INVESTIGATIONS ON FUNGAL LEAF SPOT DISEASES OF COTTON AND ITS MANAGEMENT

THESIS

Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
in partial fulfilment of the requirments
for the degree of

MASTER OF SCIENCE
IN
AGRICULTURE
(PLANT PATHOLOGY)

By
PRIYANKA PRIYADARSHINI

SECTION OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, NAGPUR

DR. PANJABRAO DESHMUKH KRISHI VIDYAPEETH,
KRISHINAGAR PO, AKOLA (MS) 444 104

Enrolment No – SS-3060 **2022**

DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the thesis entitled **"INVESTIGATIONS ON FUNGAL LEAF SPOT DISEASES OF COTTON AND ITS MANAGEMENT"** or part thereof has neither been submitted for any other degree or diploma of any university, nor the data have been derived from any thesis/publication of any university or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Nagpur **PRIYANKA PRIYADARSHINI**

Date: 17/10/2022 (Enrolment No.: SS-3060)

CERTIFICATE

This is to certify that the thesis entitled "**INVESTIGATIONS ON FUNGAL LEAF SPOT DISEASES OF COTTON AND ITS MANAGEMENT"** submitted in partial fulfilment of the requirement for the degree of **"Master of Science in Agriculture (Plant Pathology)"** of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **PRIYANKA PRIYADARSHINI** under my guidance and supervision.

The subject of the thesis has been approved by the student's advisory committee.

Place: Nagpur
Date: 17/10/2022

(Dr. K. D THAKUR)
Chairman
Advisory committee

Countersigned

Associate Dean,
College of Agriculture, Nagpur
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE INCLUDING EXTERNAL EXAMINER (AFTER VIVA – VOCE)

1.	Chairman	(Dr. K. D Thakur)	________________
2.	Member	(Dr. Shailesh P. Gawande)	________________
3.	Member	(Dr. Tini Pillai)	________________
4.	Member	(Dr. R. M Wadaskar)	________________
5.	External Member	(Dr. R. W. Ingle)	________________

ACKNOWLEDGEMENT

Omm Sai Ram. Being thankful and grateful is the least I can do in comparison to the immense help and support I have received throughout the study. I project my humble gratitude to each and every soul connected with the study and beyond.

It is a great sense of privilege to express my deepest gratitude and sincere thanks to the chairman of the advisory committee Dr. K.D Thakur, Ex-Associate Dean, College of Agriculture, Nagpur, Professor and Ex-Head, Section of Plant Pathology, College of Agriculture, Nagpur for his incredible support, valuable suggestions, guidance, and most importantly a positive outlook towards the research study. Indeed, I am happy to have been blessed with such a fatherly figure during the course of investigation. He has bestowed upon me his treasure of experience and teachings to which I am highly obliged. His constant support to carry out novel attempt in the research has pushed my talents to its limits and I am highly obliged for providing me the necessary facilities for the study which thereby has shaped the successful completion of the project.

Undoubtedly I am lucky to have been guided by Dr. S. P. Gawande, Senior-Scientist (Plant Pathology), Division of Crop Protection, ICAR-Central Institute for Cotton Research, Nagpur, co-chairperson of my advisory committee for his inspiring and ingenious guidance and for enlightening me with his enormous knowledge, wisdom and above all for being calm, composed and very supportive throughout the entire journey, without whom it would have been barely possible to carry out the research. I am thankful for his commendable and critical examination of the thesis and care for originality and quality work throughout the study. His dedication towards research motivates me to work for the scientific community and the society.

I heartily shower my gratitude and love to Dr. Tini Pillai, Assistant Professor, Section of Plant Pathology, College of Agriculture, Nagpur, for her support, guidance and care no less than a mother. Her love and affection has helped me to carry forward the research with courage and enthusiasm. I am thankful for her support during the study.

My deepest and sincere tribute to Dr. R.M Wadaskar, Assistant professor, Section of Agricultural Entomology, College of Agriculture, Nagpur for being the guiding light and providing constant support. I am thankful for his vast knowledge, wisdom, advises of the subject matter and do sincerely thank him for giving me his valuable time and care. He has been very supportive, calm, composed and has always helped me with queries related to the research.

My warmest tribute to Dr. R.W Ingle, Professor and Head Section of Plant Pathology, for imparting in depth analysis of the subject matter, whose guiding hand over my head was no less than a family. I thank him for enriching the study with his impeccable experience and careful analysis. He has always been a great source of wisdom during the entire journey and has enriched my capabilities and knowledge to a higher extent through his experience and teachings.

My sincere thanks to Mrs. Mithila Meshram, Technical Officer (T3) at CICR, for guidance in molecular research work during the course of investigation. She has made me feel homely and has been a constant guidance in the research. I am deeply thankful to her for sharing with me her knowledge and experience in the field of molecular biology. My heartfelt thanks to Mr. Hemant Bawankar at CICR, for constant guidance about the laboratory techniques, support and for being amiable, calm, composed and above all for being patient throughout the investigation.

I take hold of the opportunity to humbly and whole heartedly thank the entire faculty members of Plant Pathology Section, College of Agriculture, Nagpur and scientists from Division of Crop Protection, CICR, Nagpur Dr. Dipak T. Nagrale, and Dr. Neelakanth Hiremani for their valuable advice and care.

I extend my gratitude to Associate Dean, Dr. P. R. Kadu, College of Agriculture, Nagpur for providing with the necessary facilities during the study.

I am blessed to have classmates like Anvesh, Nikita, Grishma, Ravi, Piyush, Rushikesh, Aniket, Rajesh, Vitthal, Ankit and seniors like Shruti, Shubham, Shittal, Snehalata and Sheersha in the division for their valuable support and advice.

The words at my command are insufficient to convey my deepest sense of gratitude to my beloved Jeja, Maa, Papa, Mama, Tiki, Rahul, Nana, Pisa, Dada, Khudi, for their constant love and support. They have been the backbone of my career and the pillar of strength for the goals of my life since my existence and for years to come.

I shall remain indebted to all the souls, who directly and indirectly have a role in such an achievement. The Almighty who has been the constant source of energy every now and then, has made the task possible.

Finally, I am thankful to all the research scholars and scientists whose literature helped me shape the thesis.

Last, but not the least, I would take a moment to congratulate myself for successfully completing the research work assigned to me by the university, within the stipulated time period, never losing hope staying calm and composed during the investigation. This journey motivates me to work for the scientific community.

Jai Jawan, Jai Kisan, Jai Vigyaan.

Place: Nagpur
Date:17/10/2022 **(PRIYANKA PRIYADARSHINI)**

TABLE OF CONTENTS

(A) LIST OF TABLES

(B) LIST OF FIGURES

(C) LIST OF PLATES

(D) LIST OF ABBREVIATIONS

%	-	Per cent
/	-	Per
@	-	At the rate
^0C	-	Degree Celsius
µg	-	Microgram
bp	-	Base pair
µl	-	Microlitre
BLAST	-	Basic Local Alignment Search Tool
CD	-	Critical difference
CICR	-	Central Institute for Cotton Research
cm	-	Centimetre
Conc.	-	Concentration
CIBRC	-	Central Insecticide Board and Registration Committee
CTAB	-	Cetyl trimethyl ammonium bromide
DNA	-	Deoxy ribonucleic acid
dNTP	-	Deoxyribonucleotide triphosphate
EC	-	Emulsifiable concentrate
EDTA	-	Ethylenediamine tetraacetic acid
EtBr	-	Ethidium bromide
et al.	-	et alia (and associate)
etc	-	et. Cetera
Fig.	-	Figure
g		Gram
HCl	-	Hydrochloric acid
$HgCl_2$	-	Mercuric chloride
hrs	-	Hour

i.e.	-	Idest (that is)
ITS	-	Internal transcribed spacer
Kg	-	Kilogram
KCL	-	Potassium chloride
Ltd		Limited
MEGA X	-	Molecular evolutionary genetic analysis
$MgCl_2$	-	Magnesium chloride
ml	-	Millilitre
Mm	-	Millimetre
NCBI	-	National Center for Biotechnology
No.	-	Number
NA	-	Nutrient Agar
PCR	-	Polymerase chain reaction
PDA	-	Potato dextrose agar
Ppm	-	Parts per million
pv	-	Pathovar
R. H.	-	Relative humidity
Rpm	-	Revolution per minute
SC	-	Soluble concentrate
SE	-	Standard error
spp.	-	Species
TAE	-	Tris-acetate EDTA
viz.,	-	Namely
WP	-	Wettable powder

(E) THESIS ABSTRACT

a) **Title of the thesis** : **“INVESTIGATIONS ON FUNGAL LEAF SPOT DISEASES OF COTTON AND ITS MANAGEMENT.”**

b) **Full name of student** : **PRIYANKA PRIYADARSHINI**

c) **Name and address of** : **Dr. K. D. Thakur**

d) **Major Advisor** : Professor, Plant Pathology Section, College of Agriculture, Nagpur
Dr. P.D.K.V., Akola.

e) **Degree to be awarded** : M. Sc. (Agriculture) in Plant Pathology

f) **Year of award of Degree** : 2022

g) **Major subject** : Plant Pathology

h) **Total number of pages in the thesis** : 152

i) **Number of words in The abstract** : 504

j) **Signature of the student** :

K) **Signature, Name and Address of forwarding Authority** :

(Dr. R. W. INGLE)
Professor and Head, Plant Pathology Section,
College of Agriculture, Nagpur

ABSTRACT

Cotton is one of the oldest fibres under human cultivation. It is found to clad the whole world and dominates the Indian textile sector. Due to several leaf spot diseases causing a significant reduction in the

yield and fibre quality of cotton, attempts have been made to study the prevalence of fungal pathogens causing leaf spot in cotton and thereby devise an integrated management practice *in vitro* for the same.

The leaves exhibiting typical leaf spot symptoms were collected from experimental cotton plots C-1 and C-17 of ICAR-Central Institute for Cotton Research, Nagpur and five different fungus isolates were identified such as *Corynespora cassiicola* (CICR-F1, F2, F5, F7 to F15), *Paramyrothecium roridium* (F10), *Rhizoctonia solani* (CICR-F6), *Macrophomina phaseolina* (CICR-F4) and *Nigrospora sphaerica* (F9). The accurate identification of the fungus was done by studying the Internal Transcribed Spacer (ITS) region of rDNA which was amplified using the primers ITS1/ITS4, along with basic morphological identification under the microscope. The pathogenicity of the fungus was proved through Koch's postulate. Then, virulent strains (CICR-F2, CICR-F4, CICR-F6, F9 and F10) were selected for further studies.

Under in vitro conditions, *Bacillus aryabhattai* was found to be most effective in controlling *Corynespora cassicola* (CICR-F2) causing target spot in cotton and *Rhizoctonia solani* (CICR-F6). *Pseudomonas fluorescens* was most effective in controlling *Paramyrothecium roridium* (F10), *Macrophomina phaseolina (*CICR-F4) and *Nigrospora sphaerica* (F9).

Further, *Trichoderma harzianum* isolated from CICR *was* most effective against the test fungal pathogen *Paramyrothecium roridium* (F10) causing Myrothecium leaf spot and *Talaromyces pinophilus* 2 (neem strain) *was* most effective against the test fungal pathogen *Rhizoctonia solani* (CICR-F6), *Macrophomina phaseolina* (CICR- F4)*,* and *Nigrospora sphaerica* (F9*). Trichoderma viride, Trichoderma longibracheatum, Talaromyces purpurogenus* were found most effective against *Corynespora cassiicola* (CICR-F2).

Similarly, carbendazim 50% WP at 0.25% (2500 ppm) was found most effective against *Corynespora cassiicola* (CICR- F2)

Paramyrothecium roridium, (F10), *Rhizoctonia solani* (CICR-F6) and *Nigrospora sphaerica* (F9) showing complete inhibition of the fungal growth over control. On the other hand, fluxapyroxad 167g/L + pyraclostrobin 33g/L at 0.06% (600 ppm), was found most effective against *Macrophomina phaseolina* (CICR-F4). In general, carbendazim 50% WP at 0.25% (2500 ppm), propineb 70WP at 0.25% (2500ppm), pyraclostrobin 5% WG +Metiram 5% at 0.25% (2500 ppm), propiconazole (T5) 0.1% (1000 ppm), fluxapyroxad 167g/L + pyraclostrobin 33g/L (T8) at 0.06% (600 ppm), were found most effective where as kresoxim-methyl 44.3% at 0.1% (1000 ppm) was recorded as the least effective fungicide against the fungal leaf spot disease complex of cotton.

In the present study, *Corynespora cassiicola* causing target spot in cotton was found to be prevalent among the fungal pathogens isolated from the cotton leaf spot samples. Fungal pathogens such as *Rhizoctonia solani* and *Macrophomina phaseolina* which were reported to cause root rot in cotton, have been isolated as leaf spot causing pathogens during the investigation. *Paramyrothecium roridium* (*Myrothecium roridium*) was found to cause target like spots in cotton leaves. *Nigrospora sphaerica*, a saprophytic fungal pathogen is found to cause leaf spot in cotton, which is the first report of its kind in the cotton crop in India.

Chapter I

INTRODUCTION

1.1 Background Information

Cotton (*Gossypium* spp.) is the king of fibres and dominates the Indian economic landscape. It is also known as the white gold and belongs to family malvaceae with diploid chromosome number 26 and tetraploid chromosome number 52. It originated around Central America and is native to tropical and subtropical regions of the world including America, Africa, Egypt and India. The different species of the cotton crop grown in the world are *Gossypium hirsutum* (American Cotton), *Gossypium barbadense* (Egyptian cotton) *Gossypium arboreum* and *Gossypium herbaceum* (Indegeneous Cotton). Global cotton production is 26.11 million tones with consumption of 25.67 million tones. India holds the largest area under cotton cultivation i.e 37% of the global area under cotton cultivation (12.0 mha to 13.5 mha) and is the largest producer (340.62 lakh bales) and consumer (345 lakh bales) (about 24% of world cotton production and consumption) of cotton in the world (Cotton Corporation of India, 2022).

In India, cotton is grown in 9 major cotton growing States *viz*. Punjab, Haryana and Rajasthan in north-western zone; Gujarat, Maharashtra and Madhya Pradesh in central zone; Andhra Pradesh, Karnataka and Tamil Nadu in southern zone and Odisha in the east zone. Maharashtra has the largest area under cotton cultivation followed by Gujarat. The crop is planted in June-July till August, in light well drained black soil and harvested from October to January. India has the advantage of growing all species of cotton i.e. short staple (less than 20mm), medium staple (20.5 to 24.5mm), medium long (25.0 to 27.0mm), long (27.5 to 32.0mm) and extra long staple cottons (32.5mm and above). Cotton nearly holds about 60 million people on its cultivation, marketing, processing and exports purpose. India is also the only country in the world that grows the four cultivated species of cotton along with their intra-and-inter-specific hybrids on a commercial scale. The textile industry, where cotton is a principal raw material, contributes about 4% to the national GDP, thereby acting as the major exchange earner for the country. Hence, growth and development of cotton and cotton based textile industry has a vital bearing on the overall development of the Indian economy (Cotton Corporation of India, 2022).

Leaves are of utmost importance to plant in terms of photosynthesis, pertaining to the yield of cotton fibre. Cotton is a standing crop for more than six months and diseases cause significant losses during cotton cultivation (Tomar, 2005). In India, loss assessment has not been attempted on a national scale nor have techniques of assessment been developed to cover the losses caused due to major diseases. The problem becomes more complicated as all the four cultivated species of *Gossypium* are being grown in India and there are a large number of varieties and hybrids under cultivation providing a favourable atmosphere for diseases and pests. Thus losses are bound to be higher. In India, foliar diseases caused by fungus, bacteria and viruses have been estimated to cause yield losses ranging from 20% to 30% (Mayee and Mukewar, 2007) and majority of released Bt hybrids fall in the category of moderately to highly susceptible diseases (Hosagoudar *et al*., 2008).

The market scenario has become competitive, hence focus has to be made on quality as well as quantity of the fibre production and approximate strategies need to be formulated to lower the cost of production along with production of good quality cotton (Basu, 2001). Plants must be kept disease free and healthy enough for sustained production. Maximum possible yield must be realized from the crop and preventable losses must be reduced. In India 18% of cotton crops' production was lost every year due to different diseases that attacked the cotton plants which had its impacts on losing almost nine hundred thousand of Indian rupees (Hevner *et al*., 2004). Hence, an attempt has been made to isolate leaf spot causing fungus at different stages of crop growth.

1.2 Importance of Study

Disease is a deviation from normal functioning of physiological processes of sufficient duration or intensity to cause disturbance or cessation of vital activities. (American Phytopathological Society,1940). Among several diseases that are known to occur on cotton in India (Srinivasan, 1994) leaf spots are one of the most destructive cotton diseases causing considerable losses in yield (Bashi *et al*., 1983). Indian cotton fields are affected by different fungal leaf spot diseases such as, Myrothecium leaf spot, Corynespora leaf spot (Sandipan *et al.,* 2017). Fungal leaf spot diseases led to 30% loss in cotton production (Monga *et al*., 2011).

Corynespora leaf spot occured throughout the year and affected both mature and immature leaves in rubber crop (Silva *et al.,*1998). Corynespora leaf spot caused by *Corynespora cassiicola* has been increasing its prevalence and severity in cotton growing areas of south-central United States (Butler *et al*., 2016). It caused yield loss of 100 to 200 lb ac^{-1} of lint in cotton (Hagan, 2012) and loss of up to 448 kg/ha of seed + lint of cotton (Bowen *et al*., 2018) with yield losses up to 1,009 kg/ha of soybean (Faske, 2017). In Central India it was responsible for 30 to 40% premature defoliation in cotton cultivar PKV-081, BG-II hybrids, and cultivar Suraj in in the Vidharbha region of Maharashtra (Salunkhe *et al.,* 2019). Corynespora leaf spot was also observed in Maharashtra and Gujarat in recent years (ICAR - AICRP, 2021). Thus, identification of target leaf spot pathogen *Corynespora cassiicola* on cotton is important as it is emerging as a dominant foliar pathogen (Bhattiprolu *et al*.,2022) and hence an efficient management strategy has to be worked out to control the pathogens.

Myrothecium leaf spot of cotton caused by *Myrothecium roridium* affected the cotton fields in southern India (Vairavan, 2021), and accounted to losses of upto 29.1% in the cotton yield (Sandipan *et al*., 2017).

Rhizoctonia solani, the fungus that caused sore shin and damping-off of cotton seedlings, has been reported to cause leaf spot on cotton in Louisiana (Blank, 1953).

Macrophomina phaseolina under high temperatures (30–35°C) and low soil moisture (below 60%), caused substantial yield losses in crops such as soybean and sorghum which had a huge impact on the income of farmers (Kaur *et al*., 2012). Due to great adaptability of the fungus *Macrophomina phaseolina* to a wide range of environmental conditions, it infected a wide range of plants (Salvatore *et al*., 2020).

Nigrospora sphaerica caused leaf spot in medicinal and aromatic grasses which led to substantial yield losses (Mane *et al.,* 2018).

A significant change in the agricultural practices, such as cultivar selection, crop succession, and the intensive use of fungicides of the same mode of action have favoured inoculum survival and build-up and the high genetic diversity provides the pathogen like *Corynespora cassiicola*, an enhanced ability to adapt to different environments, infect a considerably broad range of plants. Drastic climatic change has a great impact on world agricultural production (Houghton *et al*., 1990) which led to increased abnormality in the functioning of the ecosystem. As a result, potential plant pathogens are emerging and causing different diseases. Leaf spot disease complex are causing major destruction to the crop biome. The limited studies assessing yield losses caused by leaf spot that affect economically important crops leave farmers with difficult decisions regarding fungicide application. Hence economically important crops like cotton demands utmost care regarding the diseases that affect it and thus the interests pertaining to this sector command a privileged status. Therefore, it becomes necessary to have a detailed study regarding the pathogens associated with the disease complex along with suitable identification methods and management strategies. Research insights focusing on integrated disease management are proposed to improve leaf spot disease management in the future.

1.3 Objective of Research

Against the backdrop of the situation, research project entitled "Investigations on fungal leaf spot diseases of cotton and its Management" was undertaken with the following objectives.

I. Morphological and molecular characterisation of fungal leaf spot pathogen of cotton.

II. *In vitro* efficacy of bio control agents and label claim fungicides against the fungal leaf spot pathogen.

1.4 Scope and Limitations

Leaf spot disease complex in cotton is a function of numerous pathogens associated with the crop. The symptoms, nature of damage vary from pathogen to pathogen. It affects the crop biome since the crop is sown in the field as in case of soil borne fungi *Rhizoctonia solani, Macrophomina phaseolina,* thereby imposing serious threats to yield and fibre quality of the crop. Fungal foliar pathogens like *Myrothecium roridium, Corynespora cassicola* are known to cause severe defoliation of the crop (Salunkhe *et al.*, 2019).

Due to magnanimous yet short term crop production resulting from green revolution, the world is trapped under the arms of synthetic compounds for crop development and protection. The control of diseases is highly dependent on fungicides and is the most widely used strategy for crop protection for over 200 years. Foliar fungicides are known to be an effective tool to control target spot; however, their extensive applications have led to the progressive development of resistance to fungicides (Tarazi *et al.,* 2019). *Corynespora cassiicola* is classified as a pathogen with a high risk of fungicide resistance development (FRAC, 2019). The entire crop biome is adulterated with the chemical compounds which possess serious threats to our food chain and the humankind. Thus, it necessitates the fact, to have a detailed integrated management package of the disease complex affecting the economically important crops such as cotton with a more nature friendly approach.

In reference to the alarming threat towards the crop biome, the current research work was planned and carried out at the Division of Crop Protection, ICAR-Central Institute of Cotton Research (CICR), Nagpur, and the Plant Pathology Section, College of Agriculture, Nagpur, during 2020-21, with the aforementioned objectives.

1.5 Hypothesis

Significant losses in cotton production are caused due to various diseases in cotton (Monga *et al.,* 2012). The demand for food and fibre to a growing world population is increasing, crop yields must increase on existing farmable land while protecting the environment (Crop Life International, 2014). Cotton is the most important fibre of Indian textile industry and leaf spot diseases affect the yield potential of the crop, ultimately leading to loss in the Indian economy. In the current scenario pathogens like *Corynespora cassiicola, Paramyrothecium roridium, Rhizoctonia solani, Macrophomina phaseolina, Nigrospora sphaerica* are found to have been associated with the leaf spot complex. They cause significant loss to the crop production during different crop stages. These pathogens are multifaceted and cause multiple losses to the crop such as *Rhizoctonia* and *Macrophomina* which cause damping off and root rot in cotton, now also has been reported to cause leaf spot diseases. Hence, molecular identification needs to be done to accurately confirm the pathogens isolated, along with basic morphological studies. *In vitro* evaluation of bioagents and fungicides against the leaf spot pathogens of cotton has been devised for their effective management. Thus, the study has been conducted by using different plant pathological tools and techniques.

Chapter II

REVIEW OF LITERATURE

Cotton (*Gossypium* spp.) owing to its numerous contribution to the Indian economy and the textile sector, demands utmost care regarding the factors that affect the yield potential of the crop and hence it is necessary to have a detailed review of the diseases reported in the crop to see as to what factors are relevant in detailed diagnosis of the leaf spot disease complex of cotton. The literature corresponding to the fungal leaf spot diseases of cotton has been reviewed here with respect to the following objectives:

- Occurrence and symptomatology of the pathogens associated with fungal leaf spot of cotton
- Isolation and pathogenicity of the pathogens
- Morphological characterisation of the associated pathogens
- Molecular characterisation of the pathogens
- *In vitro* efficacy of bioagents against the pathogens isolated
- *In vitro efficacy* of label claim fungicides against the pathogens isolated from the leaf spot complex disease in cotton

2.1 *Corynespora cassicola*

2.1.1 Occurence and symptomatology

Jones (1961) documented the first occurrence of *Corynespora cassicola* in cotton leaves from Mississippi.

Sarbhoy *et al.* (1971) recorded the occurrence of target leaf spot of cotton caused by *Corynespora cassicola* from southern India.

Jacob (2006) reported that since the 1980's, the severity of Corynespora leaf fall disease has been on the increasing trend, and the disease reached an epidemic scale which affected huge areas of rubber plantations and caused severe economic losses.

Schlub *et al*. (2007) reported that *Corynespora cassiicola* infected economically important plants such as rubber, tomato, cucumber, cotton, tobacco and soybean. Eventually the lesions developed outward, with light-brown to greyish centre, dark-brown margins and chlorotic halos. It caused blighting and defoliation that went undetected by growers leading to unperceived crop damage. Under suitable environmental conditions, lesions coalesced, forming large blighted areas on the leaves which led to premature leaf drop.

Godoy *et al.* (2015) reported that in cotton *Corynespora cassicola* affected all above ground parts of plant, and formed small circular to irregular spots and reddish-brown in colour.

Onofre *et al.* (2016) reported that *Corynespora cassiicola* caused leaf spot on blueberry plant (*Vaccinium corymbosum*) in florida.

Xu *et al*. (2016) reported that *Corynespora cassicola* caused leaf spot on sweet potato (*Ipomoea batatas*) in China.

Khaerati *et al. (2018)* reported that leaf fall disease in rubber caused by *Corynespora cassiicola* significantly decreased rubber productivity and caused leaves to fall all year round. This led to delayed tapping of immature rubber plants, decreased yield and death of susceptible clones.

Salunkhe *et al*. (2019) documented the first occurrence of Corynespora leaf spot caused by *Corynespora cassiicola* on cotton in central India.The disease was first observed in 10 to 12 leaf-stage from the lower canopy, which progressed upward to cover the entire plant until harvest. Initially, the lesions were circular to irregular, dark red, small and numerous, later, as lesions matured, alternating rings of light and dark brown bands developed and most mature lesions were seen as a target-type appearance.

Bhattiprolu *et al.* (2022) reported the first occurrence of *Corynespora cassiicola* from south India causing target leaf spot on cotton. Symptoms appeared in about 60-65 days after planting, with minute pinhead size light orange to brick red spots that gradually expanded as circular, oval or irregular concentric spots and finally developed as target spot with yellow halo.

