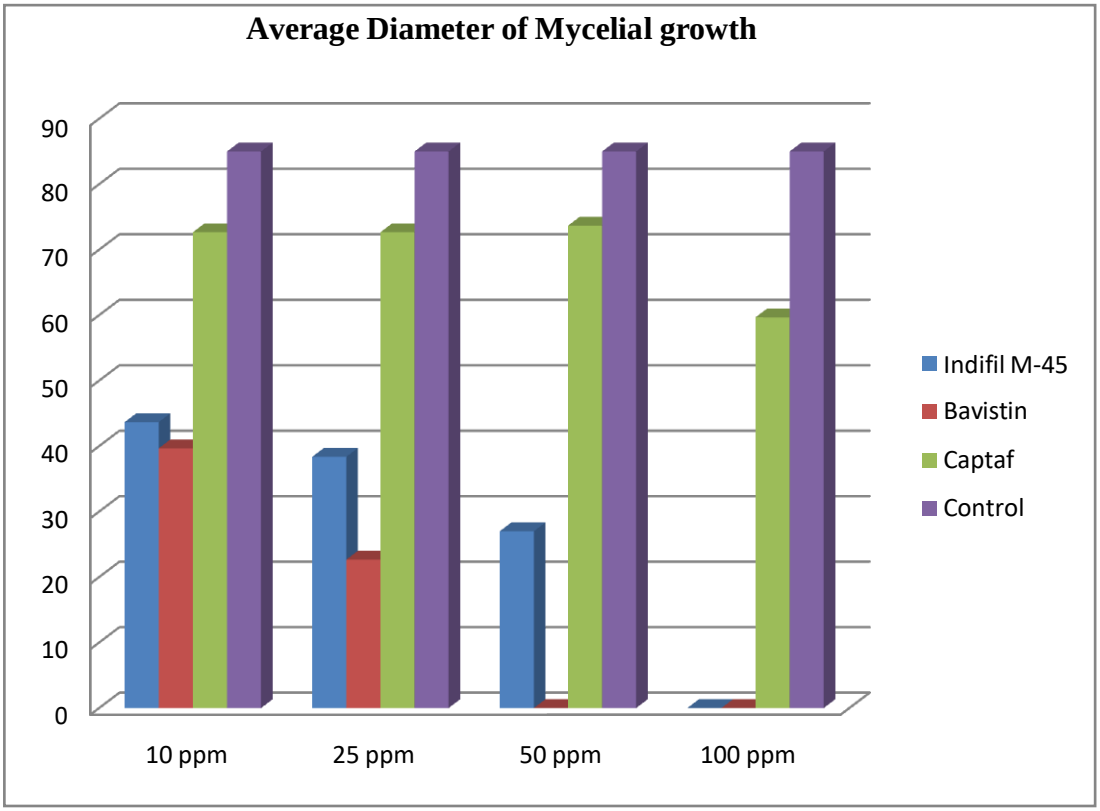


Fig: 5.13: Average diameter of mycelial growth of *Pestalotiopsis algeriensis*

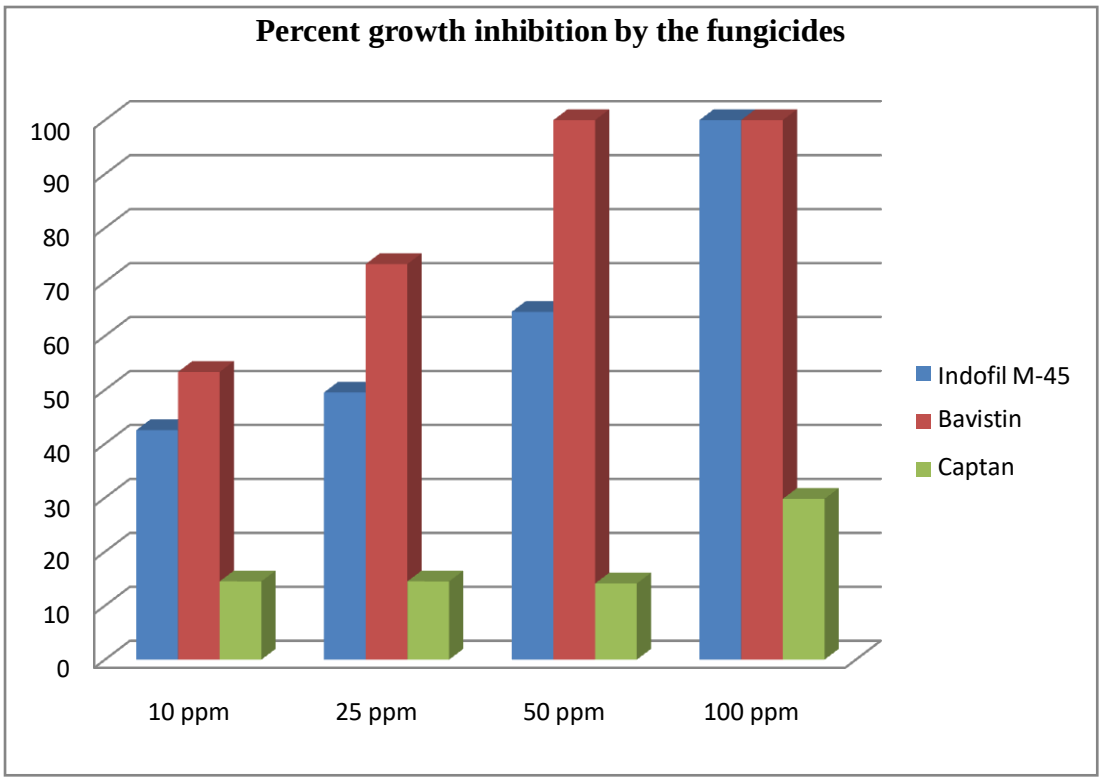


Fig: 5.14: Percent growth inhibition of the pathogen i.e. *Pestalotiopsis algeriensis*

DISCUSSION

CHAPTER VI - DISCUSSION

The present investigation on "Etiology and Management of Leaf Spot Disease in *Magnolia champaca"* was carried out for the scientific study of the pathogen and efficacy of three fungicides (Mancozeb 75% WP, Carbendazim 50% WP and Captan 50% WP) at different concentration for the management of the disease.

Infected leaves of *Magnolia champaca Linn* were collected and symptoms were recorded. The initial symptoms appeared on the leaves of the seedlings of *Magnolia champaca Linn* as tiny grayish brown dots which later increased in size and become large brown to black colored spots. The spots were light brown in the center with a black color margin. The tissues around the spots turned yellowish in color. When the neighboring spots are developed they coalesced to from large infected area.

The isolation was done from the infected leaf of *Magnolia champaca Linn.* The isolated fungus was identified on the basis of the fungal culture and microscopic observation. The Fungus produces smooth, wooly-cottony, white mycelial colony. The conidia were five celled, fusiform, four septate, middle cells were colored and the apical and basal cells were hyaline, most of the apical cells have two hyaline appendages and rarely showed three. On the basis of the above mentioned characters and confirmed identification result was given by Indian Agricultural Research Institute (IARI) the isolated fungus was identified as *Pestalotiopsis algeriensis.* (I.D. No. 11690.22).