Thus, the fungal foliar pathogen *Corynespora cassiicola* formed target spot on cotton leaves which led to yield loss and premature leaf drop. The pathogen had a wide host range and has been reported worldwide to cause significant damage at different stages of the cotton crop.

2.1.2 Isolation and pathogenicity

Silva *et al.* (1998) reported that *Corynespora cassiicola* isolates from rubber leaves produced water soaked lesions 48 hours after inoculation on tomato. These lesions later became necrotic, and the heavily infected leaves abscissed within five days.

Poltronieri *et al.* (2003) inoculated Malpighia glabra leaves using *Corynespora cassiicola* isolates obtained from several hosts and the isolates from Rhododendrum, tomato, squash and *Malpighia glabra* induced typical symptoms on *Malpighia glabra*.

Dixon *et al.* (2009) identified 50 isolates of *Corynespora cassiicolla* from nine distinct crop species that caused leaf spot disease on cucumber.

Bhattiprolu *et al.* (2022) conducted pathogenicity test on popular hybrid of cotton, Jaadoo BG II using three isolates Lam (CGGL 19001), Koniki (CPIK 19009) and Venkatapuram (CKMB 19011). After seven days of incubation, inoculated plants developed brick red spots initially which later developed into target spot.

Thus *Corynespora cassiicola* was isolated from cotton crop as well other host range and has been reported to be pathogenic in accordance with the Koch's postulate.

2.1.3 Morphological characterisation

Ellis (1957) reported that the conidia of *Corynespora cassiicola* were isolated from leaves and stems of pineapple, chilli, soybean, rubber, lettuce, bean and cowpea. Conidia developed from the apex of conidiophores with an indeterminate growth habit. Conidiophores were distinctively melanized, subhyaline to dark brown, smooth, and septate. Hilum, or the point of conidiophore attachment was distinct and appeared thicker and darker than the rest of the spore.

Qi *et al.* (2011) reported that conidial shapes of *Corynespora cassiicola* isolated from different hosts were obclavate, cylindrical, or oval and curved or straight in shape.

Godoy *et al.* (2015) reported that conidiophores of Corynespora isolates from soybean were erect, branched, brown- colored, single or in clusters with swollen basal cells. Conidia were solitary or in chains (2–6), and measured 7–22 x 39–520 µm.

Ruggiero *et al.* (2015) classified the genus Corynespora into domain eukaryota, kingdom fungi, subkingdom dikarya, phylum ascomycota, class dothideomycetes, subclass pleosporomycetidae, order pleosporales and family corynesporascaceae.

Lopez *et al.* (2018) reported that colonies of *Corynespora cassiicola* initially exhibited whitish grey mycelium and turned dark grey with age on PDA medium.

Bhattiprolu *et al*. (2022) reported that purified colonies of *Corynespora cassicola* isolated from cotton leaves were flat to round and had fibrous texture with dense mycelium measuring 83.43±31.73 x 7.61±1.43 µm.

Thus, the available literature identified the morphological characters of *Corynespora cassiicola* causing target spot in cotton.

2.1.4 Molecular characterisation

Hieu *et al.* (2014) reported that amplification of rDNA ITS for *Corynespora cassicola* from a total of 38 isolates collected from several rubber clones and other hosts in different geographical locations in Vietnam was done using universal primers ITS1 as the forward primer and ITS4 as the reverse primer. PCR amplifications of total genomic DNA primer pair ITS1–ITS4 produced a single PCR product which included ITS1-5.8S rDNA-ITS2 region in all studied isolates and DNA sequencing confirmed that the DNA fragments generated from all isolates were equal in length with 559 bp. The BLAST search results of the nucleotide sequences of isolates showed high similarity between the sequences deposited and identified as *Corynespora cassiicola* in NCBI GenBank database.

Onofre *et al.* (2016) reported that ITS region sequences of *Corynespora cassicola* isolated from blueberry showed 99% identity with several *Corynespora cassicola* accessions in Genbank.

Salunkhe *et al.* (2019) reported that the internal transcribed spacer region of rDNA of *Corynespora cassiicola* from cotton was amplified with ITS-1/ITS-4 universal primers and resulting sequences submitted to GenBank (MG976657, MG976661, and MG976664) showed 100% identity with *Corynespora cassiicola* from avocado (KU593529).

Sushmita *et al.* (2021) reported that the DNA of pathogen causing fungal leaf spot in mungbean and urdbean was amplified using published primers ITS and β-tubulin region. Sequencing and phylogenetic analysis of ITS sequence of the Jhansi isolates along with the reference sequences from NCBI, GenBank revealed maximum identity (98.53 per cent) of isolate with *Corynespora cassiicola* of cowpea reported from Ghana.

Bhattiprolu *et al.* (2022) identified *Corynespora cassicola* from cotton leaves using ITS primers with CTAB method. ITS region of three isolates were amplified using ITS1 and ITS4 primers and obtained amplicon (550 bp) were used for sequencing. BLAST search in GeneBank revealed 100% homology with *Corynespora cassiicola* in cotton having accession numbers of MN393238, MN228955, MN733135, MN288934 and MN393238.

Thus, PCR amplification using universal primers for fungus identification *i.e* ITS-1 and ITS-4 and gel documentation for analysis of base pairs has accurately identified *Corynespora cassiicola.*

2.1.5 *In vitro* efficacy of bioagents

Manju *et al.* (2019) reported that among the bio-agents tested against *Corynespora cassicola* isolated from rubber leaves, *Trichoderma viride* and *Trichoderma harzianum* were found most effective and gave fungal growth inhibition of 66.50% and 65.80% respectively. Similar trend of growth inhibition was observed in Bacillus *subtilis* (60.58%) followed by the least growth inhibition (25.67%) observed for *Pseudomonas fluorescens* against *Corynespora cassicola*

Thus, bioagents were effective in controlling *Corynespora cassicola in vitro.*

2.1.6 *In vitro* efficacy of label claim fungicides

Xavier *et al.* (2013) reported that carbendazim (benzimidazole) at 0.5 µg/ ml showed a complete inhibition of *Corynespora* isolate MES 649 from soybean plant.

Manju *et al.* (2019) reported that complete (100%) mycelial growth inhibition in *Corynespora cassiicola in vitro* was observed using carbendazim 50%WP @ 250 ppm and 500 ppm on rubber plant.

Ishwari *et al.* (2020) reported that among the systemic fungicides, complete (100%) inhibition of mycelial growth of *Corynespora cassicola* isolated from soybean plant were recorded at all the three concentrations of carbendazim i.e at 250, 500 and 1000 ppm.

Yamuna *et al.* (2020) documented that carbendazim @ 0.1% completely inhibited the mycelial growth of *Corynespora cassiicola* isolated from cotton leaves. Further, kresoxim methyl @ 0.1% and azoxystrobin @ 0.05% were found to be less effective with radial growth of 4.46 and 3.50 cm, respectively. Mycelial inhibition ranged from 48.91% (kresoxim methyl @ 0.1%) to 100% (carbendazim @ 0.1%) *in vitro.*

Thus, the available literature documented the efficacy of fungicides against *Corynespora cassicola in vitro*.

2.2 *Paramyrothecium roridium (Myrothecium roridium)*

2.2.1 Occurence and symptomatology

Ciccarone (1953) reported that *Paramyrothecium roridum* caused leaf spot on tomato and gardenia.

Munjal (1960) recorded the first report of Myrothecium leaf spot on cotton. The disease in cotton appeared as small, circular, tan coloured spots with broad violet to brown margin surrounded by zones of translucent areas forming concentric rings. Later dark green sporodochia surrounded by a rim of white hair like mycelia were formed and the spots coalesced resulting in blighting of the affected tissues.

Chauhan *et al*. (1976) observed that prominent symptom of Myrothecium leaf spot of cotton was mainly confined to the leaves when the plant were 30-45 days old in the field.

Raut *et al.* (1980) observed Myrothecium leaf spot on cotton from Vidharbha region of Maharashtra and recorded formation of shot holes when the spots became old.

Srinivasan (1994) observed that Myrothecium leaf spot on cotton caused by *Myrothecium roridum* was prevalent in most countries of Europe, the Middle East, Africa, South Asia, the Far East, New Zealand, China and USA.

Tomar (2005) reported that in central India the disease was well established particularly in the states of Maharashtra, Gujarat and Madhya Pradesh and during 2002-03 and 2003-04, the incidence of *Myrothecium* leaf blight was 41.71% and 38.28 % respectively. The symptoms occurred on cotton leaves, bracts and green bolls. The principal part affected was the leaf on which brown centered spot developed with a dark brown or purple margin. The spots coalesced to from large patches and later the centre dropped leaving a shot hole appearance. There was a conspicuous ring or more than one concentric ring of sporodochia on the spot.

Ingole and Ingle (2011) reported that Myrothecium leaf spot occurred in the vicinity of Akola on Bt Cotton paras atal, Ankur 3028 and also in areas of Buldhana district of Vidharbha. The percent disease intensity on leaves were 1.5 to 2.5%. The disease were observed in foliage, petioles, bracts and bolls.

Li *et al.* (2014) reported that *Myrothecium roridum* leaf spot occurred on many horticulture plants in China, such as *Zantedeschia aethiopica.*

Lombard *et al*. (2016) reported that the former genus, Myrothecium, was subjected to a profound revision which resulted in the recognition of 13 new genera on the basis of the polyphyletic origin of its species, and more than 15 species have been reported within two renamed genera, *Paramyrothecium* and *Albifimbria. Paramyrothecium roridum* was one of the important plant pathogens among them.

Farr and Rossman (2018) reported that *Paramyrothecium roridum* affected various economic crops, including tomato, cucurbits, cotton, soybean.

Matic *et al.* (2019) observed that symptoms of *Paramyrothecium roridium* occurred on leafy vegetables and ornamental crops were initially circular, small 1- to 2-mm grey to brown spots with well defined borders. Spots gradually expanded and coalesced to form necrotic lesions (around 30 mm) with concentric rings, and the affected leaf tissue had a water-soaked consistency.

Kumar and singh (2021) documented that the fungus *Myrothecium roridum* caused leaf spot on bael plant.

Thus, the fungus *Paramyrothecium* roridium has been reported as a leaf spot fungus in cotton and in other host plants.

2.2.2 Isolation and pathogenicity

Munjal (1960) reported that when the cotton plants were inoculated during summer or rainy season infection took place at temperatures ranging from 25°C to 38°C and a high relative humidity was necessary for successful infection, development and sporulation of *Myrothecium roridum* in cotton.

Raut *et al.* (1980) reported that the pathogencity of *Myrothecium roridium* was proved by inoculating foliage of six week old cotton plants of *Gossypium hirsutum* by atomising spore suspension. The fungusproduced typical leaf spots within 8-10 days after inoculation.

Tomar (2005) reported that the symptoms of the disease on cotton began to appear on the third/ fourth day in the form of small spots, which later formed typical zonate leaf spots and the sporodochia were visible from the seventh day.

Zhao *et al.* (2010) isolated *Myrothecium roridium* from common bean which produced colonies with black concentric rings on PDA medium.

Rattan *et al*. (2012) reported that isolates of *Myrothecium roridium* from Mulberry, paraspipal, shisham and dodak produced symptoms on poplar and cotton leaves within 6-7 days after inoculation.

Ning *et al.* (2018) reported that seven days after inoculation on *Brassica oleracea*, symptoms similar to those that occurred under natural conditions were observed on 100% of inoculated plants, whereas the control plants remained disease free and further, re-isolation and identification confirmed Koch's postulates.

Thus, *Paramyrothecium roridium* has been proved to be pathogenic on a wide range of host plants according to Koch's postulates of pathogenicity.

2.2.3 Morphological characterisation

Saccardo (1886) was the first to give a definite description of the fungus *Myrothecium roridum* in the terms "sporodochia discoid or flat, black, white fringed, conidia cylindrical with blunt ends, 8-10 x 2u. biguttulate, pale olivaceous".

Preston (1943) elaborated that the morphology of *Myrothecium roridium* was sessile sporodochia, discoid, circular or irregular in surface view, often confluent in larger masses and without setae. It was green at first, then became black white rimmed. Conidia was cylindrical or very slightly tapering with rounded cells, continuous, 2 to 3 guttulate, hyaline at first, then became became pale green and measured 5- 9×1-2.5 µ,

Ruggiero *et al.* (2015) classified *Paramyrothecium roridum* Basionym: *Myrothecium roridum* into domain eukaryota; kingdom fungi; subkingdom dikarya; phylum ascomycota, subphylum pezizomycotina; class sordariomycetes; sub class hypocreomycetidae; order hypocreales; and family stachybotryaceae

Matic *et al.* (2019) reported that the isolates of *Paramyrothecium roridium* from leafy vegetables and ornamental crops showed nonseptate conidia with a cylindrical to ellipsoidal shape, hyaline to pale green colour, and an approximate size of 6 × 2 µm. Abundant white aerial mycelium and sporodochia had developed in the form of concentric rings that contained dark green to black conidial masses.

Thus, the available literature on *Paramyrothecium roridium* facilitated clear morphological identification of the pathogen.

2.2.4 Molecular characterisation

Kwon *et al.* (2014) reported that PCR amplification was performed using genomic DNA extracted from the prepared fungal mycelium mat of *Myrothecium roridium* isolated from anthurium plant using primers ITS1 and ITS4 to amplify ITS rDNA region and a sequence of 473 bp fragment of the ITS rDNA PCR product was determined, which showed 100% sequence similarity with that of *Myrothecium roridum* BBA71015 (AJ302001).

Naz (2017) reported that PCR DNA amplification was performed using universal primer pairs, ITS4 and NS1 following standard PCR procedures with minor modifications and the sequences obtained showed 99% similarity BLAST search with *Myrothecium roridum* gb: strains BBA 71015 (AJ3020010) and BBA 67679 (AJ301995).

Ning *et al.* (2018) reported that molecular identification of *Myrothecium roridium* isolated from *Brassica* leaves using the rDNA ITS region was done using universal PCR primers ITS-4 and ITS-5 and the amplified product of 624bp (GenBank Accession No. MH050392) was sequenced and identified with BLAST analysis in the GenBank databases which showed a 99% homology to the reported *Myrothecium roridum* isolate BBA 71015 (AJ302001) from GenBank.

Chavhan *et al.* (2018) reported that a highly sensitive real-time PCR (qPCR) assay was used to detect *Paramyrothecium roridum* from pure culture and infected samples of cotton plants using a specific set of primer pair pMyro F/R to target the 185 bp ITS region of rDNA of *Paramyrothecium roridum* species and validated the result using qPCR.

Thus, adoption of molecular techniques like PCR has accurately identified *Paramyrothecium roridium*.

2.2.5 *In vitro* efficacy of bioagents

Ranjini and Naika (2019) reported that the fungal isolates of *Trichoderma harzianum* (CRF-1) and *Trichoderma viride* (CRF-2) were the most effective bioagents against *Myrothecium roridium* isolated from coffee plant, with pathogen inhibition rate up to 100% and 85.90% respectively and it showed hyper-parasitism along with inhibition of the pathogen development. *Bacillus subtilis* (CRB-2) and *Pseudomonas fluorescens* (CRB-5) were more effective in inhibiting the mycelial growth of the pathogen *Myrothecium roridum* up to 55.50% and 50.60% respectively.

2.2.6 *In vitro* efficacy of fungicides

Singh and Shukla (1986) found that benlate ceresan wet, carbendazim 50% WP and thiram completely inhibited the growth of *Myrothecium roridium* isolated from cowpea *in vitro.*

Singh and Narain (2008) reported carbendazim 50% WP completely inhibited (100%) the growth of the fungus *in vitro*.

Sultana and Ghaffar (2009) reported that carbendazim 3% effectively reduced the incidence of *Myrothecium roridum* leaf spot over control.

Ingole and Ingle *et al.* (2011) reported that carbendazim significantly reduced radial growth of *Myrothecium roridum* isolated from cotton leaves as compared to growth in control (7.06 cm) *in vitro*.

Rattan *et al*. (2012) reported that complete mycelial inhibition of *Myrothecium roridium* was recorded with carbendazim 50% WP at a concentration of 50 microgram/ millilitre under *in vitro* condition.

Yamuna *et al*. (2020) reported that carbendazim 50% WP significantly reduced radial growth of *Myrothecium roridum* isolated from cotton leaves compared to control (7.06 cm).

Vishwakarma *et al*. (2021) reported that complete (100%) mycelial growth inhibition was recorded with carbendazim (12%) + mancozeb (63%) @ 0.2%, propiconazole (25%) EC @ 0.1%, and carbendazim 50% WP @ 0.1%.

2.3 *Rhizoctonia solani*

2.3.1 Occurence and symptomatology

Donk (1956) reported that *Rhizoctonia solani* acts as the imperfect stage of *Thanatephorous cucumeris* and caused widespread destructive diseases of cotton in India.

Sneh *et al*. (1996) reported that *Rhizoctonia solani* isolate AG-2-2 and AG-3 were the main causal agent of target leaf spot in tobacco.

Elliott *et al*. (2008) reported that target spot was a foliar disease that first appeared in USA in the 1980s and caused economically important losses in tobacco production. The disease was caused by infection with basidiospores of *Thanatephorous cucumeris* that were produced from hymenia on the soil surface or infected plant tissue.

Gonzalez (2011) reported that Rhizoctonia leaf spot symptoms on tobacco leaves began as small water-soaked lesions on leaves which expanded to large circular spots with concentric rings in high-humidity environments, particularly when leaves grew close together to form a canopy in glasshouse environments. In severe cases, the pathogen might grow from leaf tissue into the stem, resulting in plant death. *Rhizoctonia solani* produced basidiospores that served as a source for rapid and long-distance dispersal of the fungus. The basidiospores germinated to produce hyphae which infected leaves during periods of high relative humidity.

Xie *et al*. (2018) reported that the first occurrence of *Rhizoctonia solani* isolate AG-1-IB caused leaf spot on *Peucedanum praeruptorum* in China. The fungus was previously reported on *Trifolium repens* and *Codonopsis lanceolata* in China. The plants presented symptoms whereby infected leaves initially exhibited circular or irregular, tiny light brown spots and water-soaked lesions. Over time, the spots expanded to become grey, often with some small black dots and dark brown margins. Under high temperature and high humidity, leaves collapsed due to soft watery rot.

Thus, *Rhizoctonia solani* has been reported in different regions with a wide host range as a fungus causing leaf spot diseases.

2.3.2 Isolation and pathogenicity

Lakhsmanan *et al*. (1988) reported that no reliable methods have been devised for inducing perfect stage under controlled condition; as a result many workers have relied on the vegetative and pathogenic characters of the imperfect stage for identification.

Xie *et al.* (2018) conducted pathogenicity test on *Peucedanum praeruptorum* leaves. Ten non-inoculated plants served as the controls. All plants were maintained in a greenhouse at 25^{o}C-30°C with natural light. Three days after inoculation, all treated leaves developed spot symptoms similar to those observed on naturally infected leaves in the field, whereas the control plants remained asymptomatic. *Rhizoctonia solani* was reisolated from all inoculated plants but not any of the control plants.

Thus*, Rhizoctonia solani* caused leaf spot diseases in several plants and has been proved to be pathogenic according to Koch’s postulates.

2.3.3 Morphological characterisation

Naito and Sugimoto (1978) reported that in sugar beet leaves basidiospores originated from basidia on the soil surface and after germination at primary infection sites, invading hyphae grew on the leaf surface and enter through stomata to create secondary lesion sites. Spores were formed, thereby completing the life cycle and generating inoculums that infected other susceptible hosts.

Keijer *et al*. (1996) reported that *Rhizoctonia solani* could survive for many years in soils by forming sclerotia or as a saprophyte and colonized soil organic matter. Sclerotia and/or mycelium found in soil and/or in plant tissue eventually activated to produce vegetative hyphae that attacked a wide range of crops.

Roberts (1999) observed that hymenia produced basidia bearing four ellipsoid to oblong, hyaline basidiospores [4–5.5(6.5) mm, 7–10 mm].

González-García *et al*. (2006) reported that sexual fruiting bodies of Rhizoctonia were typically characterized by the presence of a hypochnoid, thin basidiomata that possessed a hymenium made up of successive layers of basidia which rose from vertical branching and a cymose hyphae just above the basal hyphae with four septate sterigmata.

Gonzalez (2011) observed that *Rhizoctonia solani* isolated from tobacco leaves had hyaline hyphae formed at right angles and later turned buff to dark brown in colour. Cells lacked clamp connections, but possessed a complex dolipore septum with continuous parenthesomes and were multinucleate. Hyphae were variable in size, ranging from 3 to 17 mm in diameter. Instead of conidial structures, ellipsoid to globose, barrel-shaped cells, named monilioid cells, 10–20 mm wide were produced in chains and gave rise to sclerotia. Sclerotia were irregularly shaped,up to 8–10 mm in diameter and light to dark brown in colour.

Ruggiero *et al.* (2015) classified the genus Rhizoctonia into domain eukaryota, kingdom fungi, subkingdom dikarya, phylum basidiomycota, subphylum agaricomycotina, class agaricomycetes, order cantharellales and family ceratobasidiaceae.

Xie *et al.* (2018) observed that all the fungal colonies were light brown in colour, exhibited fast growth, and formed circular shapes with radial margins on PDA. Sclerotia were dark brown, irregularly shaped, and produced after 5 days on PDA. Hyphae were branched at right-angle with a slight septal constriction at their bases.

Yadav (2020) reported that *Rhizoctonia solani* isolated from roots of cotton was a non-spore forming soil borne fungus and its propogules i.e sclerotia were evenly distributed in the soil.

Morphological identification of *Rhizoctonia solani* has been done according to the available literature.

2.3.4 Molecular characterisation

Chakrabarty (2007) conducted PCR amplification of rDNA sequences of *Rhizoctonia solani* isolated from cotton using ITS1 and ITS4 primers. Analysis of rDNA fragments from fungal strains revealed the presence of partial sequences of 18S and 28S rRNA genes and complete sequences of ITS 1 and ITS 2 along with 5.8S rRNA gene. The obtained was *Rhizoctonia solani* DQ339103 which confirmed the presence of the pathogen.

Dragana *et al.* (2007) conducted sequencing of *Rhizoctonia solani* rDNA isolates RhKZ, RhBG, RhGL and RhVR from sugarbeet roots which showed that sizes of the ITS region varied from 713 to 716 bp and their sequences were identical (100%) in the ITS region during BLAST search.

Al-Fadhal *et al.* (2009) observed that PCR products with sizes ranging between 600 and 650 had PCR-amplified ITS region (ITS1, 5.8S rDNA, and ITS4) of *Rhizoctonia solani* isolated from cucumber. The isolates were sequenced and the generated nucleotide sequences were subjected to a BLAST search which showed that the highest genetic similarity was 97% and 96% with the *Rhizoctonia solani* isolates previously identified in Iraq (KX828173.1 and KF372660.1 respectively).

Xie *et al*. (2018) documented the PCR amplification of rDNA ITS region of *Rhizoctonia solani* isolated from Peucedanum leaves with primers ITS1 and ITS4. Sequence analysis revealed that the isolate QH1 (GenBank accession no. MF447834) shared 100% identity with *Rhizoctonia solani* AG-1-IB (HF678122).

Wallon *et al*. (2020) conducted sequencing of the 60 *Rhizoctonia solani* isolates collected from infected lettuce. Isolates AG-BI 370-P3-A and 523-1 showed 100% and 99.8% identity with AG-BI MK583630, respectively.

Thus, molecular techniques like PCR using universal primers ITS- 1 and ITS-4 have been used to accurately identify *Rhizoctonia solani.*

2.3.5 *In vitro* efficacy of bioagents

Behere *et al.* (2003) observed that the maximum percent growth inhibition of *Rhizoctonia solani* isolated from cotton roots was recorded from *Trichoderma viride* (GTB) 79.44%, closely followed by *Trichoderma harzianum* (GTB) 77.88% and *Trichoderma viride* (CICR) 75.22.

Yadav (2020) observed that maximum and significant per cent inhibition of *Rhizoctonia solani* isolated from cotton roots was recorded by *Trichoderma viride* (T-5) to be 84.5% in dual culture method, followed by *Trichoderma viride* (T-3) with 80.0% growth inhibition while *Trichoderma harzianum* (Th.J. 89-2) showed 77.7 % mycelial growth inhibition.

2.3.6 *In vitro* efficacy of label claim fungicides

Chandra *et al.* (2016) reported that propiconazole, carbendazim and hexaconazole completely inhibited the mycelial growth of *Rhizoctonia solani* isolated from rice *in vitro.*

Yadav (2020) observed that carbendazim was found most effective which completely (100%) inhibited the mycelial growth of *Rhizoctonia solani* isolated from cotton roots at all concentrations (50, 100, 250 and 500 ppm).

Usendi *et al.* (2020) observed that carbendazim 50% WP completely inhibited the radial growth of the fungus *Rhizoctonia solani* isolated from rice *in vitro.*

2.4 *Macrophomina phaseolina*

2.4.1 Occurence and symptomatology

Smith and Carvil (1997) reported that typical symptoms of *Macrophomina phaseolina* infection on soybean were yellowing and senescence of leaves that remained attached to the stems by the petioles. The cortical tissues sloughed off from the lower stem and taproot which appeared grey in colour due to the abundance of microsclerotia resulting in a premature death of the host plant.

Dadwal and Savitri (2012) reported that *Macrophomina phaseolina* caused leaf spot disease on *Chlorophytum borivilianum* in Jabalpur .

Ghosh (2018) reported that *Macrophomina phaseolina* was a generalist soil-borne fungus affecting at least 500 plant species in more than 100 families. It caused diseases such as stem and root rot, charcoal rot and seedling blight.

Huda- shakira and Rahim (2019) reported that *Macrophomina phaseolina* caused leaf spot in *Crinum asiaticum* and *Hymenocallis* *littoralis plants*. The symptom appeared as irregular brown to reddish lesions surrounded by yellow halos. As the disease progressed, the infected leaves became blighted, dried, and fell off with the presence of black microsclerotia and pycnidia on the lession.

Meena and Vasudha (2022) reported that *Macrophomina phaseolina* caused leaf blight disease on Chlorophytum leaves. The affected leaves showed characteristic symptom of necrosis spots on tips and outer margin of leaves, which gradually progressed and cover large portion of leaf lamina. Severe infection caused premature drying of leaves, which gave blighted appearance as observed on safed musli plant in ICAR- Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat.

Thus, *Macrophomina phaseolina* has been reported worldwide as a fungus that caused leaf spot on a wide range of host.

2.4.2 Isolation and pathogenicity

Fuhlbohm *et al.* (1996) isolated *Macrophomina phaseolina*, causing leaf spot of mungbean in Australia. Koch’s postulates were fulfilled and inoculum source was considered to be microsclerotia of the fungus in soil which splashed onto the leaves.

Dadwal and Savitri (2012) observed that symptoms consisted of small brown spots on the leaf surface of *Chlorophytum borivillianum*, which resulted in transparent lesions at the advanced stage. Black pycnidia developed on the margin of the spot. Severely infected leaves dried prematurely and the plants failed to produce healthy tubers, resulting in a 40% yield loss in severely infected fields.

Huda-shakirah and Rahim (2019) conducted the pathogenicity test and proved the role of the fungus *Macrophomina phaseolina* in causing leaf blight on *Crinum asiaticum* and *Hymenocallis littoralis* plants.

Salvatore *et al*. (2020) reported that phytotoxic metabolites were produced by *Macrophomina phaseolina* isolated from *Eucalyptus globulus* were phaseolinon, botryodiplodin and patulin, which played a significant role in the initial stages of infection and caused wilting of seedlings along with formation of necrotic lesions on leaves and roots.

Ekhuemelo and Chikwado (2020) observed leaf spot lesions on sweet potato plants inoculated with *Macrophomina phaseolina*.

Thus, *Macrophomina phaseolina* was proved to be pathogenic on a wide range of host.

2.4.3 Morphological characterisation

Ruggiero *et al.* (2015) classified the genus *Macrophomina* into domain eukaryota, kingdom fungi, subkingdom dikarya, phylum ascomycota, subphylum pezizomycotina, class dothideomycetes, subclass dothideomycetes, order Botryosphaeriales, and family Botryosphaeriaceae.

Lakhran (2018) observed that fungus *Macrophomina phaseolina* isolated from chickpea roots was characterized by hyaline hyphae with thin walls to light brown or dark brown hyphae with septa. Branches from the main hyphae were generally formed at right angle on parent hyphae with constriction at the point of origin. Microsclerotia were spherical, oval or oblong, light brown in the young stage which became darker (brown to black) with ageing. Pycnidia were observed under natural conditions and were dark brown to black in colour with globose or irregular in shape.

Meena and Vasudha (2022) observed that mycelium of *Macrophomina phaseolina* isolated from chlorophytum leaves showed dark olive black colonies which ultimately turns black and were found embedded with abundant of microsclerotia. Morphologically microsclerotia were black, smooth, round to oblong, 49.88 - 99.74 ± 5.62 μm in diameter. Pycnidia were found exclusively on the infected leaves of *Chlorophytum borivilianum and were* dark to greyish, globose and membranous measuring 83.62-123.88 ± 3.77 µm in diameter.

The literature reviewed on morphology has been used to identify the fungus *Macrophomina phaseolina.*

2.4.4 Molecular characterisation

Almomani *et al.* (2013) conducted the PCR amplification of the ITS region and indicated that the different isolates of Macrophomina belonged to one single species *Macrophomina phaseolina*.

Huda-shakirah and Rahim (2019) reported that DNA sequences of ITS region and translation elongation factor 1-alpha (TEF1-α) gene accurately identified *Macrophomina phaseolina* as causal pathogen of leaf spot of lilies.

Pandey *et al.* (2020) observed that sequence of ITS portion of 18S rRNA of all the three isolates isolated from mungbean, urdbean, and vegetable soybean were identical. These sequences showed 99% similarity with the ITS sequences of M. phaseolina isolates from common bean, (KU831500.1), cowpea, (KF951783.1), mungbean (KF951636.1),urdbean (KF951637.1), potato (KU721993.1), and cotton (KX270356.1) in BLAST search.

Meena and Vasudha (2022) confirmed that the fungus isolated from Chlorophytum leaves were identified as *Macrophomina phaseolina* by molecular sequencing of ITS of rDNA region and actin gene.

Molecular characterisation techniques like PCR have been used to accurately identify *Macrophomina phaseolina.*

2.4.5 *In vitro* efficacy of bioagents

Khan and Gupta (1998) reported that radial growth of *Macrophomina phaseolina* on PDA isolated from eggplant was greatly suppressed by *Trichoderma harzianum and Trichoderma viride*.

Khalili *et al*. (2016) reported that T2, T10 and T12 isolates of *Trichoderma harzianum* exhibited growth inhibition of *Macrophomina phaseolina* isolated from soybean *in vitro* as 72.31%, 45.23% and 44.13% respectively and showed exceptional hyperparasitism against the colonies of *Macrophomina phaseolina*. A closer examination revealed significant growth of the *Trichoderma harzianum* hyphae over the pathogen growth and new colonies of the antagonists had overlapped the colonies of the phytopathogen *Macrophomina phaseolina*

Ahmed *et al.* (2021) reported that *Trichoderma viride* was found to be the most effective biocontrol agent against *Macrophomina phaseolina* isolated from green gram roots, followed by *Trichoderma harzianum. Pseudomonas fluorescens* was found to be the least effective against the fungus.

Nagrale *et al.* (2022) conducted bi-compartmental petri plate assay against *Macrophomina phaseolina* isolated from cotton roots which showed that the CICR-D3 strain (*Bacillus cereus*) was the most effective in inhibiting the mycelial growth (86.70 ± 0.50%), followed by the CICR-D5 (*Bacillus aryabhattai*) (69.27±0.17%) and CICR-H3 (*Bacillus tequillensis*) (62.84±0.50%) strains.

2.4.6 *In vitro* efficacy of label claim fungicides

Iqbal and Mukhtar (2020) reported that carbendazim, propineb and meatalaxyl + mancozeb proved to be effective against *Macrophomina phaseolina in vitro.*

Savaliya *et al.* (2020) reported that propiconazole and tebuconazole gave 100% inhibition against *Macrophomina phaseolina* isolated from sesame. Carbendazim 12% + mancozeb 63% (2000 ppm) and pyraclostrobin 13.3% + epoxyconazole 5% (500 ppm) also gave 100% inhibition of mycelial growth.

2.5 *Nigrospora sphaerica*

2.5.1 Occurence and symtomatology

Farr and Rossman (2013) reported that *Nigrospora sphaerica* was frequently encountered as a secondary invader or as a saprophyte on many plant species and also as a causative organism of foliar disease on several hosts worldwide.

Abass and Najlaa (2014) observed *Nigrospora sphaerica as a* true pathogen of date palm trees which exhibited severe symptoms of leaf and stem spot diseases. Most severe symptoms were observed on young date palm leaves and stem.

Dutta (2015) documented the first report of *Nigrospora sphaerica* as a foliar pathogen of *Camellia sinensis* in Darjeeling, West Bengal, India. The foliar symptoms were characterized by greyish to brown, semicircular or irregular shaped lesions, often surrounded by pale yellow zones up to 9 mm in diameter. The lesions later expanded and the affected leaves turned greyish to dark brown and eventually the dried tissue dropped leading to complete defoliation of the plant. The disease caused damage to leaves of all ages and was severe in young leaves.

Chen *et al.* (2016) documented the first report of *Nigrospora sphaerica* as a leaf spot pathogen of kiwi fruit in China.

Ismail and Nur (2021) documented first report of *Nigrospora sphaerica* causing leaf spot of watermelon in Malaysia which reduced fruit quality and caused premature defoliation that reduced watermelon productivity.

Qiu (2022) observed leaf spot symptom in false daisy *Eclipta prostrata* in China. The isolated and purified fungus CQLC820 was confirmed as *Nigrospora sphaerica.*

Thus, *Nigrospora sphaerica* has been reported as leafspot causing pathogen.

2.5.2 Isolation and pathogenicity

Wright *et al.* (2008) isolated *Nigrospora sphaerica* from leaves of blueberry plants.

Abass and Najlaa (2014) isolated *Nigrospora sphaerica* from heavily infected date palm leaves. 30 days post-inoculation, infection with *Nigrospora sphaerica* produced 1.42 cm lesions on leaves.

Dutta (2015) proved the pathogenicity of *Nigrospora sphaerica* on tea leaves.

Chen *et al.* (2016) reported that no symptoms developed on leaves which were treated with sterile agar pieces without fungal culture while *Nigrospora sphaerica* was consistently reisolated from the margins of artificially inoculated experimental leaves.

Hence, *Nigrospora sphaerica* has been proved to be pathogenic in accordance with Koch’s postulate.

2.5.3 Morphological characterisation

Ruggiero *et al.* (2015) classified the genus Nigrospora into domain eukaryota, kingdom fungi; subkingdom dikarya, phylum ascomycota, subphylum pezizomycotina, class sordariomycetes, subclass xylariomycetidae, order xylariales and family apiosporaceae.

Dutta (2015) reported that conidia were spherical to sub spherical, single-celled, black, 19 to 21 μm in diameter, and were borne on a hyaline vesicle at the tip of each conidiophores.

Mane (2020) reported that fungal colonies were initially white, becoming light to dark grey with the onset of sporulation with black, spherical to subsphaerical conidia that measured 18.11 to 21.61 × 15.16 to 19.08 µm and were borne on a hyaline vesicle at the tip of each conidiophores.

Thus, *Nigrospora sphaerica* was identified in accordance with basic literature available on morphological characterisation.

2.5.4 Molecular characterisation

Abbas and Najlaa (2014) conducted molecular characterization of *Nigrospora* species isolated from leaves of date palm.The ITS region of rDNA was amplified with ITS-1 and ITS-4 primers and ITS sequence analysis showed 99% of identity with a total of 500 bp for *Nigrospora sphaerica.*

Dutta (2015) reported that ITS region of the ribosomal DNA isolated from tea leaves was amplified by using primers ITS-1 and ITS-4 and sequence results (GenBank Accession No. KJ767520) showed 100% similarity to *Nigrospora sphaerica* (KC519729.1).

Chen *et al.* (2016) reported that ITS region of the ribosomal DNA isolated from kiwi leaves were amplified using universal ITS-1 and ITS-4 primers followed by the sequencing of the PCR products and the ITS sequences showed 99% homology with that of *Nigrospora sphaerica* (GenBank accession no. KJ767121. 1).

Hiremani (2020) reported that the ITS region analysis was used for species-level identification of the fungal endophytes in cotton, wherein Nigrospora was found dominant.

Thus, molecular characterisation by PCR techniques has accurately identified *Nigrospora sphaerica*.

2.5.5 *In vitro* efficacy of bioagents

Mane *et al.* (2018) observed that in dual culture technique, maximum growth inhibition of *Nigrospora sphaerica* (67.88%) was observed with *Trichoderma viride* followed by *Pseudomonas fluorescens* and *Bacillus subtilis*.

2.5.6 *In vitro* efficacy of label claim fungicides

Mane *et al.* (2018) reported that carbendazim (0.1%) was recorded effective against *Nigrospora sphaerica.*

Chapter III

MATERIAL AND METHODS

The study on "Investigations on Fungal Leaf spot Diseases of Cotton and its Management" was carried out during *Kharif* 2021-2022 in the laboratory of Division of Crop Protection, ICAR–Central Institute for Cotton Research, Nagpur and Plant pathology laboratory, Plant Pathology Section, College of Agriculture, Nagpur. Experiments were conducted in the glasshouse as well as in the laboratory. The materials used and methods followed in the research work are described in this chapter. The standard laboratory techniques followed were according to the standard procedures given by Dhingra and Sinclair (1985).

3.1 Materials

3.1.1 Collection of the diseased sample

Fresh specimens of cotton leaves showing typical symptoms of leafspot were collected from experimental plots C-1 and C-17 of ICAR-Central Institute for Cotton Research, Nagpur in the *Kharif* season of year 2021-22 from mid to late season cotton (*Gossypium hirsutum*) variety RCH-659 and Suraj NBt (non Gm).

3.1.2 Glasswares

The glasswares used in the investigation were conical flask (100ml, 250ml, 500ml), petriplates (90mm)(Borosil); beaker (Tarsons), testtubes (Genaxy), measuring cylinder, microscope slides and cover slips.

3.1.3 Equipments

Equipments used during the course of investigation includes Hot Air Oven, Autoclave, Microwave (Kenstar), Digital Weighing Balance, Laminar Flow Ultra clean Air Unit, Refrigerator (4^oC, BPL,LG), Deep Freezer (-20^oC, Vest Frost), Ultra Low Temperature Freezer (-80^oC, Eppendorf), Incubator, Micropipette, Pipette tips, Centrifuge (Eppendorf), PCR Machine (ABI Thermocycler), PCR Tubes, Water Bath, Vortex Shaker, Gel Casting Tray, Gel Electrophoresis Apparatus, Gel Documentation Unit (Biorad), Ice flecking Machine (Ziegra), Computer (Acer).

3.1.4 Miscellaneous material

Inoculating needle, Scalpel, Forceps, Spirit lamp, Cotton, Sodium Hypochlorite (0.1 %), Dessicator, Cork borer, Label, Polythene bags, Permanent marks, Blotting paper, Tissue paper, Ethanol (70 % and 100%), Sterile distilled water.

3.1.5 Sterilisation

Sterilisation was done to remove any forms of living cell, spores, virus from the working area, and glasswares. Chemical washing gel (Hiclean cleaning agent, Himedia) was used to clean the glasswares and further it was thoroughly cleaned with tap water. The glasswares were sterilised in hot air at 160^{o}C for 2 hours and nutrient media were sterilised in an autoclave at 15 PSI with 121^{o}C for 20 minutes. Before inoculation, the laminar chamber was sterilised by UV Light for 20 minutes. During inoculation, laminar airflow bench was surface sterilised by wiping with tissue paper dipped in Ethanol (70%). Inoculation needle and loop, scalpel, cork borer were sterilised by dipping in ethanol followed by flame sterilisation.

3.1.6 Culture media

3.1.6.1 Preparation of 1Litre Potato Dextrose Agar (PDA):

I. Potato (peeled) : 200.00 g
II. Dextrose : 20.00 g
III. Agar Agar : 20g
iv. Distilled Water : 1000ml

3.1.6.2 Preparation of 1Litre Potato Dextrose Broth (PDB):

I. Potato (peeled) : 200.00 g
II. Dextrose : 20.00 g
III. Distilled water : 1000 ml

3.1.6.3 Preparation of 1Litre Nutrient Agar:

I. Nutrient Agar Powder : 28 g
II. Distilled water : 1000 ml

3.1.7 DNA extraction and purification buffer

I. Extraction Buffer

Sr. No.	Materials for DNA ExtractionBuffer	Concentration (ingrams) for 200 ml	Concentration (in grams) for 100 ml
1.	Tris	20	10
2.	EDTA	8	4
3.	NaCl	56	28
4.	CTAB	40	20
5.	PVP	2	1
6.	Dicea	0.2	0.1
7.	Glucose	18	9

II. Liquid Nitrogen

III. Chloroform/Isoamyl mixture

IV. Isopropanol

V. Wash Buffer : 70% Ethanol

3.1.8 PCR master mix

Regeants 1 Sample (in microlitre)

I. Buffer : 5

II. dNTP : 0.4

III. Mgcl$_2$: 1.5

IV. Pri F : 1

V. Pri R : 1

VI. Taq : 0.3

VII. Nuclease Free Water : 15.8

3.1.9 Agarose gel electrophoresis

I. 1.5 % Agarose (1.5 g Agarose powder)

II. Ethidium Bromide (4 µl)

III. TAE buffer (100 ml)

IV. 6X Gel loading dye

V. Ladder (500 bp)

3.1.10 Culture of bacterial bioagents and fungal bioagents

The pure culture of different bacterial and fungal bioagents were obtained from the culture collection of Plant Pathology Section, Division of Crop Protection, ICAR–Central Institute for Cotton Research, Nagpur and are mentioned in Appendix I.

3.1.11 Fungicides

The fungicides used for poison food technique (Grover and Moore, 1962) against different pathogens isolated are mentioned in Appendix I.

3.2 Methods

3.2.1 Collection of diseased samples

The fresh specimen of cotton leaf samples showing typical symptoms of fungal leaf spot were collected from the cotton experimental fields of ICAR-Central Institute for Cotton Research, Nagpur, in a separate polythene bags to avoid contamination. It was brought in a box packed with ice pack to the plant pathology laboratory of ICAR-CICR, Nagpur for further study.

3.2.2 Preparation of media

Potato Dextrose Agar (PDA) medium:

Potato pieces (disease free and properly peeled-200 g) were boiled in 500ml of distilled water taken in a glass beaker (1000ml). Then the resultant potato infusion was filtered through a muslin cloth and the filtrate was collected in a separate glass beaker (1000ml). In another 500 ml of distilled water taken in glass beaker (1000ml), 20g of Agar-Agar (solidifying agent) was added and dissolved by boiling. The filtrate and dissolved agar solution were mixed, 20g of Dextrose (to encourage luxuriant fungal growth) was added to the above mixture and volume was made upto 1000ml using distilled water. The lid of the flask was covered with cotton plug, wrapped with paper, tied and labelled as PDA along with the date of preparation of the media.

3.2.3 Isolation of fungi

The leaves showing typical symptoms were examined under the microscope for the presence of fungal spore and mycelium. The standard procedure for tissue isolation was followed. The leaf samples were then rinsed with sterile water and sterilised with 70% ethanol.The infected tissues along with small adjacent unaffected healthy tissue were cut into small pieces (2-3 mm length) with the help of a flame sterilized scalpel. Then, the tissues were further transferred into sterile petri dish containing 0.1% sodium hypochlorite for a period of 20-30 seconds in laminar air flow cabinet. Then, the samples were rinsed in three changes of sterile distilled water to remove the traces of sodium hypochlorite and were dried on sterilized blotting paper to remove excess moisture. Thereafter, the small tissues (3 bits per plate) were aseptically placed on potato dextrose agar (PDA) medium already poured and solidified in sterilized petri plates. The plates were wrapped with parafilm followed by incubation at 27 ± 2^0C and were observed periodically for the growth of fungal pathogen on the basis of visible colony appearing around the tissues.

3.2.4 Pure culture of the isolated organisms

Pure Culture of fungal isolates was done by transferring a small bit of hyphae to PDA slants in aseptic condition.

3.2.5 Maintenance of cultures

The cultures of fungi were maintained on PDA slants as well as PDA petriplates and stored at low temperature in the refrigerator at 5^0C. The culture isolates were sub cultured once in a month to maintain the viability of the organisms.

3.2.6 Identification of the isolated organisms

The fungal isolates were identified by observing the growth on media, colony characteristics and observations made from the slides (prepared directly from the active cultures) viewed under microscope. The morphological features like colour, branching and septation of hyphae, conidiophores and conidia of the isolates were studied under compound microscope(10x, 40x magnification). The growth characters and morphology were compared with available descriptions from mycological books such as Hyphomycetes (Subramaniam, 1971), Morphology and taxonomy of fungi (Bessey, 1950), Manual of soil fungi (Gilman, 1957). The fungal isolates were further undergone through the process of molecular identification.

3.2.7 Morphology

The Morphological characters of different fungal pathogens isolated from the affected spots in the leaves were studied. Seven days old cultures grown on the (PDA) media incubated at $27\underline{+}2^0$C were used. The slides of each pathogen were prepared aseptically in laminar air flow cabinet by using lactophenol (more for dark coloured fungal colony) and cotton blue stain (more for light coloured fungal colony) followed by observation under compound microscope for morphological features like colour, branching, septation of hyphae, conidiophores and conidia of the pathogens under 10X and 40X magnification. Size of the conidia was measured using the stage and ocular micrometer. Stage and ocular micrometer was set in the microscope and their lines of coincidence were marked. Calibration factor (F) was calculated by the formula,

$$\mathrm{F} = \frac{\text{No of stage divisions between lines of coincidence}}{\text{No of ocular divisions between lines of coincidence}} \times 10\ \mu\text{m}$$

The reading observed in the ocular was multiplied with calibration factor to get the diameter of the conidia.

3.2.8 Pathogenicity test of the isolates

3.2.8.1 Inoculation of fungi

The pathogenicity test was carried out in the healthy cotton leaves. Healthy cotton leaves were collected from the fields of CICR and were kept in a 2ml vial containing sucrose solutions and surface sterilised with 70% ethanol and then the leaves were injured using silicon carbide powder. With the help of sterile cork borer, 5 mm diameter fungal disc of pure culture of fungal isolate was placed over the injuries made, by keeping fungal growth side towards injuries and then covered it with sterile cotton swab. The leaves inoculated with sterilized PDA media disc were used as a control. The inoculated leaves were then placed in the aseptic moisture chamber for observation upto 7 days. The moisture chamber was poured with nearly 0.5 litre of sterilized water in the bottom of chamber to maintain humidity and temperature was maintained at 27 ± 2^0C. Observations of pathogenicity and appearance of the leaf spot caused by pathogenic isolates were recorded.

3.2.8.2 Reisolation

Reisolation of the inoculated fungal isolates causing leaf spots were carried out on PDA medium from the artificially inoculated bolls showing typical symptoms of leaf spot infection and disease.

3.2.9 Molecular identification of fungal pathogens

3.2.9.1 Preparation of potato dextrose broth

24g PDB powder added to 1000ml distilled water taken in a glass jar was heated. Then poured in conical flask of 250ml, wrapped the lid in cotton plug and paper followed by tieing. The flask was put to autoclave for sterilisation.

3.2.9.2 Inoculation and incubation in broth culture

After preparation of PDB, it was cooled down to room temperature and 5 mm disc of culture cut by cork borer were put in the PDB using sterilised inoculation needle, under laminar air flow cabinet. Then, the lid was plugged with cotton and kept in BOD Incubator at 27 ± 2^0C with 80 ± 5% R.H for desired growth of the fungi.

3.2.9.3 Harvesting of mycelia

Seven days after incubation, fully grown mycelia of fungal isolates were harvested. The medium from the conical flask was drained out by using funnel lined with a filter paper, the mycelia was washed with 3-4 changes of sterilized distilled water to remove the spores and traces of medium from the mycelia mat. Pre-sterilized blotting paper was used to remove the excess moisture from the mycelia. The dried mycelia was then wrapped in an aluminium foil and kept at -20^{0}C in deep freezer overnight.

3.2.9.4 DNA extraction of fungal pathogens

DNA extraction was done by using the standard CTAB method. The following protocol (Aamir *et al.,*2015 and Tripathy *et al.,* 2017) was used for DNA extraction.

Protocol

1. The fresh fungal mycelia mat was crushed in sterilised mortar and pestle using liquid nitrogen (N_2).
2. 1 ml of extraction buffer was added to the mortar and pestle. The crushed samples were added to the vials and were kept in water bath at 65^{0}C for 1 hour for complete removal of the cell membranes and nuclear membrane.
3. Then, equal volume of phenol chloroform: isomyl alcohol mixture (24:1) was added to the samples to separate the protein.
4. The samples were then centrifuged at 10,000 rpm for 10 min at room temperature.
5. The supernatant was collected in vials of 1-5 ml.
6. 0.5 ml volume of Isopropanol was added to the above samples, and the vials kept in stand were given a gentle shake for the solution to mix properly.
7. DNA gets precipitated during this step.
8. The samples were then kept in refrigerator (4^{0}C) overnight for further precipitation.

9. The DNA was spooled with pipette tip or alternatively centrifuged at 10,000 rpm for 10 min for precipitation.
10. The supernatant were decanted, the pellets were restored and 100 µl of 70% ethanol was added to wash the pellet. Then the lids were dried using tissue paper.
11. After drying, TE buffer was added to dissolve the pellet.
12. Once the pellet was dissolved, DNA was used for PCR amplification.

3.2.9.5 PCR amplification by using ITS primers

The PCR amplification of the fungal DNA was done by using universal primers ITS-1 (5”-TCCGTAGGTGAACCTGCGG-3”) and ITS-4 (5”-TCCTCCGCTTATTGGTAT-3”) [White *et al*., 1990]. The PCR was carried out in a 25 µl PCR master mix containing 5X PCR buffer 5 µl, purified DNA template 2 µl, $MgCl_2$ 1.5 µl, dNTPs 0.4 µl, primer ITS-F 1 µl, ITS-R 1 µl, taq polymerase 0.3 µl and the final volume was adjusted by adding 15.8 µl of nuclease free water. The PCR was performed in applied biosystems thermal cycler (ABI Thermo Cycler). The PCR amplifications condition includes an initial denaturation at 94^0C for 4 min and 35 cycles, each cycle consisting of a denaturation at 94^0C for 45 sec followed by annealing at 57^0C for 1 min and extension at 72^0C for 2 min; a final extension step at 72^0C for 7 min. The resulting PCR products were analyzed on1.5 %agarose gel. (Erlich, 1989)

3.2.9.6 Preparation of 500 ml TAE buffer

TAE buffer is a solution containing mixture of tris base, acetic acid and EDTA.

1. 121 g Tris HCl, 9.305 g disodium EDTA and 28.5 ml glacial acetic acid were measured and added to 300 ml distilled water.
2. The solution was mixed with magnetic stirrer until the EDTA dissolves completely (p^H 8) and final volume was made to 500 ml.

3.2.9.7 Preparation of 1.5% agarose gel

1. 1.5 g of agarose powder was added in 100 ml 1XTAE buffer.
2. The content was melted by heating in oven and the solution was stirred for proper mixing and complete dissolution of agarose.
3. The solution was then allowed to cool down to 40-45ºC and then 3 µl of Ethidium bromide (EtBr) was added.
4. Prepared gel was used for gel electrophoresis.

3.2.9.8 Gel electrophoresis

Amplified DNA products were separated by agarose gel electrophoresis.

1. Prepared agarose gel was poured into the casting platform after inserting the comb in tray.
2. The gel was allowed to solidify and the comb was removed. Then, solidified gel was placed in the electrophoresis apparatus containing 1X TAE buffer.
3. About 1 µl of loading dye was added to each tube containing amplified DNA.
4. The amplified products (20 µl) were carefully loaded into the sample wells. A 500 bp ladder was added for comparison. Electrophoresis was carried out at 102 volts until the tracking dye migrated to the end of the gel.
5. Gel was visualized in the gel documentation unit (BioRad) for DNA amplification analysis.