Michelia sp. was reported with various kinds of disease caused by different microorganisms. Mahrotra, (1989) reported leaf spot and blight in *Michelia champaca* caused by *Rhizoctoina solani* in the nurseries of Dehradun. Zhang *et al*., (2018), they observed necrotic spots on the mature and young leaves of *Michelia champaca* in China which was caused by *Colletotrichum fioriniae.* Mitra *et al*., (2014) reported that *M. champaca* could be infected by fungus *Phomopsis micheliae* which possesses some harmful effects on *M. champaca*. However, the literatures reveal that the pathogen i.e. *Pestalotiopsis algeriensis* has not been reported earlier on *M. champaca* as a pathogen. Hence it forms a new host record on *M. champaca* and this is the first report from India.

During this study, antifungal potential of three commercially available fungicides with trade name Mancozeb 75% WP (Indofil M-45), Carbendazim 50% WP (Bavistin) and Captan 50% WP (Captaf) were evaluated against leaf spot pathogen of *M. champaca*

Pestalotiopsis algeriensis by poisoned food method. Maximum growth of the mycelial colony of the pathogen was recorded in the control plates. In contrast, maximum percent growth inhibition was observed in the concentration of 25 ppm of Carbendazim 50%WP (Bavistin) followed by 50 ppm of Mancozeb 75% WP (Indofil M-45) which was accounted for 73.32% and 64.47% respectively. Therefore among the three fungicides, Carbendazim 50%WP (Bavistin) was found to be most effective for control the disease followed by Mancozeb 75% WP (Indofil M-45). Therefore Carbendazim 50%WP (Bavistin) may be recommended for the field trial for control of the leaf spot disease of *M. champaca*.