3.2.9.9 DNA sequencing

DNA sequencing of the PCR products was done by using nucleotide sequencing of the amplified ITS-rDNA region. The pathogenic fungal isolates causing leaf spot resulted from the pathogenicity tests were sent for DNA sequencing. The samples were sent for outsourcing to Barcode Biosciences Pvt. Ltd., Hyderabad (India). The sequence homology search was performed by using BLAST through NCBI (National Center for Biotechnology Information) database (webpage: https:// blast.ncbi.nlm.nih.gov.).The consensus sequence was obtained using CAP3 sequence assembly program (Huang and Madan, 1999).

3.2.10 *In vitro* evaluation of bioagents

3.2.10.1 Preparation of Nutrient Agar Medium

1 Litre of Nutrient Agar media was prepared in 2 slots of 500 ml distilled water taken in a plastic jar. 14 g of Nutrient Agar and 2 g of bacteriological grade agar was added to 500 ml of distilled water and heated in a microwave for 5 mins. Then 250 ml each was poured in a 500 ml conical flask. The lid of the flask was covered with a sterilised cotton plug, wrapped with paper, tied and labelled as NA along with the date of preparation.

3.2.10.2 Study for bioefficacy of the bioagents against the fungus.

The antagonistic studies between isolated pathogens and bioagents were carried out under *in vitro* condition. The antagonistic effects of different bioagents were carried out in petri plates containing sterilized PDA medium. The fungal pathogens and bioagents were grown separately on the potato dextrose agar. The disc of 5 mm diameter of seven days old culture of antagonistic fungi bioagents were cut with the help of sterilized cork borer and placed onto sterilized PDA media. The 5 mm diameter disc of the test pathogen of seven days old culture was placed exactly opposite to the disc of the bioagents culture. The plates with only fungal test pathogen isolate culture were served as a control. The inoculated petri plates were then incubated at 27 ± 2^0C in BOD incubator. The observations regarding the area covered by the pathogens and the bioagents were recorded after 6 days of inoculation. (Dennis and Webster, 1971).

The percent growth inhibition (PGI) was calculated by using the formula. (Vincent 1927).

$$\mathrm{PGI} = \frac{\text{colony growth in control} - \text{colony growth in interaction pathogen}}{\text{colony growth in control}} \times 100$$

3.2.11 *In vitro* evaluation of fungicides

The efficacy of fungicides listed in Appendix I was determined by poison food technique (Nene and Thapliyal, 1993). The PDA media was distributed as 100 ml in each 250 ml flasks and sterilized in autoclave at 121^0C under 15 psi steam pressure for 15 min. The recommended dose of fungicides was added in the flasks after sufficient cooling so as to get the required final concentration and then it was thoroughly mixed by stirring the flasks. The medium was poured into petri plates with three replications for each treatment. Seven days old culture grown on potato dextrose agar (PDA) medium was used as an inoculum. The plates were inoculated with 5 mm diameter fungal disc with the help of the sterilized cork borer and transferred aseptically at the centre of the petri plates containing PDA media along with the measured quantities of fungicides. Petri plates with potato dextrose agar media without fungicide served as a control and then incubated at 27 ± 2^0C for seven days. The percent inhibition of the mycelial growth of inoculated pathogens was calculated by using the formula given by Vincent (1927).

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

Where,

I = Percent inhibition on fungal growth

C = Growth in mm on the 7th day after inoculation in control

T = Growth in mm on the 7th day after inoculation in treatment

Chapter IV

RESULTS AND DISCUSSION

Cotton is known as the king of fibres. India is a global leader in cotton production and the domestic textile industry is one of the largest textile industries in the country. The study on leaf spot disease complex of cotton was carried out as it caused significant reduction in the yield and fibre quality of the crop which ultimately led to serious loss in the Indian economy and most importantly the current scenario of climate change makes it necessary to investigate the prevalence of disease causing pathogens in the present context. At the backdrop of the situation, attempts were made to investigate the pathogens associated with leaf spot disease complex of cotton with respect to isolation, identification, pathogenicity, morphological characteristics, molecular identification, *in vitro* efficacy of bioagents and label claim fungicides. This chapter concludes the results obtained during the investigation.

4.1 Isolation, identification and pathogenicity of the pathogens

4.1.1 Isolation

The leaf spot disease samples of cotton, *Gossypium hirsutum* variety RCH-659 and Suraj NBt (non Gm) were collected from experimental fields of ICAR-Central Institute for Cotton Research (CICR), Nagpur (table 1, plate 1 (a)). Isolation of pathogen from affected leaf samples showing typical leaf spot symptoms were made on PDA by tissue isolation method (plate 1(b)). The pure cultures of the different fungal pathogens were obtained and maintained for further studies (plate 2).

Table 1. Source modifier of the collected leaf spot samples

GPS coordinate: 21deg 02’23.5”N 79deg 03’37.0”E

Host: *Gossypium hirsutum*

Source - Cotton leaf

Spc.ID	Date	Isolate	Specimen voucher
CICR-F1	27/8/21	LS1R1C(L)	CICR-F1
CICR-F2	27/8/21	LS-2	CICR-F2
CICR-F4	27/8/21	LS-2R1(A)	CICR-F4
CICR-F5	27/8/21	LS-6(A)	CICR-F5
CICR-F6	27/8/21	LS-3R2(B)	CICR-F6
CICR-F7	27/8/21	LS-1R1(C)	CICR-F7
CICR-F8	27/8/21	LS-1R1(A)	CICR-F8
CICR-F9	27/8/21	LS-4R1(A)	CICR-F9
CICR-F10	27/8/21	LS7A(I)	CICR-F10
CICR-F11	27/8/21	LS-3R2(A)	CICR-F11
CICR-F12	27/8/21	LS-5(A)	CICR-F12
CICR-F13	27/8/21	LS-1R1(B)	CICR-F13
CICR-F14	27/8/21	LS-5(B)	CICR-F14
CICR-F15	27/8/21	LS-7(A)	CICR-F15
F9	9/9/21	LS-16	F9
F10	9/9/21	LS-17	F10

Spc. ID- Specimen Identity

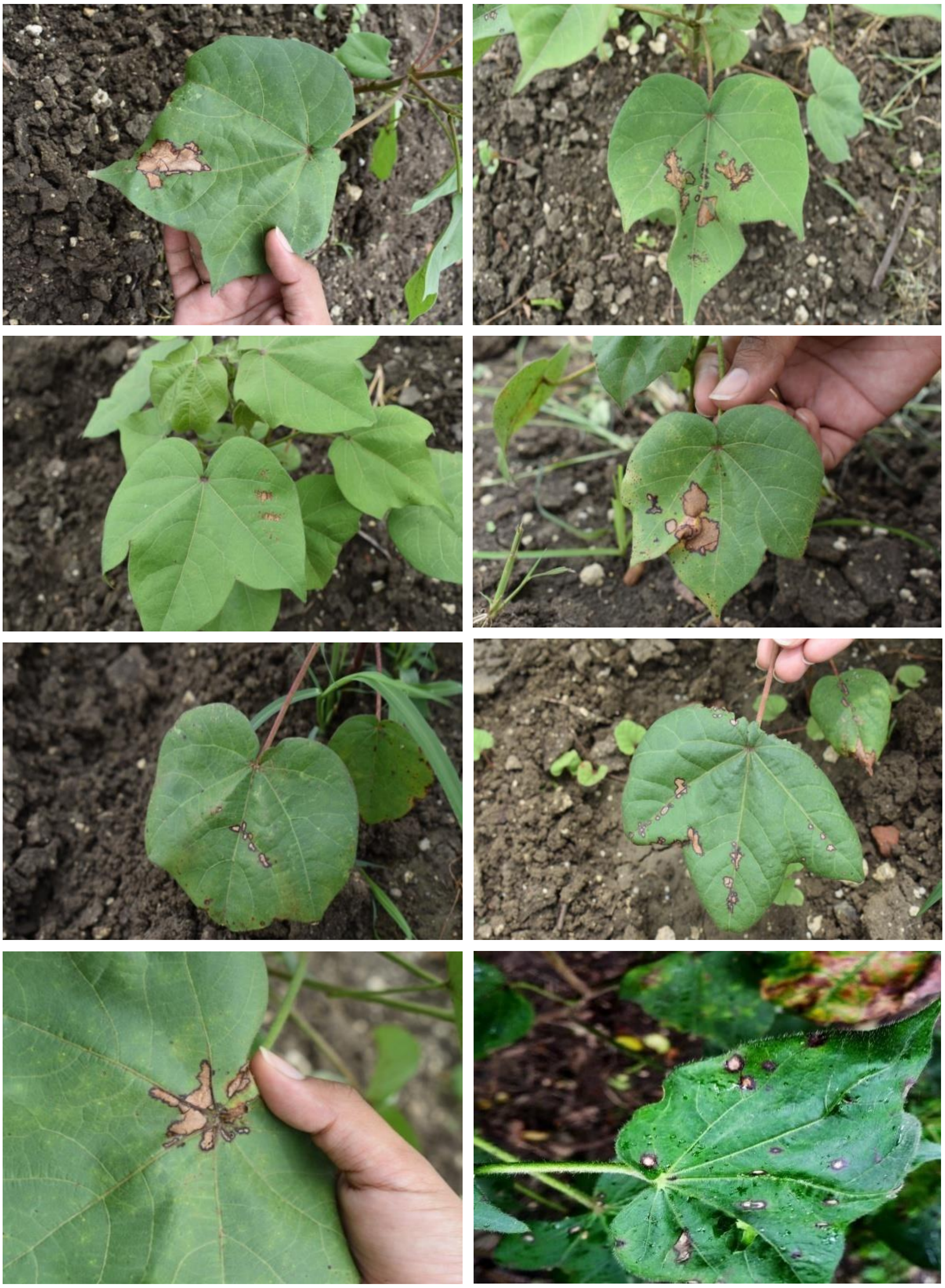


Plate1 (a). Symptoms of cotton leaf spot disease samples collected from experimental field ICAR-CICR

Table 2. Leaf spot symptoms associated with the pathogen

Isolate	Pathogen	Symptom
CICR-F1, F2,F5,F7-15	*Corynespora cassiicola*	Circular to irregular shaped, red lessions. Later turned brown with dark border. Alternating rings of light and dark brown bands developed target spot.
F10	*Paramyrothecium roridium*	Circular to irregular tan coloured lessions with broad violet to brown marginsurrounded by translucent area like a concentric ring. It coalesced to form bigger spot, the entire leaf dries and shed, pin head sized sporodochia seen.
CICR-F6	*Rhizoctonia solani*	Irregular, tiny light brown spots and water-soaked lesions, with small black dots and dark brown margins, leaves collapse with soft watery rot.
CICR-F4	*Macrophomina phaseolina*	Yellowing and senescence of leaves, damage of cortical tissues from the lower stem, presence of microsclerotia were seen.
F9	*Nigrospora sphaerica*	Greyish to brown, semicircular to irregular shaped lesions, surrounded by pale yellow zones, later turn greyish to dark brown.

Corynespora cassiicola

Paramyrothecium roridium

Rhizoctonia solani

Macrophomina phaseolina

Nigrospora sphaerica

Plate 1(b). Symptoms of cotton leaf spot disease samples collected from experimental field ICAR-CICR

Corynespora cassiicola *Paramyrothecium roridium*

Rhizoctonia solani *Macrophomina phaseolina*

Nigrospora sphaerica

Plate 2. Pure cultures of leaf spot isolates collected from cotton (*Gossypium hirsutum*)

4.1.2 Identification of the cultures

The fungal cultures were examined under microscope of magnification 10X and 40X for morphological studies and identified (table 3).

Table 3. Identification of the fungal pathogen isolated

Isolate	Isolate ID	Identification
1	CICR-F1	*Corynespora cassiicola*
2	CICR-F2	*Corynespora cassiicola*
3	CICR-F4	*Macrophomina phaseolina*
4	CICR-F5	*Corynespora cassiicola*
5	CICR-F6	*Rhizoctonia solani*
6	CICR-F7	*Corynespora cassiicola*
7	CICR-F8	*Corynespora cassiicola*
8	CICR-F9	*Corynespora cassiicola*
9	CICR-F10	*Corynespora cassiicola*
10	CICR-F11	*Corynespora cassiicola*
11	CICR-F12	*Corynespora cassiicola*
12	CICR-F13	*Corynespora cassiicola*
13	CICR-F14	*Corynespora cassiicola*
14	CICR-F15	*Corynespora cassiicola*
15	F9	*Nigrospora sphaerica*
16	F10	*Paramyrothecium roridium*

The results are in confirmation with Salunkhe *et al.* (2019) and Bhattiprolu *et al.* (2022) where *Corynespora cassicola* has been found as prevalent leaf spotting fungus in cotton.

The results are in agreement with Raut *et al.* (1980), Tomar (2005), Ingole and Ingle (2011) where *Myrothecium roridium* has been found to cause leaf spot in cotton.

The fungus generally reported as root rot causing pathogen such as *Rhizoctonia solani* and *Macrophomina phaseolina* are found to cause leaf spot in cotton in the present study. Hence the result is in accordance with Blank (1953) where *Rhizoctonia solani* has been found to cause leaf spot in cotton. *Rhizoctonia solani* caused leaf spot in other crops such as tobacco (Sneh *et al.,*1996; Gonzalez, 2011) and *Peucedanum praeruptorum* (Xie *et al*., 2018).

The results are confirmed as *Macrophomina phaseolina* caused leaf spot in Chlorophytum (Dadwal and Savitri , 2012), mungbean (Fuhlbohm *et al.,* (1996) and sweet potato (Ekhuemelo and Chikwado 2020).

Saprophytic pathogens such as *Nigrospora sphaerica* (Farr and Rossman,2013) is found to cause leaf spot in cotton in the present study. Thus, the result is in accordance with Abbas and Najlaa (2014) where *Nigrospora sphaerica* caused leaf spot in date palm. Nigrospora leaf spot in tea was confirmed by Dutta (2015).

4.1.3 Pathogenicity

Pathogenicity test according to koch's postulate under *in vitro* condition of different fungal pathogens were determined (Plate 3(a)). The typical leaf spot symptoms were observed at the infection sites similar to those observed on naturally infected cotton leaves. The fungal cultures were tried for pathogenicity tests and only 5 fungal cultures had developed the typical symptoms of different leaf spot diseases and were proved to be pathogenic. The fungal cultures were

Corynespora cassiicola (CICR-F2), *Paramyrothecium roridium* (*F10*), *Rhizoctonia solani* (*CICR-F6*), *Macrophomina phaseolina* (*CICR-F4*) *and Nigrospora sphaerica* (*F9*) (Plate 2). Therefore, these five fungal cultures were taken for detailed studies (Plate 3(b)). Symptoms associated with the causal organisms are described in the table 3.

4.1.4 Reisolation

Reisolations were made from artificially inoculated leaves of cotton showing typical symptoms of leaf spots, which yielded fungal pathogens to those of the orginal organisms.

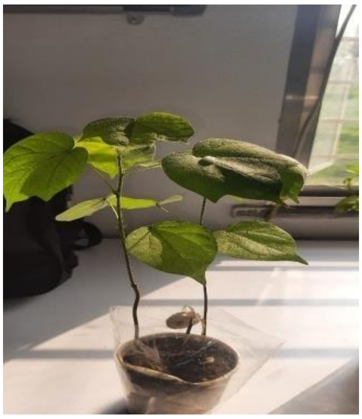
The results are in confirmation with those reported by Salunkhe *et al*. (2019) who proved the pathogenicity of *Corynespora cassiicola* in cotton leaves, and Mattic *et al.* (2019) for *Paramyrothecium roridium* in leafy vegetables and ornamental crops and further, Xie *et al*. (2018) for *Rhizoctonia solani* in leaves of *Peucedanum praeropturum.* Similarly, Huda-Shakirah and Rahim (2019) proved the pathogenicity of *Macrophomina paseolina* in leaves of lily plant and Dutta (2015) proved the pathogenicity of *Nigrospora sphaerica* in tea leaves.

Artificially inoculated cotton leaves placed in incubator

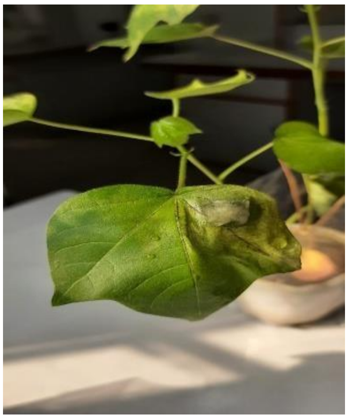
Plate 3(a). ***In vitro*** **method for evaluating the pathogenicity of organisms associated with the leaf spot disease**

Corynespora cassiicola

Paramyrothecium roridium



Rhizoctonia solani



Macrophomina phaseolina

Nigrospora sphaerica

Plate 3(b). ***In vitro*** **method for evaluating the pathogenicity of organisms associated with the leaf spot disease**

4.2 Morphological Studies

The final five pathogens were selected for morphological characterization. Morphological characters of the pathogen were taken from 7 days old culture growing on PDA medium. Morphological observations of the selected phytopathogens were recorded (table 4, plate 4).

4.2.1 *Corynespora cassiicola*

Pure cultures of *Corynespora cassiicola* exhibited white to dark grey profuse mycelia growth. Conidiophores were pale brown, simple, cylindrical and septate with intermittent branching, conidia were subhyaline to olivaceous, solitary or in chains, straight to slightly curved, obclavate to cylindrical, 40 to 128 × 5 to 12 µm. (table 4, plate 4(a)).

Morphological characteristics were consistent with the original descriptions of morphology of *Corynespora cassiicola* on cotton (Jones, 1961) and more recent reports of cotton leaf spot (Salunkhe *et al.,* 2019; Bhattiprolu *et al.,* 2022).

4.2.2 *Paramyrothecium roridium*

Conidia was nonseptate with a cylindrical to ellipsoidal shape, one celled, both ends rounded with oil globules, hyaline to pale green colour, and an approximate size of 6 × 2 µm. Abundant white aerial mycelium and sporodochia developed in the form of concentric rings that contained dark green to black conidial masses (table 4, plate 4(a)).

The results are in agreement with the original description of morphology of *Myrothecium roridium* that caused leaf spot on cotton (Saccardo 1886) and recent reports of leaf spot caused by *Paramyrothecium roridium* on leafy vegetables and ornamental crops (Matic *et al.,* 2019).

4.2.3 Morphology of ***Rhizoctonia solani***

The fungal colonies were light brown in colour and exhibited vigorous growth. Ellipsoid to globose, barrel-shaped cells,10-20 mm wide, were produced in chains forming sclerotia. Sclerotia were dark brown, irregularly shaped, and produced after 5 days on PDA with 8-10 mm in diameter (table 4, plate 4(b)).

The results are in confirmation with similar description of morphology of *Rhizoctonia solani* that caused leaf spot in Tobacco by Gonzalez (2011) and Peucedanum by Xie *et al*., (2018).

4.2.4 Morphology of ***Macrophomina phaseolina***

Mycelium of the fungus was dark olive black colonies that ultimately turn black, embedded with microsclerotia. Morphologically microsclerotia were black, smooth, round to oblong. Pycnidia were dark to greyish, globose, membranous and 83-124μm in diameter. (table 4, plate 4(b)).

The results are in agreement with Meena *et al.* (2021) which had similar morphological description of the fungus *Macrophomina phaseolin*a that caused leaf spot in Chlorophytum leaves.

4.2.5 Morphology of ***Nigrospora sphaerica***

The fungal colonies were fast growing, initially white, becoming light to dark grey with the onset of sporulation with black, spherical to subsphaerical conidia A light orange colour secretion could be seen in the fungal cultures at seven days of incubation. Conidia were spherical to sub spherical, single-celled, black, 19 to 21 μm in diameter (table 4, plate 4(c)).

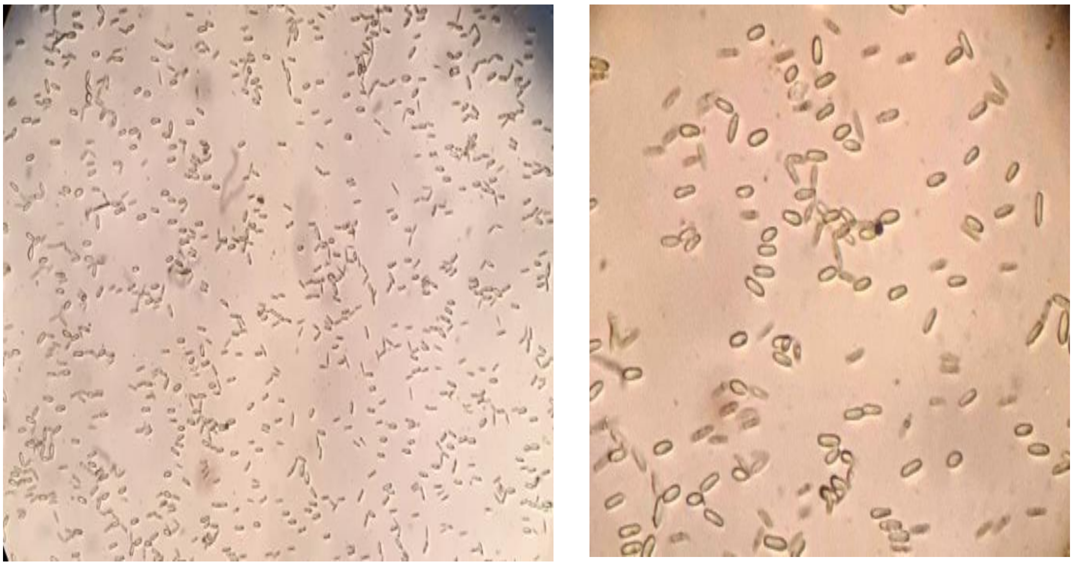
The results are consistent with morphological characteristics of *Nigrospora sphaerica* that caused leaf blight on tea by Dutta (2015) and leaf spot of medicinal and aromatic grasses by Mane (2020).

Table 4. Morphological observations of the selected phytopathogens causing leaf spot

Sr. No.	Pathogen	Average colony diameter (8 DAI)	conidia size (μm)	Shape of spore/conidia	Colour of the spore/conidia
1.	*Corynespora cassiicola*	57	40 -128 × 10 -12	Obclavate-cylindrical,	Subhyaline- dark brown conidia
2.	*Paramyrothecium roridium*	49.34	6 × 2	Discoid. circular - irregular	Conidia Initially palegreen-black
3.	*Rhizoctonia solani*	87	8-10	Ellipsoid –globose	Dark brown sclerotia
4.	*Macrophomina phaseolina*	87	83-124	Obovoid	Black pycnidia
5.	*Nigrospora sphaerica*	87	19-21	Subsphaerical	Black conidia

Mycelium at 10X **Conidia at 40X**

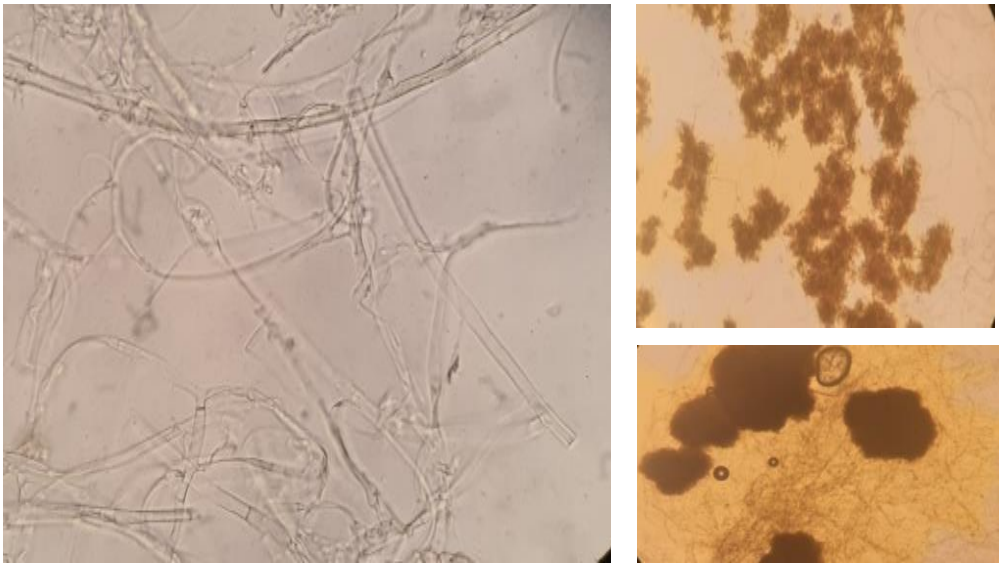
Corynespora cassiicola



Mycelium at 10X **Conidia at 40X**

Paramyrothecium roridium

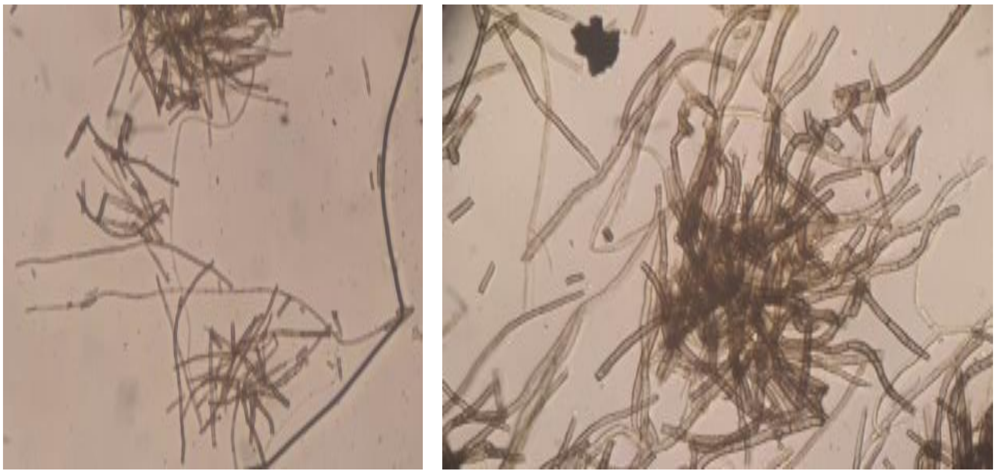
Plate 4(a). Isolated fungal mycelium and spore at magnification 10X and 40X respectively of different fungal pathogens associated with cotton leaf spot



Mycelium at 10X

Sclerotia at 40X

Rhizoctonia solani

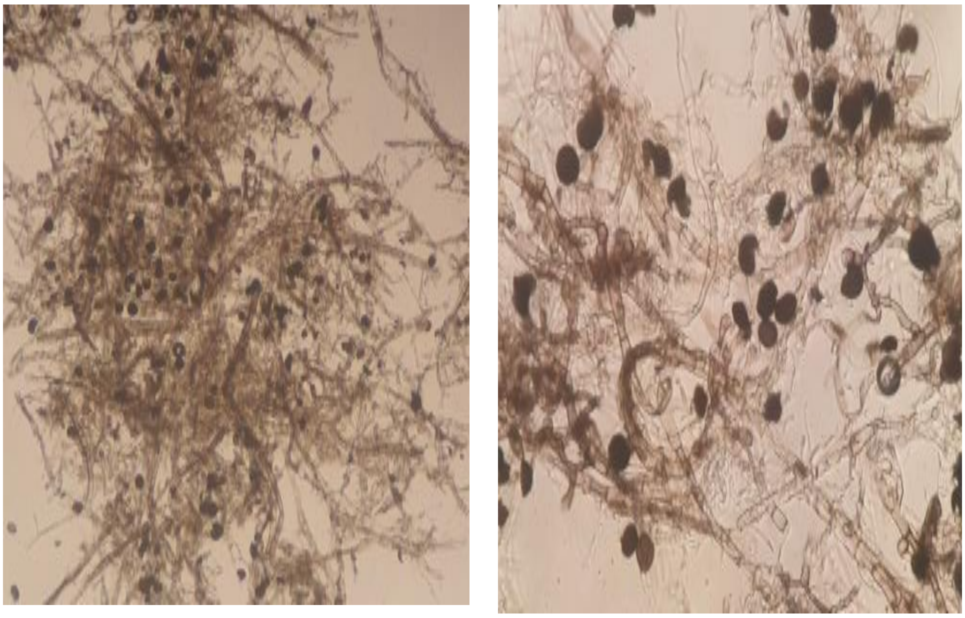


Mycelium at 10X

Pycnidia at 40X

Macrophomina phaseolina

Plate 4(b). Isolated fungal mycelium and spore at magnification 10X and 40X respectively of different fungal pathogens associated with cotton leaf spot



Mycelium at 10X **Conidia at 40X**

Nigrospora sphaerica

Plate 4(c). Isolated fungal mycelium and spore at magnification 10X and 40X respectively of different fungal pathogens associated with cotton leaf spot

4.3 Molecular identification of different fungal pathogens

The selected five different fungal cultures of different leaf spot fungal pathogens were examined for molecular identification by using ITS-rDNA sequence analysis for accurately identifying the pathogen causing leaf spot of cotton.

4.3.1 Sequencing of PCR products

The PCR amplified products produced a range of 500-600 bp amplicon by using ITS1 and ITS4 universal primer (plate 5). Total 16 PCR products of different fungal cultures were sent for sequencing to Barcode Biosciences Pvt. Ltd., Hyderabad (India). DNA sequencing revealed that these fragments ranged in size from 500-568 bp. The source modifier is provided in table 1 and NCBI GenBank accession numbers for all the ITS sequences determined in the study are provided in the table 5.

Table 5. NCBI GenBank accession numbers of ITS sequences determined inthe study

Sr. No.	Isolate ID	Fragment size (bp)	NCBI Genbank accessionno.	Identification	Per cent Identity
1.	CICR-F1	194	ON384549	*Corynespora cassiicola*	100
2.	CICR-F2	504	ON384550	*Corynespora cassiicola*	100
3.	CICR-F4	530	ON384551	*Macrophomina phaseolina*	100
4.	CICR-F5	525	ON384552	*Corynespora cassiicola*	100
5.	CICR-F6	488	ON384553	*Rhizoctonia solani*	100
6.	CICR-F7	567	ON384554	*Corynespora cassiicola*	100
7.	CICR-F8	528	ON38455	*Corynespora cassiicola*	100
8.	CICR-F9	510	ON384556	*Corynespora cassiicola*	100
9.	CICR-F10	505	ON384557	*Corynespora cassiicola*	100
10.	CICR-F11	568	ON384558	*Corynespora cassiicola*	100
11.	CICR-F12	510	ON384559	*Corynespora cassiicola*	100
12.	CICR-F13	507	ON384560	*Corynespora cassiicola*	100
13.	CICR-F14	466	ON384561	*Corynespora cassiicola*	100
14.	CICR-F15	485	ON384562	*Corynespora cassiicola*	100
15.	F9	430	ON406213	*Nigrospora sphaerica*	100
16.	F10	532	ON406214	*Paramyrothecium roridium*	100

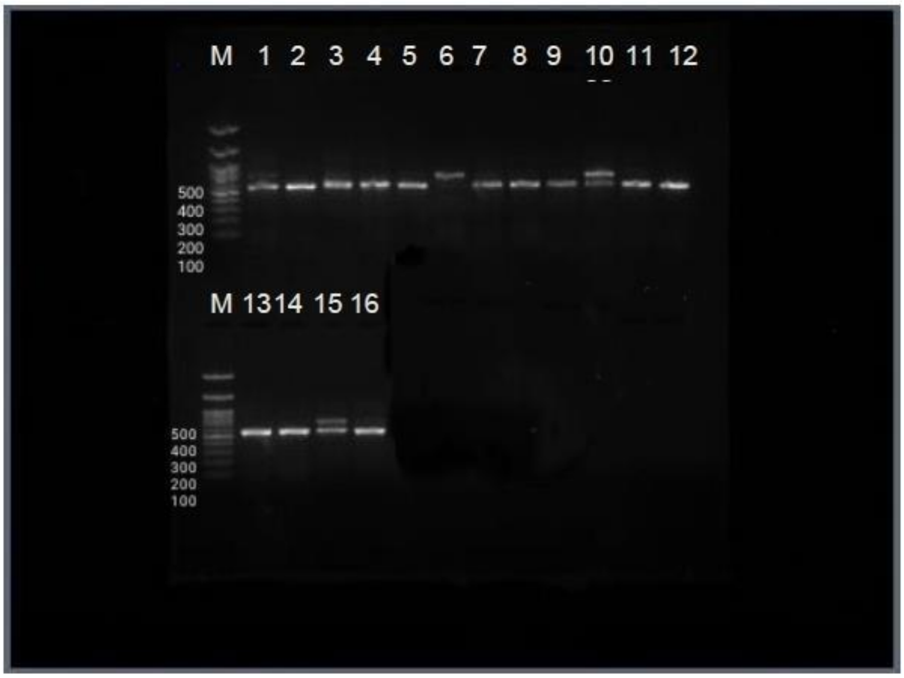


Plate 5. PCR amplification of genomic DNA from selected phytopathogens causing leaf spot in cotton using primers ITS1/ITS4

1- Corynespora cassiicola

2- Corynespora cassiicola

3- Macrophomina phaseolina

4- Corynespora cassiicola

5- Rhizoctonia solani

6- Corynespora cassiicola

7- Corynespora cassiicola

8- Corynespora cassiicola

9- *Corynespora cassiicola*

10- *Corynespora cassiicola*

11- *Corynespora cassiicola*

12- *Corynespora cassiicola*

13- *Corynespora cassiicola*

14- *Corynespora cassiicola*

15- *Nigrospora sphaerica*

16-*Paramyrothecium roridium*

4.3.2 Identification through Blast n tool

The sequences were subjected to online BLAST-nucleotide tool in NCBI for analysis to identify the different fungal cultures.The ITS-1/ ITS-4 rDNA sequences were compared with those available in the NCBI GenBank database. The results showed that 5 different fugal cultures were identified as *Corynespora cassiicola* (CICR F1, CICR F2, CICR F5, CICR F7 TO CICR F15); *Paramyrothecium roridium* (F10), *Rhizoctonia solani* (CICR F6), *Macrophomina phaseolina* (CICR F4), and *Nigrospora sphaerica (*F9) with NCBI Genbank accession numbers: ON384549, 0N384550, ON384552, ON384554 to ON384562; ON406214, ON384553, ON384551, and ON406213 respectively with 100% sequence identity (table 5). The phylogenetic tree was constructed using MEGA X Software (Kumar *et al.,* 2018) with maximum likelihood parameter for *Corynespora cassiicola* CICR F1, CICR F2, CICR F5, CICR F7 TO CICR F15, *Paramyrothecium roridium* F10, *Rhizoctonia solani* CICR F6, *Macrophomina phaseolina* CICR F4 and *Nigrospora sphaerica* F9 (plate 6- plate 10). The isolates showed 100% identity with the sequences present in NCBI Genbank.

Similar experiment was carried out by Salunkhe *et al*. (2019) for the identification of *Corynespora cassiicola* from cotton leaves causing Corynespora leafspot by using ITS-1 and ITS-4 universal primers and the resulting sequences showed 100% similarity with *Corynespora cassiicola* from avocado.

A sensitive real-time PCR (qPCR) assay was conducted by Chavhan *et al.* (2018) detect *Paramyrothecium roridum* from pure culture and infected samples of cotton plants using a specific set of primer pair pMyro F/R designed to target the 185 bp ITS region of rDNA of *Paramyrothecium roridum* species and validated using qPCR.

PCR amplification was performed by Xie *et al.* (2018) on the rDNA ITS region with primers ITS-1 and ITS-4 of fungal culture causing leaf spot on *Peucedanum praeruptorum* and the sequence analysis revealed that the isolate QH1 (GenBank accession no. MF447834) shared 100% identity with *Rhizoctonia solani* AG-1-IB (HF678122).

The sequencing of the ITS region with ITS-4 and ITS-5 primers was done by Meena and Vasudha (2022) and the obtained sequences were 99% identical to the reference sequence of *Macrophomina phaseolina* (GenBank accession KF951698) on *Phaseolus vulgaris* from Mexico.

ITS region of the ribosomal DNA of Nigrospora leafspot of kiwi was amplified by Chen *et al.* (2016) using universal ITS-1 and ITS-4 primers followed by the sequencing of the PCR products and the ITS sequences showed 99% homology with that of *Nigrospora sphaerica* (GenBank accession no. KJ767121.1) and hence the isolates were confirmed as *Nigrospora sphaerica.*

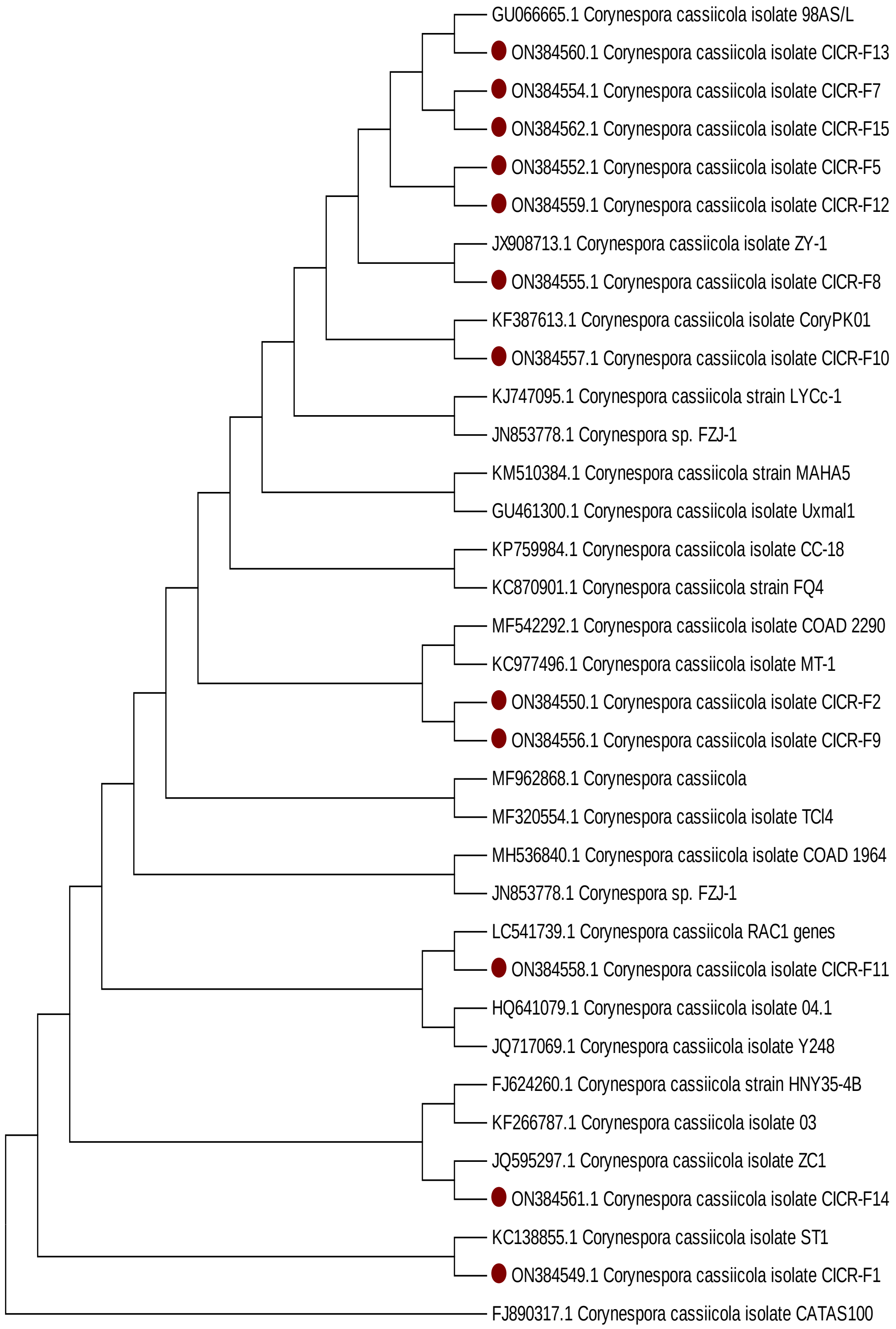


Plate 6. Maximum Likelihood (ML) tree or Phylogeny tree based on ITS-rDna sequences (ITS-1 and ITS-4 region) of *Corynespora cassiicola* isolate CICR-F1, F2, F5, F7 TO F15

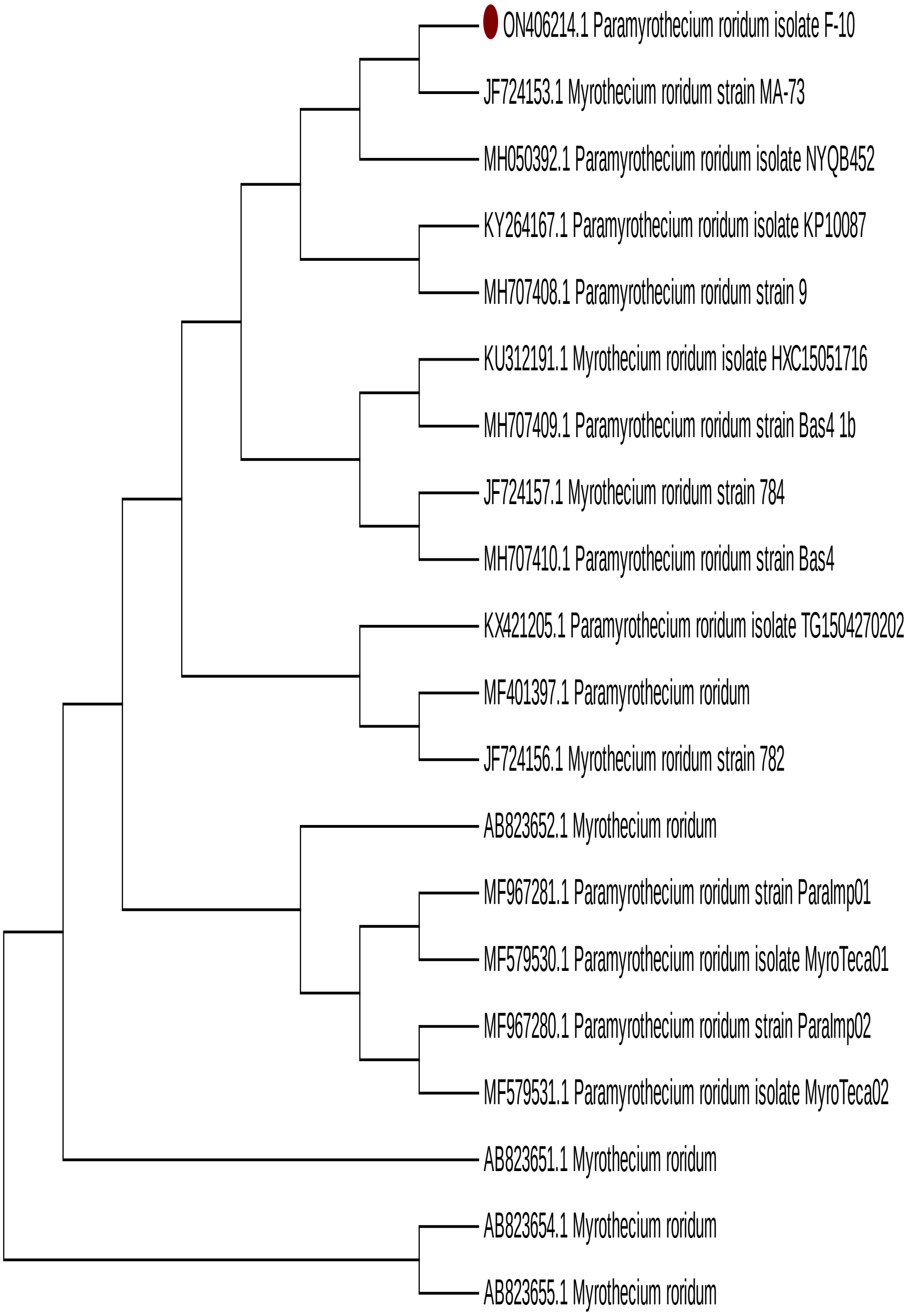


Plate 7. Maximum Likelihood (ML) tree or Phylogeny tree based on ITS-rDna sequences (ITS-1 and ITS-4 region) of *Paramyrothecium roridium* isolate F10

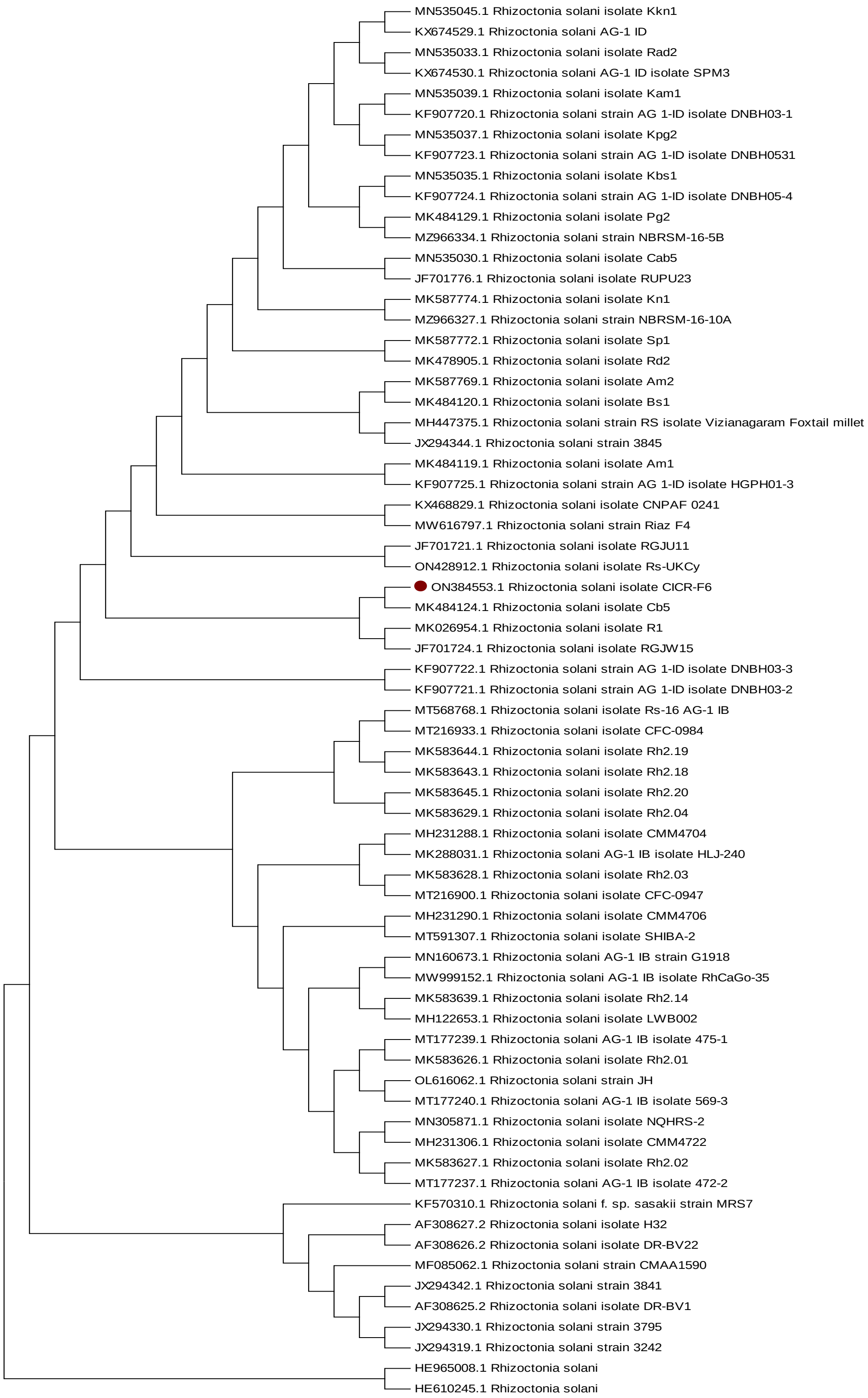


Plate 8. Maximum Likelihood (ML) tree or Phylogeny tree based on ITS-rDna sequences (ITS-1 and ITS-4 region) of *Rhizoctonia solani* isolate CICR-F6

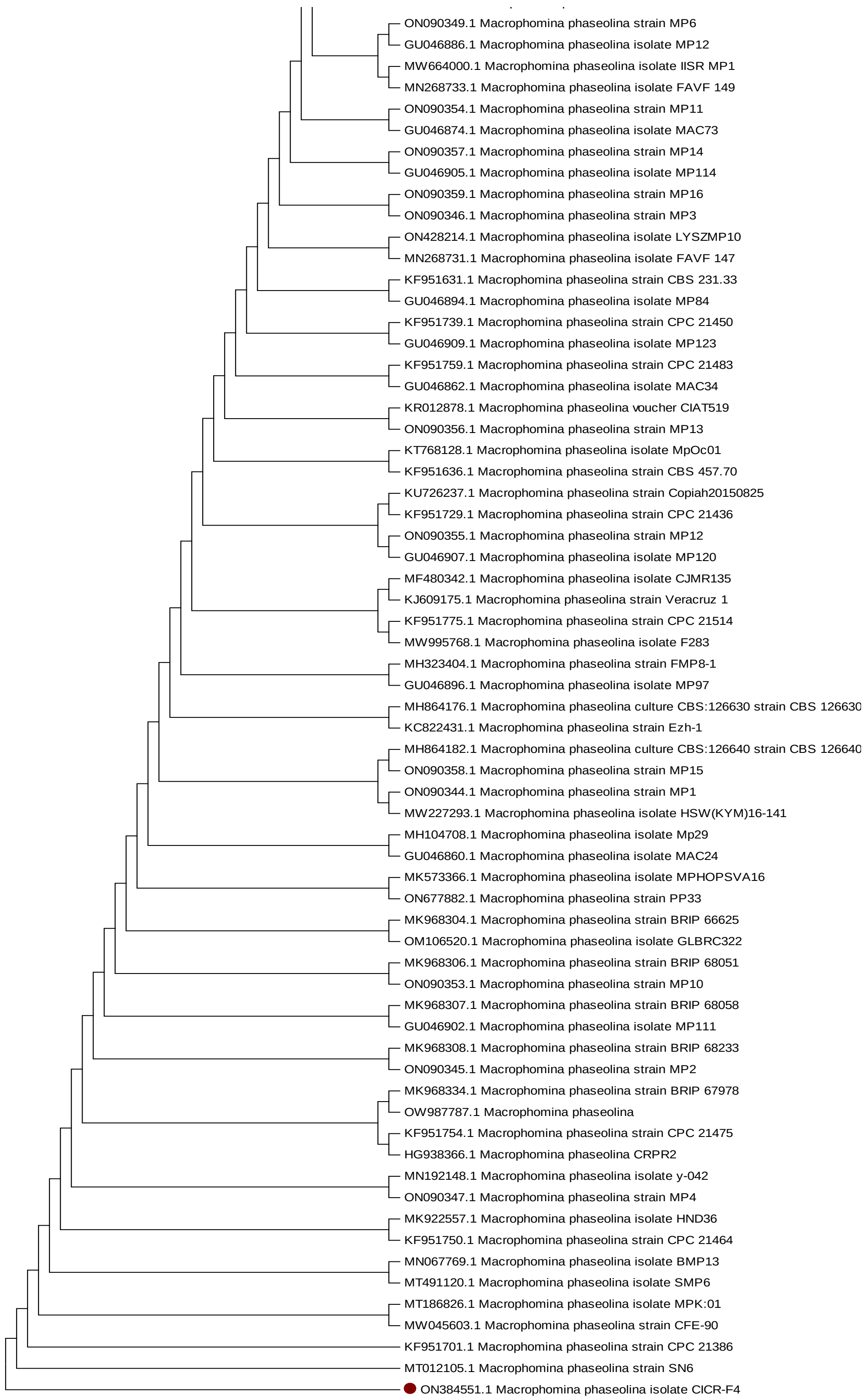


Plate 9. Maximum Likelihood (ML) tree or Phylogeny tree based on ITS-rDna sequences (ITS-1 and ITS-4 region) of *Macrophomina phaseolina* isolate CICR-F4