The leaf spot diseases are known as very minor disease but this disease is occur in the nurseries at a very large scale. *Pestalotiopsis algeriensis* produces abundant spores that can be easily dispersed by wind and water movement (splashing from rain or irrigation), so sanitation and water management are critical. Wounds or plant damage often seems to be a prerequisite for disease development, naturally or human activity. Water management requires limiting the length of time when the leaves are wet or exposed to high humidity levels. This includes elimination of overhead irrigation or irrigating in the early morning hours when dew is already present. Leaf wetness can also be decreased by increasing air circulation and increasing plant spacing. In a nursery situation, severely diseased leaves should be pruned and destroyed to reduce spores available to infect healthy tissue. While fungicides may be useful to prevent further spread of the disease. Fungicides alone will not solve the problem. Fungicides are used to prevent further spread of the disease by protecting leaf tissue that has not been infected by the fungal pathogen. In the nursery situation, prune severely diseased leaves prior to fungicide application. These leaves need to be removed anyway, and this will reduce the amount of fungicide used in the process.

SUMMERY AND CONCLUSION

CHAPTER-VII -SUMMERY AND CONCLUSION

The present study was carried out to study the diseased samples of *Magnolia champaca* in nursery at Rajabhatkhawa, West Bengal. The diseased seedlings were brought to the Rain Forest Research Institute for further study. The pathogen was isolated from the infected leaves of the plant on Potato Dextrose Agar. The pure culture of the pathogen was obtained by transferring the radial hyphae of the isolated pathogen on PDA media. The pathogen was observed under microscope and identified as *Pestalotiopsis algeriensis* (I.D No 11690.22) based on its conidial morphological characteristics. The conidia were five celled, fusiform, 4 septate, the middle cells were colored (the upper most two cells were slightly darker then the lower one) and the apical and basal cells were hyaline, most the apical cells had two hyaline appendages and rarely three. The pathogenicity was confirmed through the Koch postulates. The pathogenicity test was performed by pin prick method. After 3 days, similar symptoms were noticed in the artificially inoculated leaves. *P. algeriensis* was re-isolated on PDA medium from the artificially infected leaves. The colony produced by the pathogen resemble with the colony produced by the pathogen of naturally infected leaves.

Three fungicides namely – Indofil M-45 (Mancozeb 75% WP), Bavistin (Carbendazim 50% WP) and Captaf (Captan 50% WP) were taken to test the inhibitory efficiency of these fungicides against the *P. algeriensis*. The fungicides amended PDA media were prepared at a specific concentrations and the pathogen was inoculated in the fungicide incorporated media and incubated for 10 days. The growth of the pathogen was recorded in every 24 hours of interval. After 10 days of incubation period the average value of the mycelial growth of three replication of each concentration were taken and the per cent growth inhibition was calculated.

In Carbendazim 50% WP (Bavistin) minimum mycelial growth was observed in 25 ppm which is accounted for 73% reduction in mycelial growth over control followed by Mancozeb 75% WP (Indofil M-45). Captan 50% WP (Captan) was recorded to have minimum inhibitory effect against the pathogen i.e. *P. algeriensis*. Therefore, the use of 25 ppm concentration of Carbendazim 50% WP (Bavistin) may

be recommended for the management of the leaf spot disease of *M. champaca* caused by *P. algeriensis* in the field.

Conclusion

The leaf spot disease is considered as minor disease in forest. However, under severe infection, it may become epidemic by spreading into new localities. Leaf spot disease affects the physiological activities of the plant; therefore scientific study of this disease is very much important so that it‟s transmission into a new area can be stopped. The fungal disease can be controlled by using suitable fungicides against the causal organism. The present study revealed that, the use of Carbendazim 50%WP (Bavistin) at a minimal concentration of 25 ppm may be recommended for the management of the leaf spot disease of *M. champaca* caused by *P. algeriensis* in nursery condition.

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CHAPTER: VIII-REFERENCE

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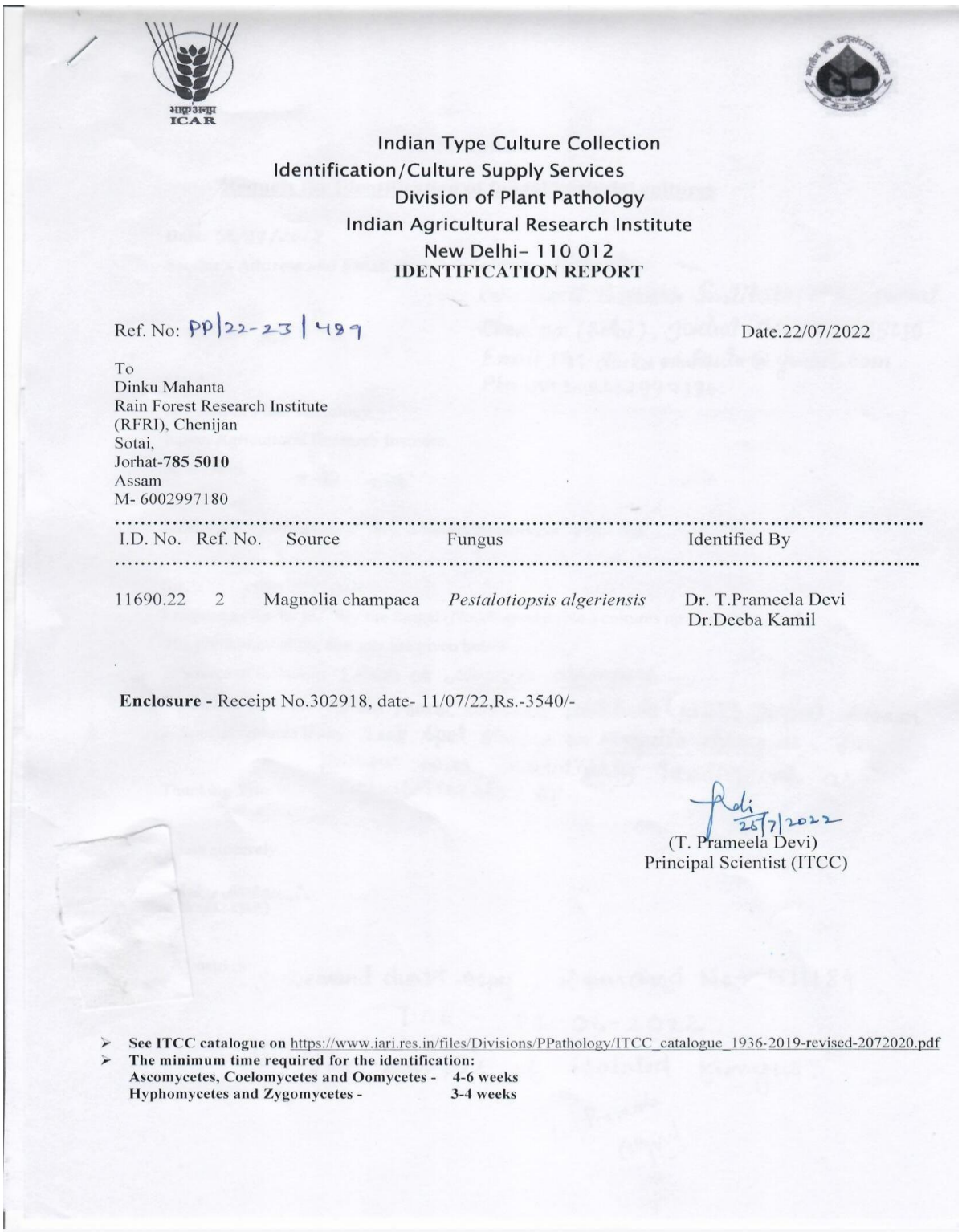
Enclosure-1: Identification Report of the Pathogen



Indian Type Culture Collection
Identification/Culture Supply Services
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Indian Agricultural Research Institute
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IDENTIFICATION REPORT

Ref. No: PP/22-23/489 Date.22/07/2022

To
Dinku Mahanta
Rain Forest Research Institute
(RFRI), Chenijan
Sotai,
Jorhat-**785 5010**
Assam
M- 6002997180

I.D. No.	Ref. No.	Source	Fungus	Identified By
11690.22	2	Magnolia champaca	*Pestalotiopsis algeriensis*	Dr. T.Prameela Devi Dr.Deeba Kamil

Enclosure - Receipt No.302918, date- 11/07/22,Rs.-3540/-

26/7/2022
(T. Prameela Devi)
Principal Scientist (ITCC)

- **See ITCC catalogue on** https://www.iari.res.in/files/Divisions/PPathology/ITCC_catalogue_1936-2019-revised-2072020.pdf
- **The minimum time required for the identification:**
  Ascomycetes, Coelomycetes and Oomycetes - 4-6 weeks
  Hyphomycetes and Zygomycetes - 3-4 weeks