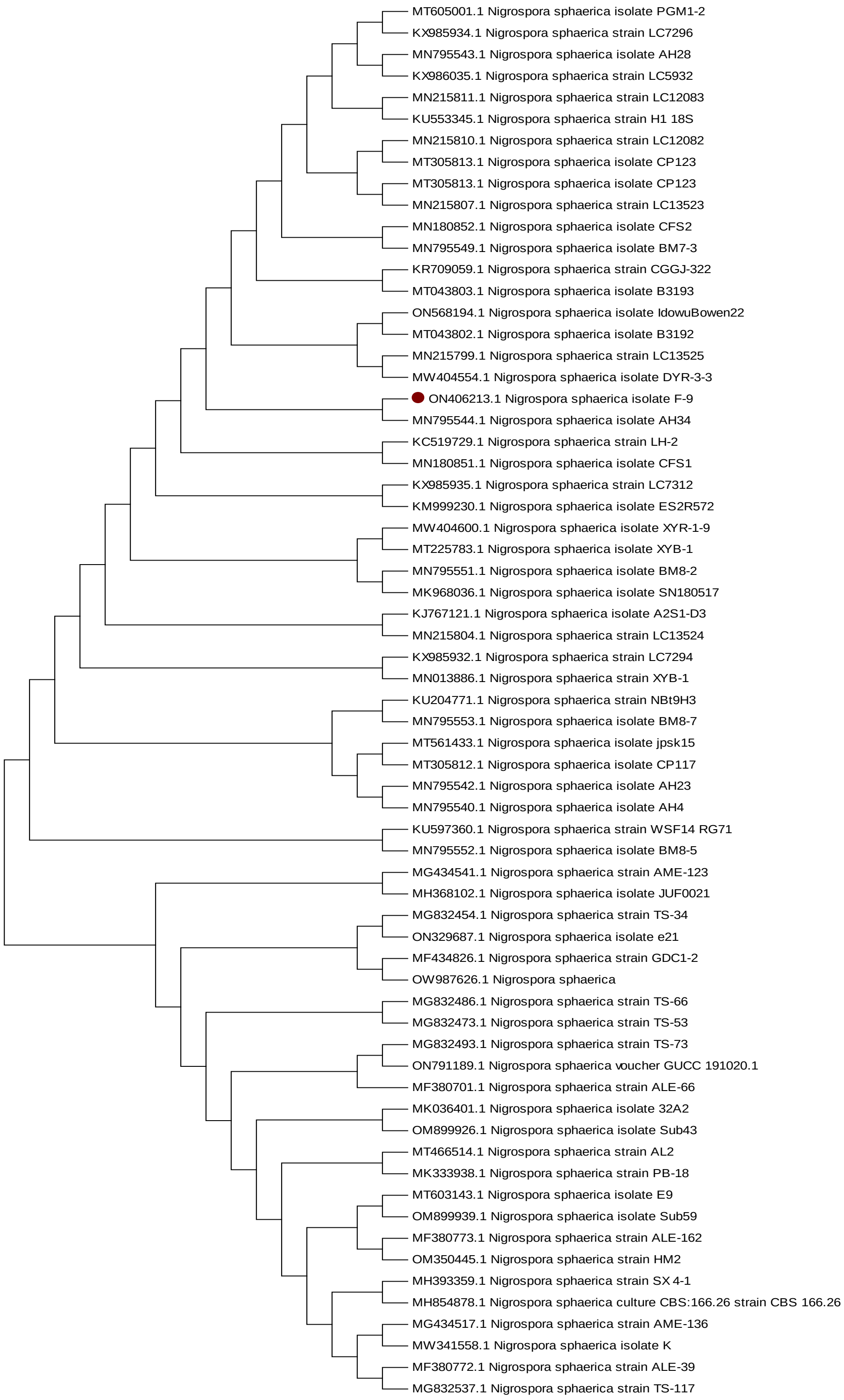


Plate 10. Maximum Likelihood (ML) tree or Phylogeny tree based on ITS-rDna sequences (ITS-1 and ITS-4 region) of *Nigrospora sphaerica* isolate F9

4.4 *In vitro* evaluation of different strains of fungal and bacterial bioagents against the pathogens identified

Seven different strains of fungal bioagents (T1-T7) were tested against the five different fungal cultures isolated from the cotton leaves by dual culture experiment (Johnson *et al.*, 1959) and observations were recorded for 3rd, 5th and 8th days after inoculation. Efficacy of two different strains of *Baciilus* i.e *Bacillus aryabhattai* (T1) *Bacillus tequilensis* (T2) and *Pseudomonas fluoroscens* (T3) were tested against the isolated fungal pathogens and observations were noted on fourth day after inoculation.

4.4.1 *Corynespora cassiicola*

Bacillus aryabhattai (T1) showed 63.86 % fungal growth inhibition (maximum) followed by *Pseudomonas fluorescens* (T3) exhibiting 48.73% fungal growth inhibition over control. *Bacillus tequilensis* inhibited (T2) 4.20% (least) of fungal growth over control (table 6a, figure 1, plate 11a). *Trichoderma viride* (T1), *Trichoderma longibracheatum* (T6), *Talaromyces purpurogenus* (T7) were found most effective among all the strains of fungal bioagent against *Corynespora cassiicola* and exhibited complete (100%) fungal growth inhibition over control and were at par with each other. *Talaromyces pinophilus* 1 (T3) Deoli strain performed least fungal growth inhibition of 6.53 % over control (table7a, figure7, plate 12 a).

The results are in confirmation with Manju *et al.* (2019) where *Trichoderma viride* was found superior over other bioagents (66.5 % inhibition of fungal growth over control).

4.4.2 *Paramyrothecium roridium*

Pseudomonas fluorescens (T3) showed 47.29% inhibition of fungal growth over control followed by *Bacillus aryabhatttai* (T1) showing 42.97% fungal growth inhibition over control. *Bacillus tequilensis* (T2) inhibited 3.51% (least) of fungal growth over control (table6b, figure 2, plate11b,). *Trichoderma harzianum* (TH- CICR) (T2) isolated from CICR was found most effective against *Paramyrothecium roridium* and showed fungal growth inhibition of 66.01% over control. *Talaromyces purpurogenus* (T7) showed least fungal growth inhibition of 2.91% over control. (table7b, figure 8, plate 12b).

The results are in agreement with Ranjini and Naika (2019) where *Trichoderma harzianum* (100% fungal growth inhibition) was the most effective bioagent against *Paramyrothecium roridium .*

4.4.3 *Rhizoctonia solani*

Bacillus aryabhatttai (T1) inhibited fungal growth upto 45.11% over control where as *Pseudomonas fluorescens* (T3) showed 30.17% of fungal growth inhibition over control. *Bacillus tequilensis* (T2) was ineffective (0%) in *inhibiting* fungal growth over control (table 6c, figure 3, plate 11c). *Trichoderma harzianum* (TH-CICR) (T2) isolated from CICR was found most effective against *Rhizoctonia solani* exhibiting highest fungal growth inhibition of 33.90 % over control and *Talaromyces purpurogenus* (T7) showed least fungal growth inhibition of 10.34 % over control (table 7c, figure 9, plate 12c).

The results are in confirmation with Yadav (2020) where *Trichoderma harzianum* (77.7% fungal growth inhibition) was effective in controlling *Rhizoctonia solani.*

4.4.4 *Macrophomina phaseolina*

Pseudomonas fluorescens (T3) showed maximum of 59.21% inhibition of fungal growth over control followed by *Bacillus aryabhatttai* (T1) showing 9.31% inhibition of fungal growth over control. *Bacillus tequilensis* (T2) was ineffective (3.64%) in inhibiting fungal growth over control (table 6d, figure 4, plate 11d). *Talaromyces pinophulus* 2 neem strain (T4) was found most effective against *Macrophomina phaseolina* inhibiting fungal growth upto 58.98% over control and *Talaromyces purpurogenus* (T7) showed least 12.26% fungal growth inhibition over control (table 7d, figure 10, plate 12d).

The results are in confirmation with Nagrale *et al*. (2022) where *Bacillus aryabhattai* (69.27 ± 0.17 % fungal growth inhibition) was the most effective in controlling *Macrophomina phaseolina.*

4.4.5 *Nigrospora sphaerica*

Pseudomonas fluorescens (T3) completely (100%) inhibited the fungal growth over control followed by *Bacillus aryabhattai* (T1) showing 53.59% inhibition of fungal growth over control where as *Bacillus tequilensis* (T2) was ineffective (2.76%) in inhibiting fungal growth over control (table 6e, figure 5, plate 11e). *Talaromyces pinophilus* 2 (T4) neem strain showed highest fungal growth inhibition upto 41.36 % over control and *Talaromyces purpurogenus* (T7) showed least 17.56% fungal growth inhibition over control (table 7e, figure 11, plate 12e).

The results are in confirmation with Mane *et al.* (2018) where *Pseudomonas fluorescens* was effective in controlling *Nigrospora sphaerica.*

Thus, *Bacillus aryabhattai* (T1) was most effective in controlling *Corynespora cassicola* and *Rhizoctonia solani. Pseudomonas fluorescens* (T3) was most effective in controlling *Paramyrothecium roridium* (Ranjini and Naika 2019), *Macrophomina phaseolina* and *Nigrospora sphaerica* (Mane *et al.,* 2019). *Trichoderma harzianum* (TH-CICR) (T2) isolated from CICR were most effective against *Paramyrothecium roridium* (F10)*, Rhizoctonia solani* CICR-F6 (Yadav, 2020, Ranjini and Naika, 2019) (table 6f, figure 6). *Talaromyces pinophulus* 2 (T4) neem strain were most effective against *Macrophomina phaseolina* (CICR-F4)*, Nigrospora sphaerica* (F9). *Trichoderma viride* (T1), *Trichoderma longibracheatum* (T6), *Talaromyces purpurogenus* (T7) were found most effective against *Corynespora cassiicola* (Manju *et al.,*2019). (table 7f, figure 12).

Table 6a. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Corynespora cassiicola in vitro* causing target spot in cotton

Treatment	Bioagent	Average colony Diameter(in mm) at 4DAI	Per cent Growth Inhibition at 4DAI
T1	*Bacillus aryabhattai*	10.75	63.86
T2	*Bacillus tequillensis*	28.50	4.20
T3	*Pseudomonas fluorescens*	15.25	48.73
T4	Control	29.75	
FTest		Sig	
SE(m)±		0.99	
CD@1%		6.46	

Table 6b. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Paramyrothecium roridium in vitro*

Treatment	Bioagent	Average colony Diameter(in mm) at 4DAI	Per cent Growth Inhibition at 4DAI
T1	*Bacillus aryabhattai*	10.55	42.97
T2	*Bacillus tequillensis*	17.85	3.51
T3	*Pseudomonas fluorescens*	9..75	47.29
T4	Control	18.50	
FTest		Sig	
SE(m)±		0.31	
CD@1%		2.07	

Table 6c. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Rhizoctonia solani in vitro* causing root rot in cotton

Treatment	Bioagent	Average colony Diameter (in mm) at 4DAI	Per cent Growth Inhibition at 4DAI
T1	*Bacillus aryabhattai*	47.75	45.11
T2	*Bacillus tequillensis*	87	0
T3	*Pseudomonas fluorescens*	60.75	30.17
T4	Control	87	
FTest		Sig	
SE(m)±		1.98	
CD@1%		12.86	

Table 6d. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Macrophomina phaseolina in vitro* causing dry root rot in cotton

Treatment	Bioagent	Average colony Diameter(in mm) at 4DAI	Per cent Growth Inhibition at 4DAI
T1	*Bacillus aryabhattai*	56	9.31
T2	*Bacillus tequillensis*	59.5	3.64
T3	*Pseudomonas fluorescens*	25.00	59.51
T4	Control	61.75	
FTest		Sig	
SE(m)±		1.20	
CD@1%		7.84	

Table 6e. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Nigrospora sphaerica in vitro*

Treatment	**Bioagent**	**Average colony Diameter (in mm) at 4DAI**	**Per cent Growth Inhibition at 4DAI**
T1	*Bacillus aryabhattai*	21	53.59
T2	*Bacillus tequillensis*	44	2.76
T3	*Pseudomonas fluorescens*	0	100
T4	Control	45.25	
FTest		Sig	
SE(m)±		2.40	
CD@1%		2.07	

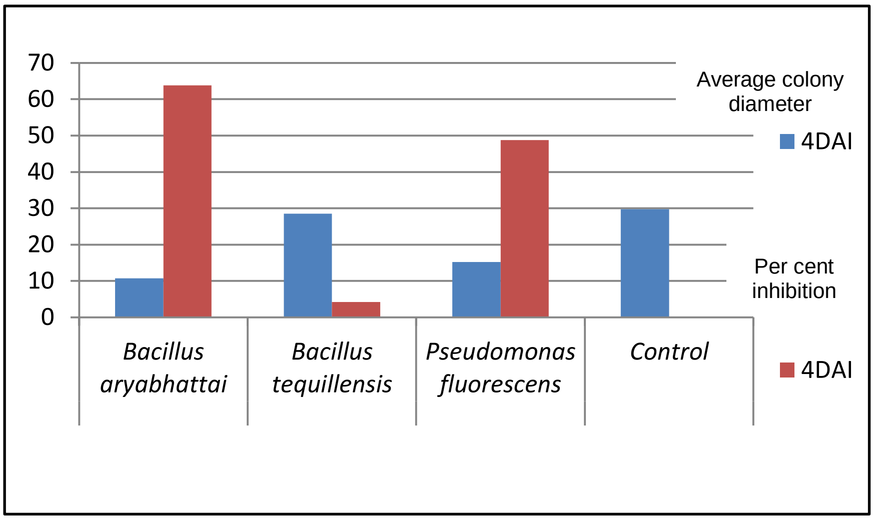


Figure 1. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Corynespora cassiicola in vitro* causing target spot in cotton

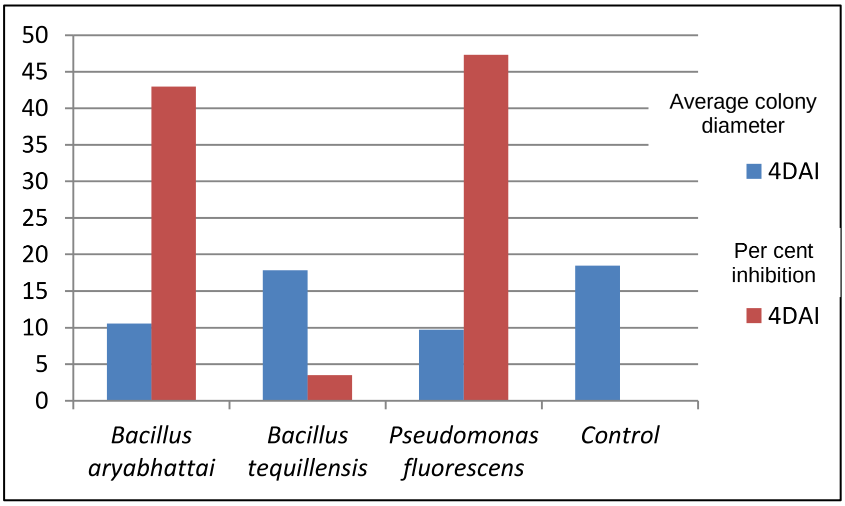


Figure 2. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Paramyrothecium roridium in vitro*

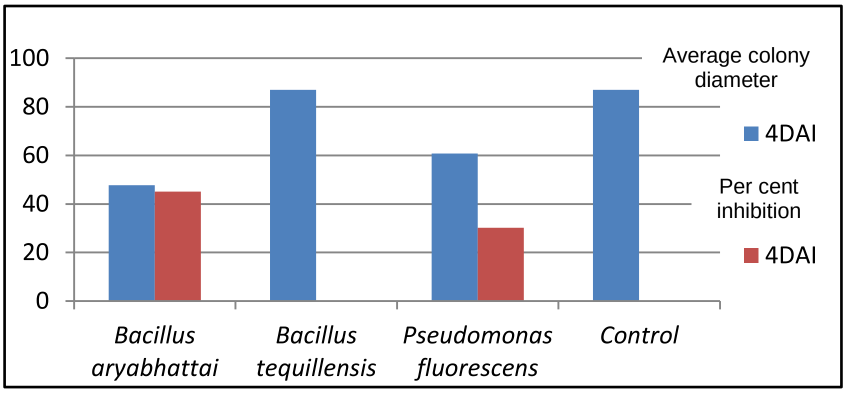


Figure 3. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Rhizoctonia solani in vitro* causing root rot in cotton

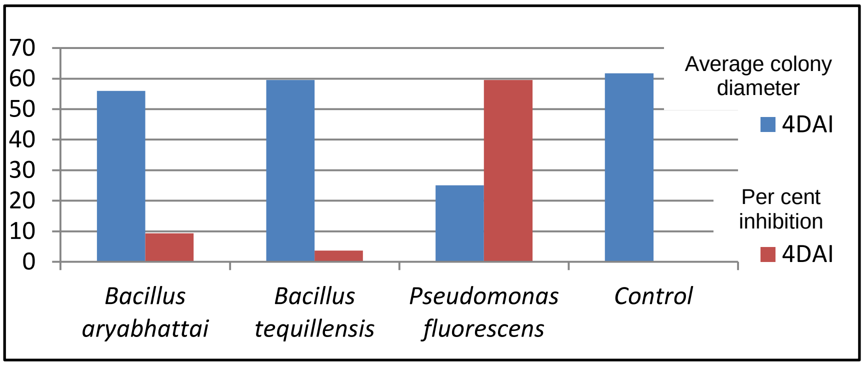


Figure 4. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Macrophomina phaseolina in vitro* causing dry root rot in cotton

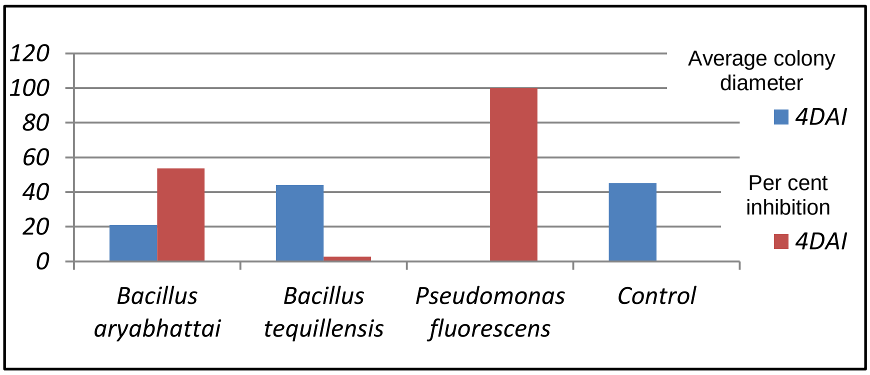


Figure 5. Effect of bacterial bioagents on average colony diameter and per cent inhibition of *Nigrospora sphaerica in vitro*

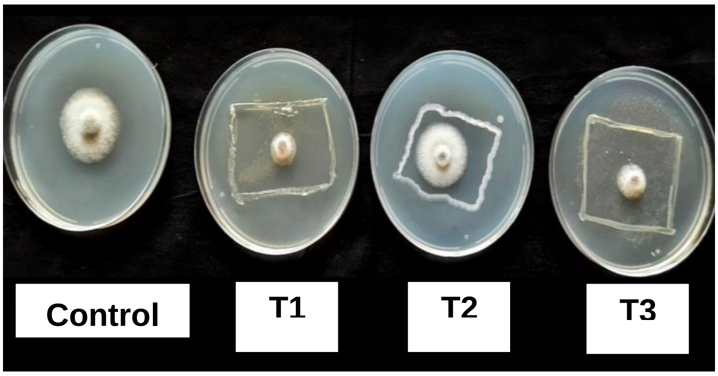


Plate 11a. Dual culture test of bacterial bioagents on growth inhibition against *Corynespora cassiicola in vitro* causing target spot in cotton

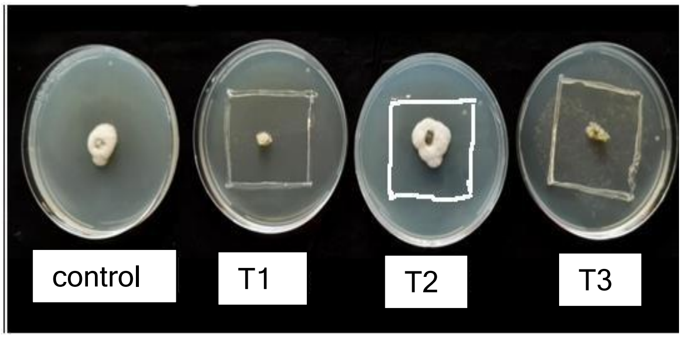


Plate 11b. Dual culture test of bacterial bioagents on growth inhibition against *Paramyrothecium roridium in vitro*

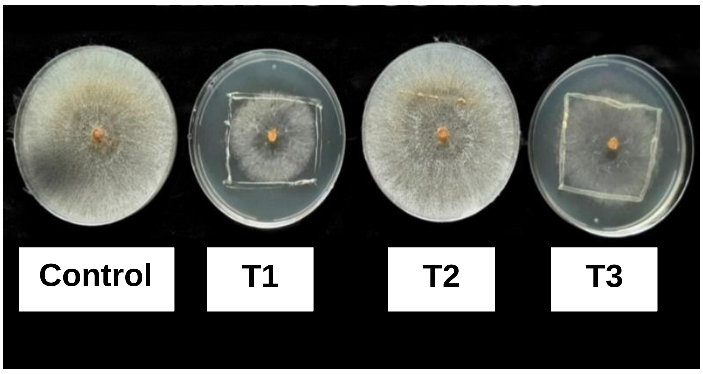


Plate 11c. Dual culture test of bacterial bioagents on growth inhibition against *Rhizoctonia solani in vitro* causing root rot in cotton

T1- ***Bacillus aryabhattai***
T2- ***Bacillus tequillensis***
T3- ***Pseudomonas fluorescens***

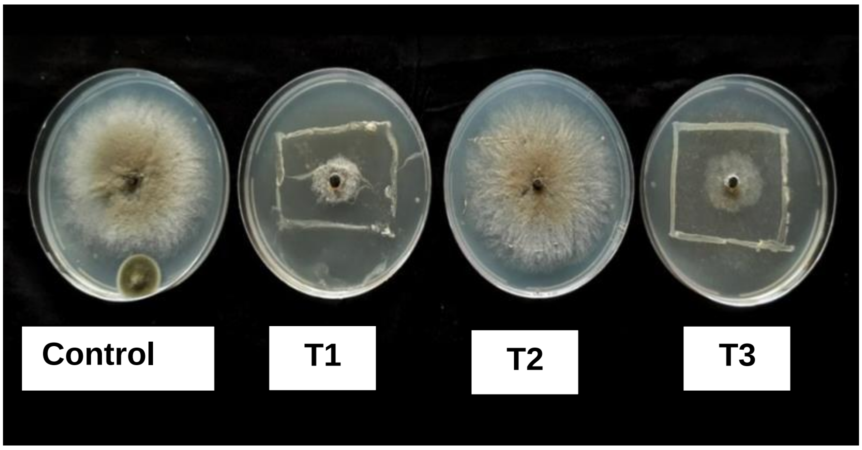


Plate 11d. Dual culture test of bacterial bioagents on growth inhibition against *Macrophomina phaseolina in vitro* causing dry root rot in cotton

Plate 11e. Dual culture test of bacterial bioagents on growth inhibition against *Nigrospora sphaerica in vitro*

T1- *Bacillus aryabhattai*

T2- *Bacillus tequillensis*

T3- *Pseudomonas fluorescens*

Table 6f. Effect of bacterial bioagents against the average colony diameter of pathogens associated with cotton leaf spot *in vitro*

Sr. No.	**Fungal pathogen**	***Corynespora cassiicola***		***Paramyrothecium roridium***		***Rhizoctonia solani***		***Macrophomina phaseolina***		***Nigrospora sphaerica***	
	Bacterial Bioagent	Average colony diameter (in mm)	Per cent inhibition (%)	Average colony diameter (in mm)	Per cent inhibition (%)	Average colony diameter (in mm)	Per cent inhibition (%)	Average colony diameter (in mm)	Per cent inhibition (%)	Average colony diameter (in mm)	Per cent inhibition (%)
1	*Bacillus aryabhattai*	10.75	63.86	10.55	42.97	47.75	45.11	56	9.31	21	53.59
2	*Bacillus tequillensis*	28.50	4.20	17.85	3.51	87	0	59.5	3.64	44	2.76
3	*Pseudomonas fluorescens*	15.25	48.73	9.75	47.29	60.75	30.17	25.00	59.51	0	100
4	Control	29.75		18.50		87		61.75		45.25	

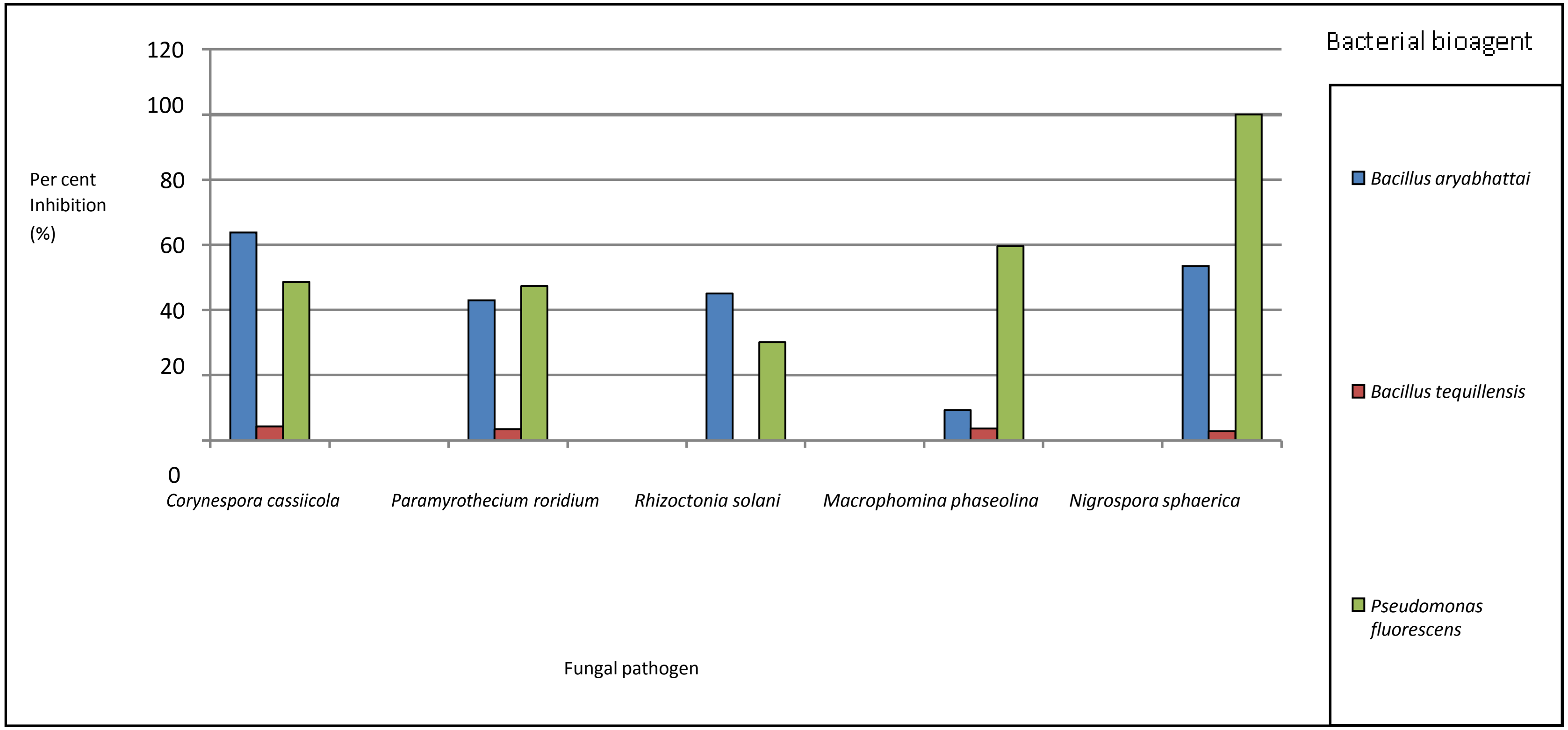


Figure 6. Effect of bacterial bioagents against the average colony diameter of pathogens associated with cotton leaf spot *in vitro*

Table 7a. Effect of fungal bioagents on average colony diameter and per cent inhibition of *Corynespora cassiicola in vitro* causing target spot in cotton

Treatment	***Corynespora casiicola***	**Average colony diameter (in mm)**			**Per cent inhibition (%)**		
	Fungal Bioagent	**3DAI**	**5DAI**	**8DAI**	**3DAI**	**5DAI**	**8DAI**
T1	*Trichoderma viride*	0	0	0	100	100	100
T2	*Trichoderma harzianum*	21.75	21.75	21.75	27.50	52.20	64.50
T3	*Talaromyces pinophilus* 1	29.00	45.00	57.25	3.34	1.10	6.53
T4	*Talaromyces pinophilus* 2	28.50	41.50	50.00	5.00	8.80	18.37
T5	*Acrimonium cellulolyticus*	28.25	38.00	40.50	5.84	16.48	33.88
T6	*Trichoderma longibracheatum*	0	0	0	100	100	100
T7	*Talaromyces purpurogenus*	0	0	0	100	100	100
T8	Control	30	45.50	61.25			
	FTest	Sig	Sig	Sig			
	SE(m)±	1.09	2.75	3.83			
	CD@1%	5.20	13.08	18.18			

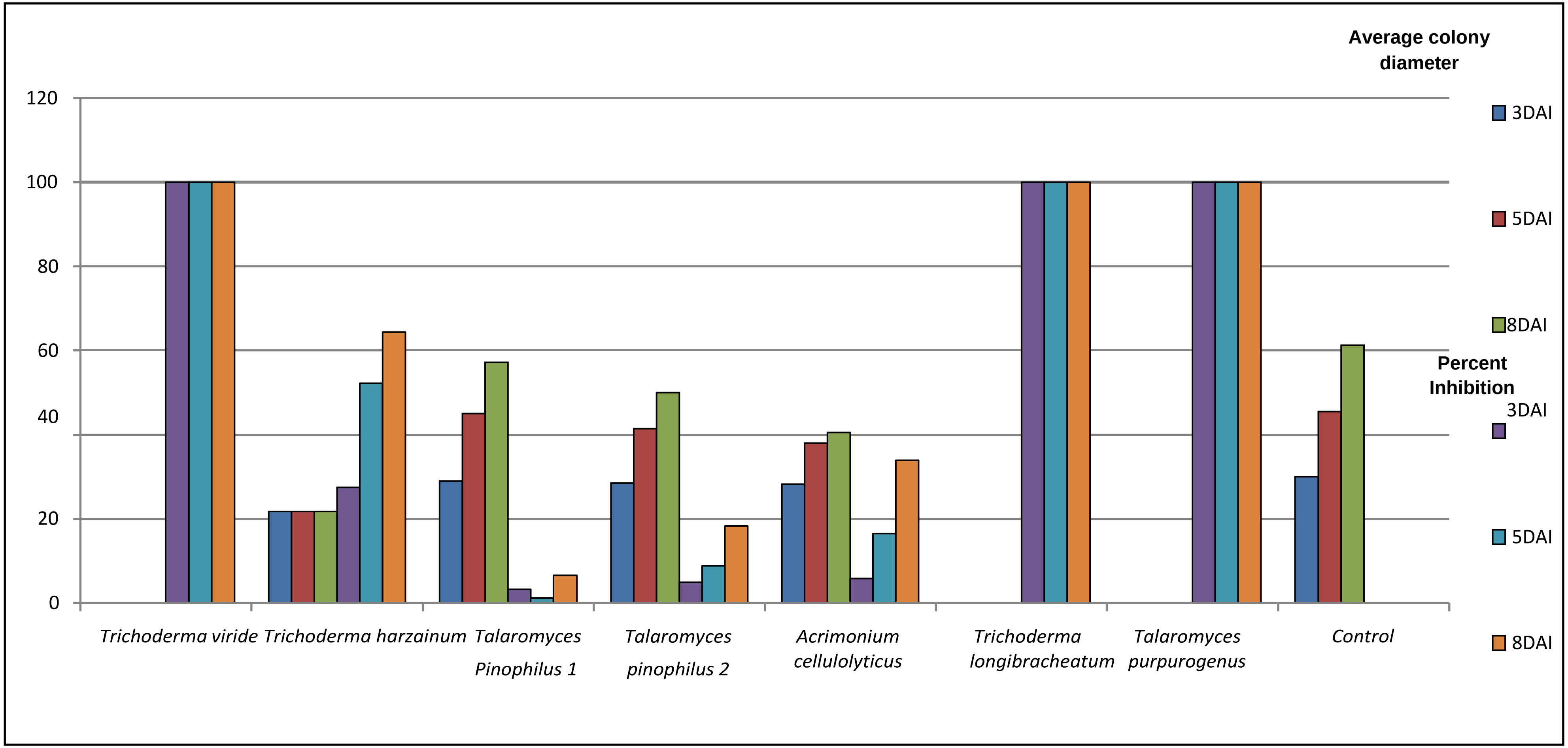


Figure 7. Effect of fungal bioagents on average colony diameter and per cent inhibition of *Corynespora cassiicola in vitro* causing target spot in cotton

Table 7b. Effect of fungal bioagent on average colony diameter and per cent inhibition of *Paramyrothecium roridium* *in vitro*

Treatment	***Paramyrothecium roridium***	**Average colony diameter(in mm)**			**Percent inhibition (%)**		
	Fungal Bioagent	**3DAI**	**5DAI**	**8DAI**	**3DAI**	**5DAI**	**8DAI**
T1	*Trichoderma viride*	14.75	14.75	18	3.13	28.05	65.04
T2	*Trichoderma harzianum*	14.25	15.50	17.50	6.06	24.39	66.01
T3	*Talaromyces pinophilus* 1	14.75	14.75	19.75	1.51	28.05	61.74
T4	*Talaromyces pinophilus* 2	14.00	17.75	21.00	6.06	13.41	61.16
T5	*Acrimonium cellulolyticus*	14.75	18.50	19.25	10.90	9.70	62.71
T6	*Trichoderma longibracheatum*	10.50	20.50	20.75	3.03	0	59.80
T7	*Talaromyces purpurogenus*	11.50	18.00	26.25	9.09	12.19	2.91
T8	**Control**	16.50	20.50	51.50			
	FTest	Sig	Sig	Sig			
	SE(m)±	0.79	1.26	1.14			
	CD@1%	3.75	6.00	5.43			

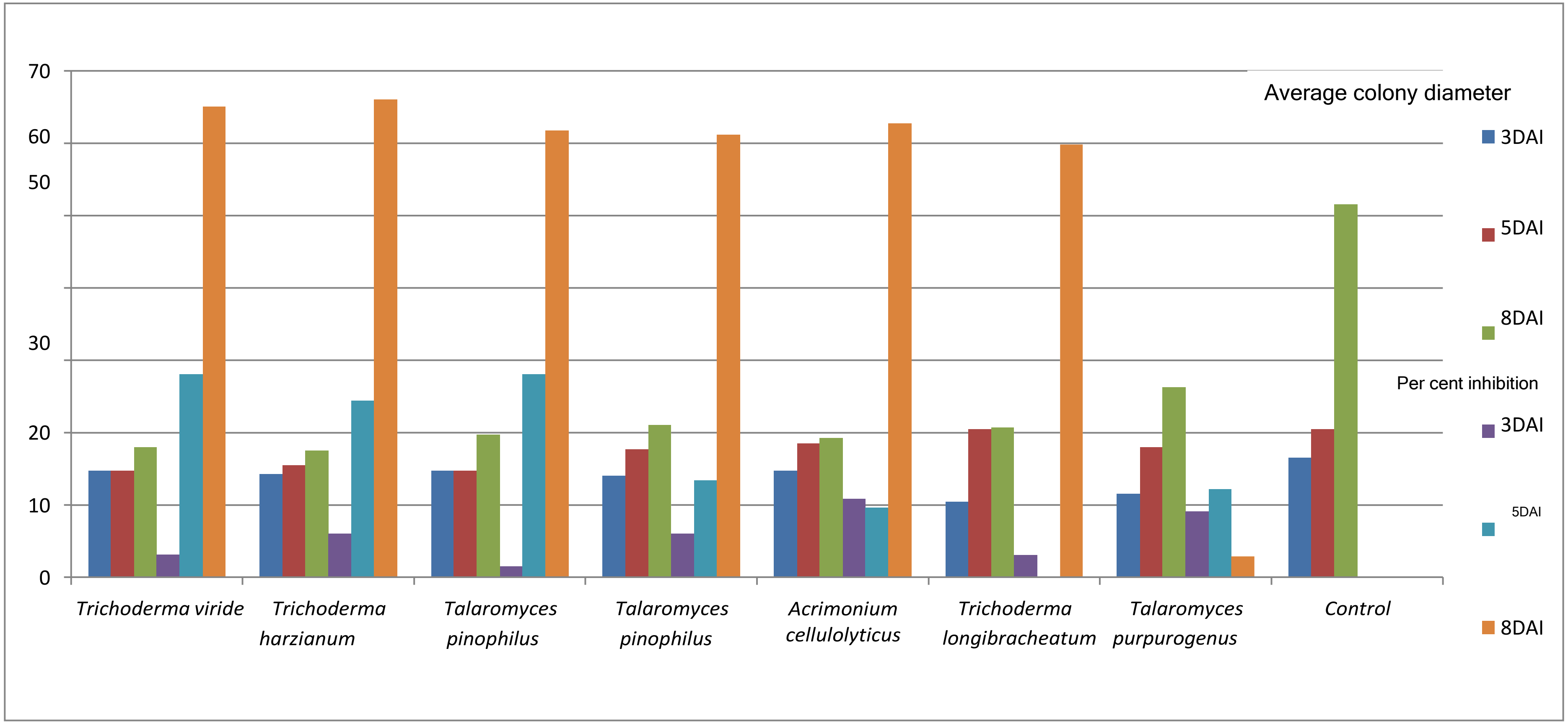


Figure 8. Effect of fungal bioagents on average colony diameter and per cent inhibition of *Paramyrothecium roridium in vitro*

Table 7c. Effect of fungal bioagents on average colony diameter and per cent inhibition of *Rhizoctonia solani in vitro* causing root rot in cotton

Treatment	***Rhizoctonia solani***	**Average colony diameter (in mm)**			**Percent inhibition (%)**		
	Fungal Bioagent	**3DAI**	**5DAI**	**8DAI**	**3DAI**	**5DAI**	**8DAI**
T1	*Trichoderma viride*	67.75	67.50	67.50	9.36	22.41	22.41
T2	*Trichoderma harzianum*	59.00	57.50	57.50	21.07	33.90	33.90
T3	*Talaromyces pinophilus* 1	62.00	61.75	61.75	17.06	29.02	29.02
T4	*Talaromyces pinophilus* 2	63.00	75.75	75.75	15.71	12.93	12.93
T5	*Acrimonium cellulolyticus*	60.50	61.25	77.25	19.07	29.65	11.20
T6	*Trichoderma longibracheatum*	63.25	67.00	75.00	15.39	22.98	13.79
T7	*Talaromyces purpurogenus*	62.75	70.00	78.50	16.06	19.54	10.34
T8	Control	74.75	87.00	87.00			
	FTest	Sig	Sig	Sig			
	SE(m)±	2.27	4.46	4.21			
	CD@1%	10.77	21.16	19.98			

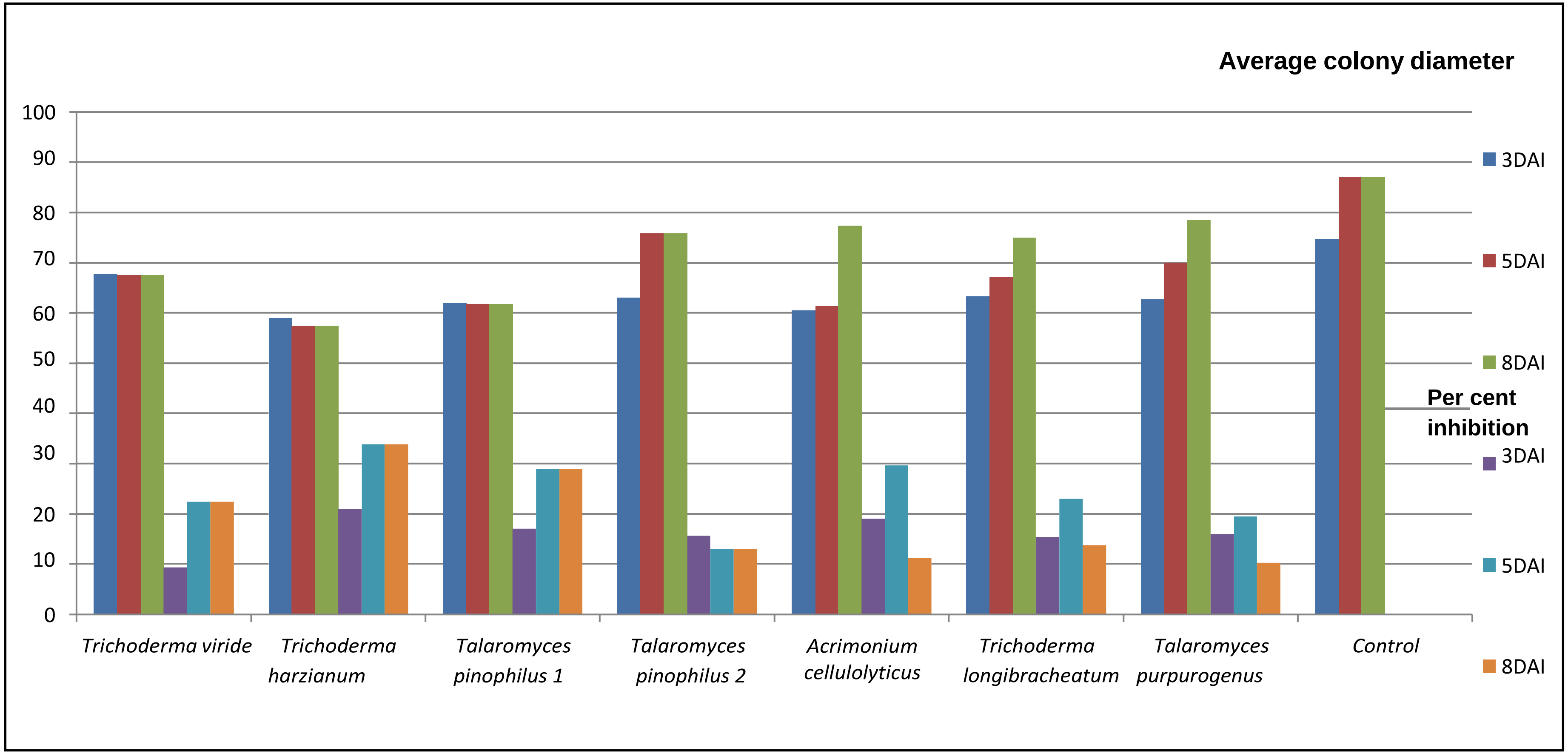


Figure 9. Effect of different fungal bioagents on average colony diameter and per cent inhibition of *Rhizoctonia solani in vitro* causing root rot in cotton

Table 7d. Effect of fungal bioagents on average colony diameter and per cent inhibition of *Macrophomina phaseolina invitro* causing dry root rot in cotton

Treatment	***Macrophomina phaseolina***	**Average colony diameter (in mm)**			**Percent inhibition (%)**		
	Fungal Bioagent	**3DAI**	**5DAI**	**8DAI**	**3DAI**	**5DAI**	**8DAI**
T1	*Trichoderma viride*	30.75	37.00	45.70	16.89	21.70	45.59
T2	*Trichoderma harzianum*	31.50	37.00	43.50	14.86	21.70	48.21
T3	*Talaromyces pinophilus* 1	33.25	37.20	41.00	10.13	21.18	51.19
T4	*Talaromyces pinophilus* 2	32.99	40.00	34.45	0.03	15.25	58.98
T5	*Acrimonium cellulolyticus*	33.00	39.00	39.00	10.81	17.37	53.57
T6	*Trichoderma longibracheatum*	33.50	43.50	48.35	9.45	7.83	42.44
T7	*Talaromyces purpurogenus*	33.00	46.00	73.70	10.81	2.54	12.26
T8	Control	37.00	47.20	84.00			
	FTest	Sig	Sig	Sig			
	SE(m)±	0.83	4.70	2.14			
	CD@1%	3.97	22.33	10.19			

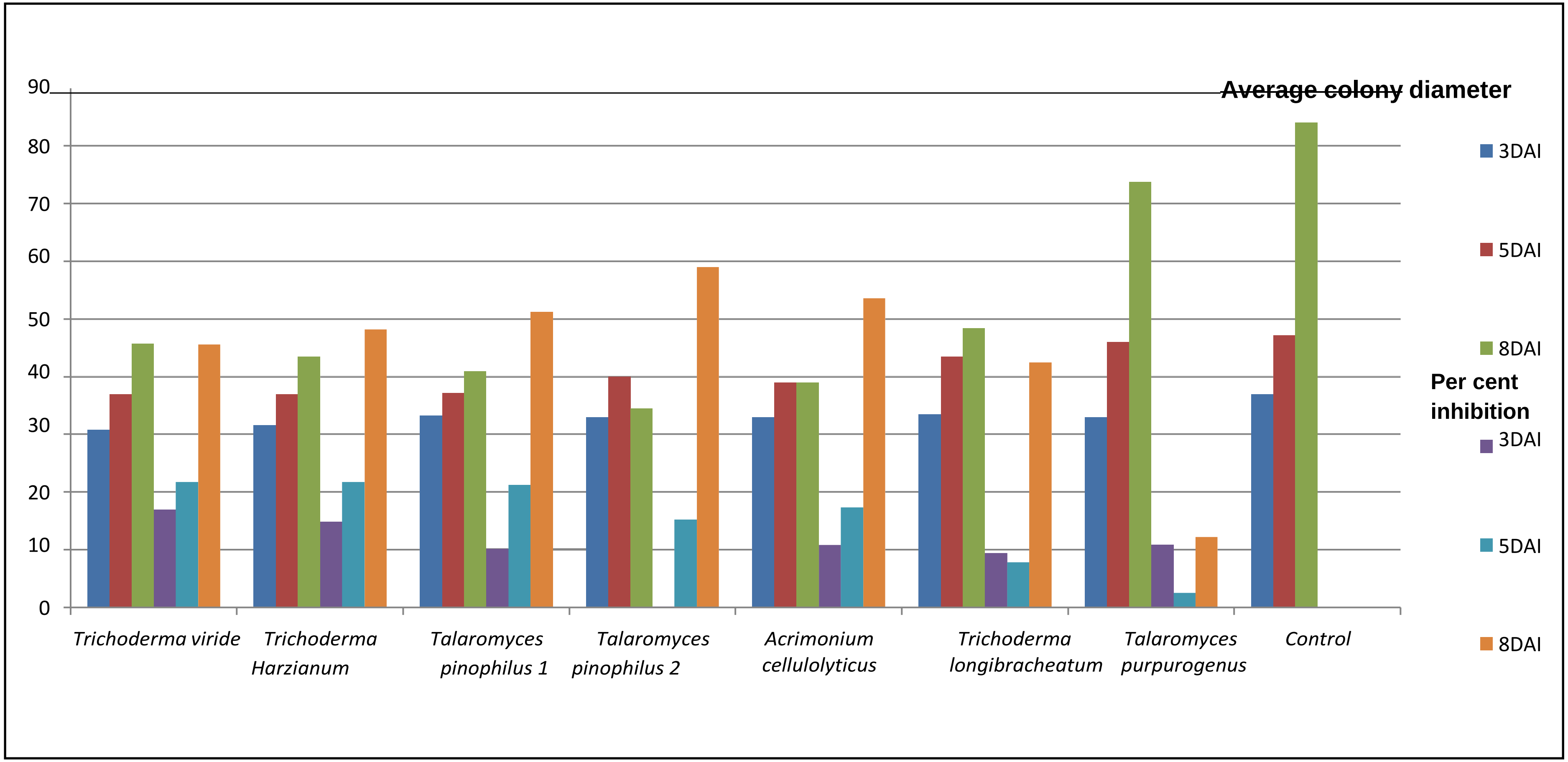


Figure 10. Effect of different fungal bioagents on average colony diameter and per cent inhibition of *Macrophomina phaseolina* causing dry root rot in cotton

Table 7e. Effect of fungal bioagents on average colony diameter and per cent inhibition of *Nigrospora sphaerica in vitro*

Treatment	***Nigrospora sphaerica***	**Average colony diameter(in mm)**			**Percent inhibition (%)**		
	Fungal Bioagent	**3DAI**	**5DAI**	**8DAI**	**3DAI**	**5DAI**	**8DAI**
T1	*Trichoderma viride*	48	60	60	27.81	22.33	28.57
T2	*Trichoderma harzianum*	50.00	60.50	60.50	24.81	27.97	33.92
T3	*Talaromyces pinophilus* 1	45.00	51.75	51.75	32.33	33.00	38.39
T4	*Talaromyces pinophilus* 2	46.25	49.25	49.25	30.45	36.24	41.36
T5	*Acrimonium cellulolyticus*	51.00	61.50	61.50	23.30	20.38	26.78
T6	*Trichoderma longibracheatum*	52.25	64.00	64.00	21.42	17.15	23.80
T7	*Talaromyces purpurogenus*	54.75	69.25	69.25	17.67	10.35	17.56
T8	Control	66.50	77.25	84.00			
	FTest	Sig	Sig	Sig			
	SE(m)±	2.55	2.68	3.16			
	CD@1%	12.11	12.74	15.02			

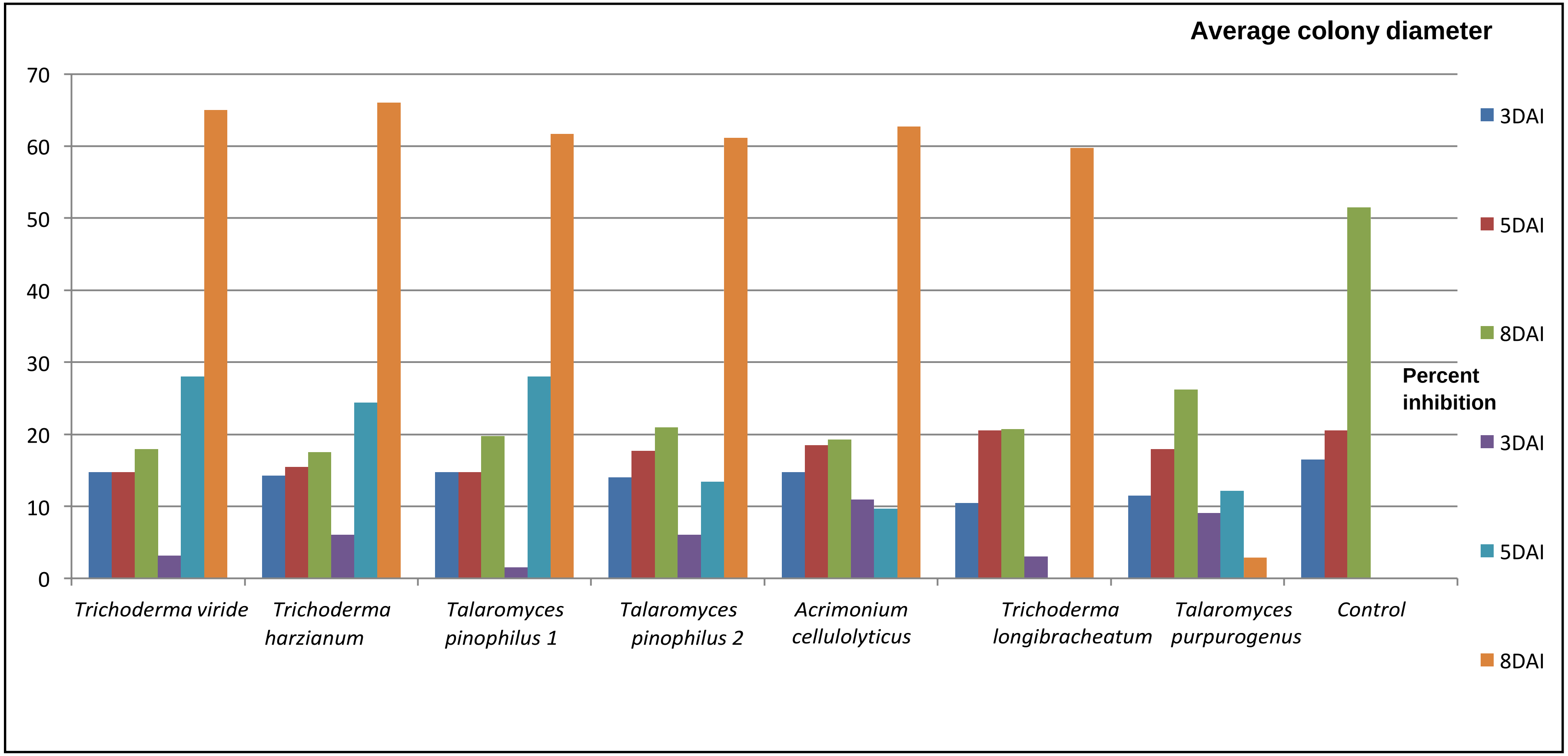


Figure 11. Effect of fungal bioagent on average colony diameter and per cent inhibition of *Nigrospora sphaerica in vitro*

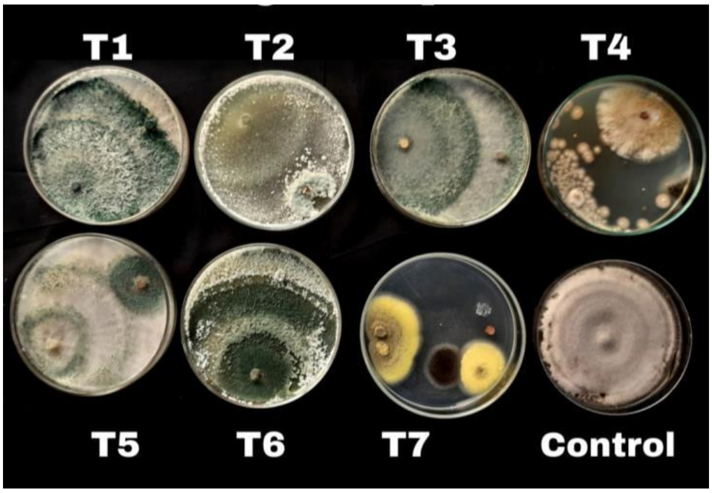


Plate 12a. Dual culture test of fungal bioagents on growth inbhibition against *Corynespora cassiicola in vitro* causing target spot in cotton

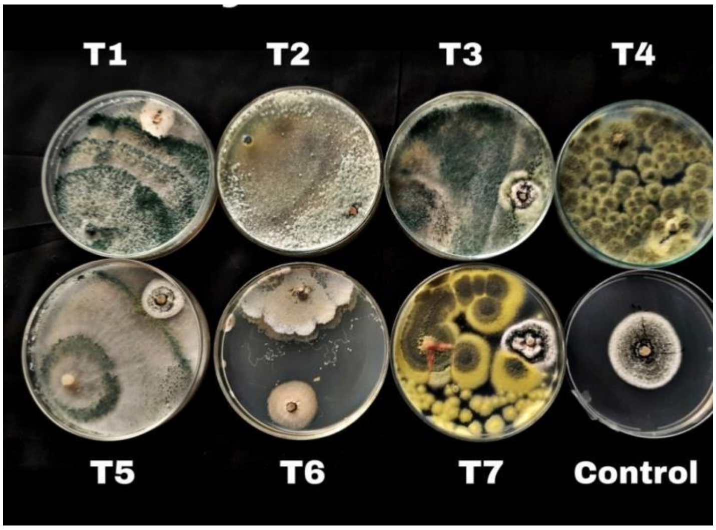


Plate 12b. Dual culture test of fungal bioagents on growth inbhibition against *Paramyrothecium roridium in vitro*

T1-*Trichoderma viride*

T2- *Trichoderma harzianum*

T3- *Talaromyces pinophilus1*

T4- *Talaromyces pinophilus2*

T5-*Acrimonium cellulolyticus*

T6- *Trichoderma longibracheatum*

T7- *Talaromyces purpurogenus*

Plate 12c. Dual culture test of fungal bioagents on growth inbhibition against *Rhizoctonia solani in vitro* causing root rot in cotton

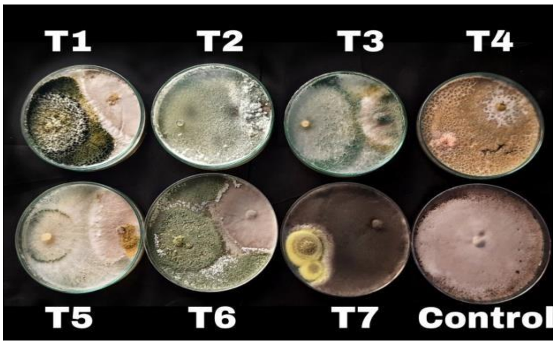


Plate 12d. Dual culture test of fungal bioagents on growth inbhibition against *Macrophomina phaseolina in vitro* causing dry root rot in cotton

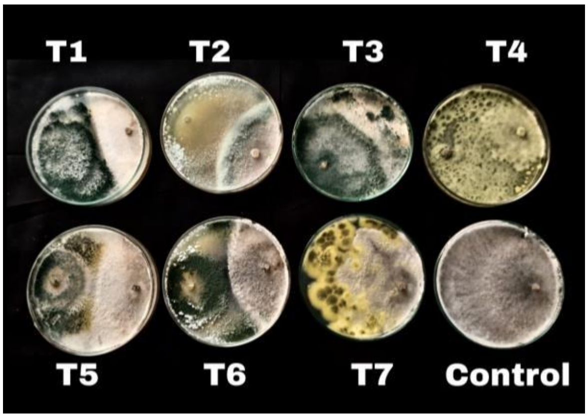


Plate 12e. Dual culture test of fungal bioagents on growth inhibition against *Nigrospora sphaerica in vitro*

T1-*Trichoderma viride*
T2- *Trichoderma harzianum*
T3- *Talaromyces pinophilus 1*
T4-*Talaromyces pinophilus 2*
T5-*Acrimonium cellulolyticus*
T6- *Trichoderma longibracheatum*
T7- *Talaromyces purpureogenus*

Table 7f. Effect of fungal bioagents against the average colony diameter of pathogens associated with cotton leaf spot *in vitro*

Sr. No.	Fungal pathogen	***Corynespora cassiicola***		***Paramyrothecium roridium***		***Rhizoctonia solani***		***Macrophomina phaseolina***		***Nigrospora Sphaerica***	
	Bacterial Bioagent	Average colony diameter (in mm)	Per cent inhibition (%)	Average colony diameter (in mm)	Per cent inhibition (%)	Average colony diameter (in mm)	Per cent inhibition (%)	Average colony diameter (in mm)	Per cent inhibition (%)	Average colony diameter (in mm)	Per cent inhibition (%)
1	*Trichoderma viride*	0	100	18	42.97	67.50	22.41	45.70	45.59	60.00	28.57
2	*Trichoderma harzianum*	21.75	64.50	17.50	3.51	57.50	33.90	43.50	48.21	60.50	33.92
3	*Talaromyces pinophilus* 1	57.25	6.53	19.75	47.29	61.75	29.02	41.00	51.19	51.75	38.39
4	*Talaromyces pinophilus* 2	50.00	18.37	21.00	65.04	75.75	12.93	34.45	58.98	49.25	41.36
5	*Acrimonium cellulolyticus*	40.50	33.88	19.25	66.01	77.25	11.20	39.00	53.57	61.50	26.78
6	*Trichoderma longibracheatum*	0	100	20.75	61.74	75.00	13.79	48.35	42.44	64.00	23.80
7	*Talaromyces purpurogenus*	0	100	28.75	61.16	78.50	10.34	73.70	12.26	69.25	17.56
8	Control	61.25		51.50		87.00		84.00		84.00	

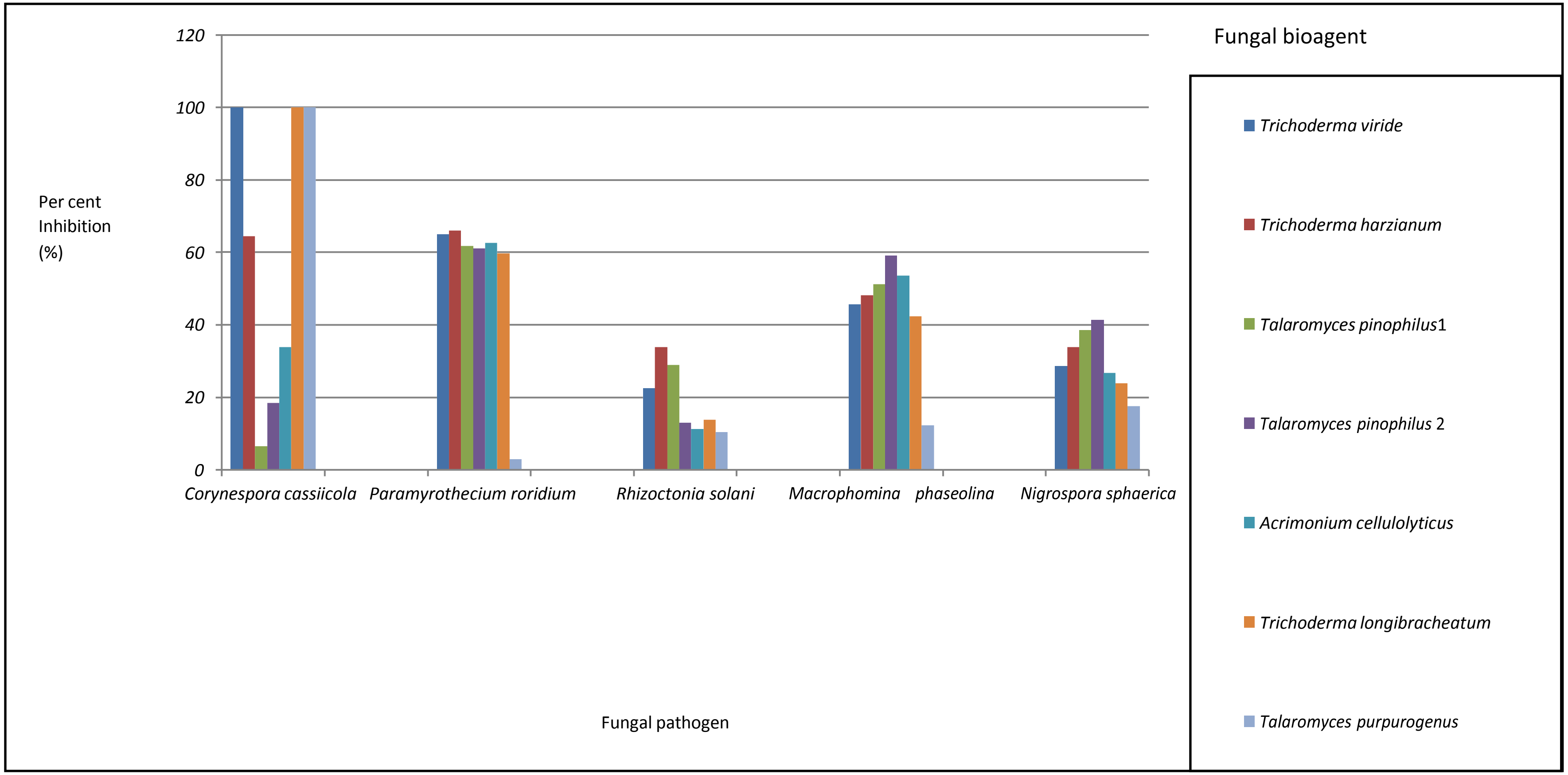


Figure 12. Effect of fungal bioagents against the average colony diameter of pathogens associated with cotton leaf spot *in vitro*

4.5 *In vitro* evaluation of different label claim fungicides against the pathogens identified

The results of *in vitro* studies on the efficacy of label claim fungicides (Appendix I) at recommended dose were presented in table8a-table 8f, plate 13a-13e and also graphically represented in figure 13-18. The treatment differences in respect of colony diameter were statistically significant. All fungicidal treatments showed significantly least colony diameter as compared to control. The efficacy of different fungicides for controlling different pathogens responsible for causing leaf spot of cotton was determined by poison food technique (Nene and Thapliyal, 1993). Eight different label claim fungicides were tested *in vitro* against the five different fungal pathogens isolated from cotton leaves.

4.5.1 *Corynespora cassiicola*

All the treatments were found significantly different from each other. Carbendazim 50% WP (T4) at 0.25% (2500ppm) was found most effective against *Corynespora cassiicola* and showed complete (100%) fungal growth inhibition over control. Fluxapyroxad 167g/L + pyraclostrobin 33g/L (T8) at 0.06% (600ppm) showed only upto 93.56% fungal growth inhibition over control. Other fungicides such as propineb 70WP (T2) at 0.25% (2500ppm), pyraclostrobin 5% WG + metiram 5% (T3) at 0.25% (2500ppm) and propiconazole (T5) 0.1% (1000ppm), recorded over 70% inhibition of fungal growth over control. The least effective fungicide was kresoxim-methyl 44.3% (T6) at 0.1%(1000ppm) exhibiting fungal growth inhibition upto only 21.92% over control (table 8a, plate 13a, figure 13).

The results are in confirmation with Manju *et al.* (2019), Ishwari *et al.* (2020), Yamuna *et al.* (2020) where carbendazim 50% WP gave complete inhibition (100%) of *Corynespora cassicola* over control.

4.5.2 *Paramyrothecium roridium*

All the test fungicides effectively inhibited fungal growth over control. Copper oxychloride 50WP (T1) at 0.25% (2500ppm), pyraclostrobin 5% WG + metiram 5% (T3) at 0.25% (2500ppm), carbendazim 50% WP (T4) at 0.25% (2500ppm) and fluxapyroxad 167g/L + pyraclostrobin 33g/L (T8) at 0.06% (600ppm) were found at par with each other (90.09% and 90.07% inhibition over control). Propiconazole (T5) 0.1% (1000ppm) and azoxystrobin 18.2% W/W + difenconazole 11.4% W/W SC (T7) at 0.1% (1000ppm) were found at par with each other (89.88% and 89.60% inhibition over control) (table 8b, plate 13b, figure 14).

The results are in confirmation with Ingole and Ingle (2011) where carbendazim 50% WP effectively controlled *Myrothecium roridium*, and Rattan *et al.* (2012), Yamuna *et al.* (2020) and Vishwakarma *et al.* (2021) where carbendazim showed complete mycelia inhibition (100%) of *Myrothecium roridium* over control.

4.5.3 *Rhizoctonia solani*

Carbendazim 50% WP (T4) at 0.25% (2500ppm) and Fluxapyroxad 167g/L + Pyraclostrobin 33g/L (T8) at 0.06% (600ppm) showed complete (100%) fungal growth inhibition over control against *Rhizoctonia solani* and were found at par with each other. Fungicides such as pyraclostrobin 5% WG + metiram 5% (T3) at 0.25% (2500ppm) and propiconazole (T5) 0.1% (1000ppm) (92.72% and 91.57% inhibition over control) were found at par with each other. In contrast, kresoxim- methyl 44.3% (T6) at 0.1% (1000ppm) exhibiting 0% fungal growth inhibition over control was recorded as the least effective fungicide against *Rhizoctonia solani* (Table 8c, Plate 13c, figure 15).

The results are in confirmation with Yadav (2020) and Usendi *et al*. (2020) where carbendazim 50% WP showed complete (100%) inhibition of *Rhizoctonia solani* over control.

4.5.4 ***Macrophomina phaseolina***

Fluxapyroxad 167g/L + pyraclostrobin 33g/L (T8) at 0.06% (600ppm) was found most effective against *Macrophomina phaseolina* showing 91.95% fungal growth inhibition over control and was found at par with Propineb 70WP (T2) at 0.25% (2500ppm), pyraclostrobin 5% WG +metiram 5% (T3) at 0.25% (2500ppm), propiconazole (T5) 0.1% (1000ppm) and azoxystrobin 18.2% W/W + difenconazole 11.4% W/W SC (T7) at 0.1% (1000ppm) showing 91.37%, 88.50% and 87.73% inhibition over control). Kresoxim-methyl 44.3% (T6) at 0.1% (1000ppm) showing 34.48% fungal growth inhibition over control was recorded as the least effective fungicide against *Macrophomina phaseolina.* (table 8d, Plate 13d, graph 16).

The results are in confirmation with Iqbal and Mukhtar (2020) and Savaliya *et al.* (2020) where carbendazim 50% WP and pyraclostrobin effectively controlled (100% inhibition) of *Macrophomina phaseolina* over control.

4.5.5 ***Nigrospora sphaerica***

Carbendazim 50% WP (T4) at 0.25% (2500 ppm) and propineb 70WP (T2) at 0.25% (2500 ppm) were most effective fungicide against *Nigrospora sphaerica* showing complete inhibition (100%) of fungal growth over control and were found at par with each other. Pyraclostrobin 5% WG + Metiram 5% (T3) at 0.25% (2500ppm) and propiconazole (T5) 0.1% (1000ppm) were found at par with each other (92.33% and 89.45% inhibition). fluxapyroxad 167g/L + pyraclostrobin 33g/L (T8) at 0.06% (600ppm) and Copper oxychloride 50WP (T1) at 0.25% (2500ppm) were found at par with each other (58.43% inhibition). kresoxim-methyl 44.3% (T6) at 0.1% (1000ppm) showing 44.64% fungal growth inhibition was recorded as the least effective fungicide against *Nigrospora sphaerica* (table 8e, plate 13e, figure 17).

The results are in confirmation with Mane *et al.* (2018) where carbendazim 0.1% effectively controlled *Nigrospora sphaerica* over control.

Thus, in general, carbendazim 50% WP (T4) at 0.25% (2500ppm), propineb 70WP (T2) at 0.25% (2500ppm), pyraclostrobin 5% WG + Metiram 5% (T3) at 0.25% (2500ppm), propiconazole (T5) 0.1% (1000ppm), fluxapyroxad 167g/L + pyraclostrobin 33g/L (T8) at 0.06% (600ppm), were found most effective and kresoxim-methyl 44.3% (T6) at 0.1%(1000ppm) was recorded as the least effective fungicide against the fungal leaf spot disease complex of cotton. (table 8f, figure 18).

Thus, the results are in confirmation with Manju *et al.* (2019), Yamuna *et al*. (2020), Ingole and Ingle (2011), Vishwakarma *et al.* (2021), Yadav (2020), Iqbal and Mukhtar (2020), Savaliya *et al.* (2020) and Mane *et al.* (2018) where similar results were obtained.

Table 8a. Effect of CIBRC label claim fungicides on average colony diameter and per cent inhibition of *Corynespora cassiicola in vitro* causing target spot in cotton

Treatment	*Corynespora casiicolla*	Conc. (%)	Average colony diameter (in mm)			Percent inhibition (%)		
	CIBRC label claim fungicides		**3DAI**	**5DAI**	**8DAI**	**3DAI**	**5DAI**	**8DAI**
T1	Copper Oxychloride50WP	0.25	10.67	13.67	17.67	63.62	69.16	69.00
T2	Propineb70WP	0.25	0	5.33	11.00	100	87.97	80.70
T3	Pyraclostrobin 5%WG+ Metiram55%	0.25	6.5	8.00	16.50	77.83	81.95	71.05
T4	Carbendazim 50%WP	0.25	0	0	0	100	100	100
T5	Propiconazole 0.01%	0.1	0	5.00	10.00	100	88.72	82.45
T6	Kresoxim-Methyl 44.3 %	0.1	14.17	27.83	44.50	51.68	37.22	21.92
T7	Azoxystrobin 18.2% W/W+ Difenconazole 11.4% W/W SC	0.1	10.17	15.67	23.17	66.32	64.65	59.36
T8	Fluxapyroxad 167g/L+ Pyraclostrobin 33g/L SC	0.06	2.83	3.50	3.67	90.35	92.10	93.56
T9	Control		29.33	44.33	57.00			
	F Test		Sig	Sig	Sig			
	SE(m)±		0.10	0.14	0.23			
	CD@1%		0.43	0.59	0.96			

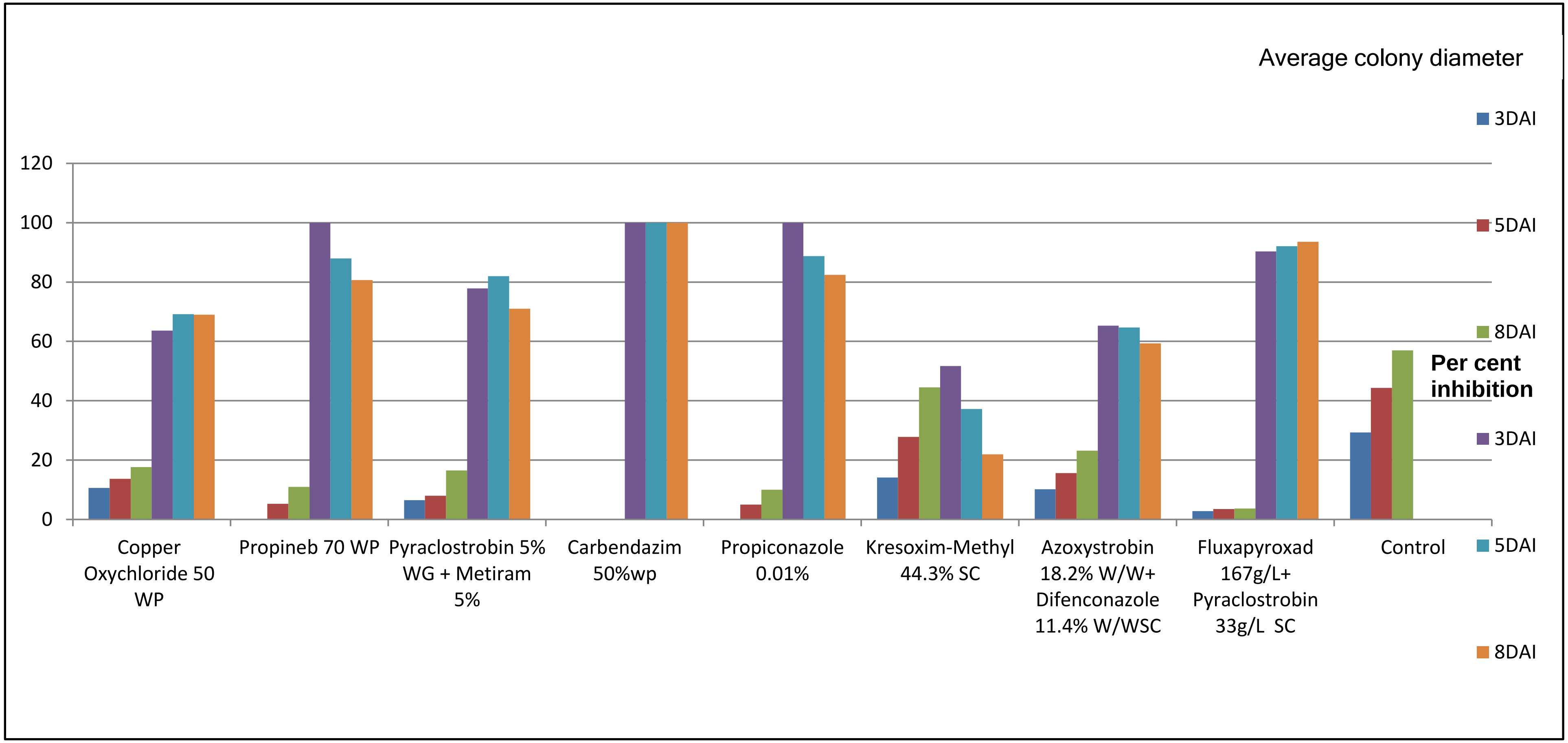


Figure 13. Effect of CIBRC label claim fungicides on average colony diameter and per cent inhibition of *Corynespora cassiicola in vitro* causing target spot in cotton

Table 8b. Effect of CIBRC label claim fungicides on average colony diameter and per cent inhibition of *Paramyrothecium roridium in vitro*

Treatment	*Paramyrothecium roridium*	Conc. (%)	Average colony diameter (in mm)			Percent inhibition(%)		
	CIBRC label claim fungicides		**3DAI**	**5DAI**	**8DAI**	**3DAI**	**5DAI**	**8DAI**
T1	Copper Oxychloride50WP	0.25	5.05	5.05	5.05	85.97	89.94	90.09
T2	Propineb70WP	0.25	7	7	7	80.55	85.41	86.27
T3	Pyraclostrobin 5%WG+ Metiram55%	0.25	5.06	5.06	5.06	85.94	89.45	90.07
T4	Carbendazim 50%WP	0.25	5.06	5.06	5.06	85.94	89.45	90.07
T5	Propiconazole 0.01%	0.1	5.3	5.3	5.3	85.27	88.95	89.60
T6	Kresoxim-Methyl 44.3 %	0.1	7.67	7.67	7.67	78.69	84.02	84.96
T7	Azoxystrobin 18.2% W/W+ Difenconazole 11.4% W/W SC	0.1	5.16	5.16	5.16	85.67	89.25	89.88
T8	Fluxapyroxad 167g/L+ Pyraclostrobin 33g/L SC	0.06	5.06	5.06	5.06	85.94	89.45	90.07
T9	Control		36	48	51			
	F Test		Sig	Sig	Sig			
	SE(m) ±		0.22	0.22	0.22			
	CD@1%		0.92	0.92	0.92			

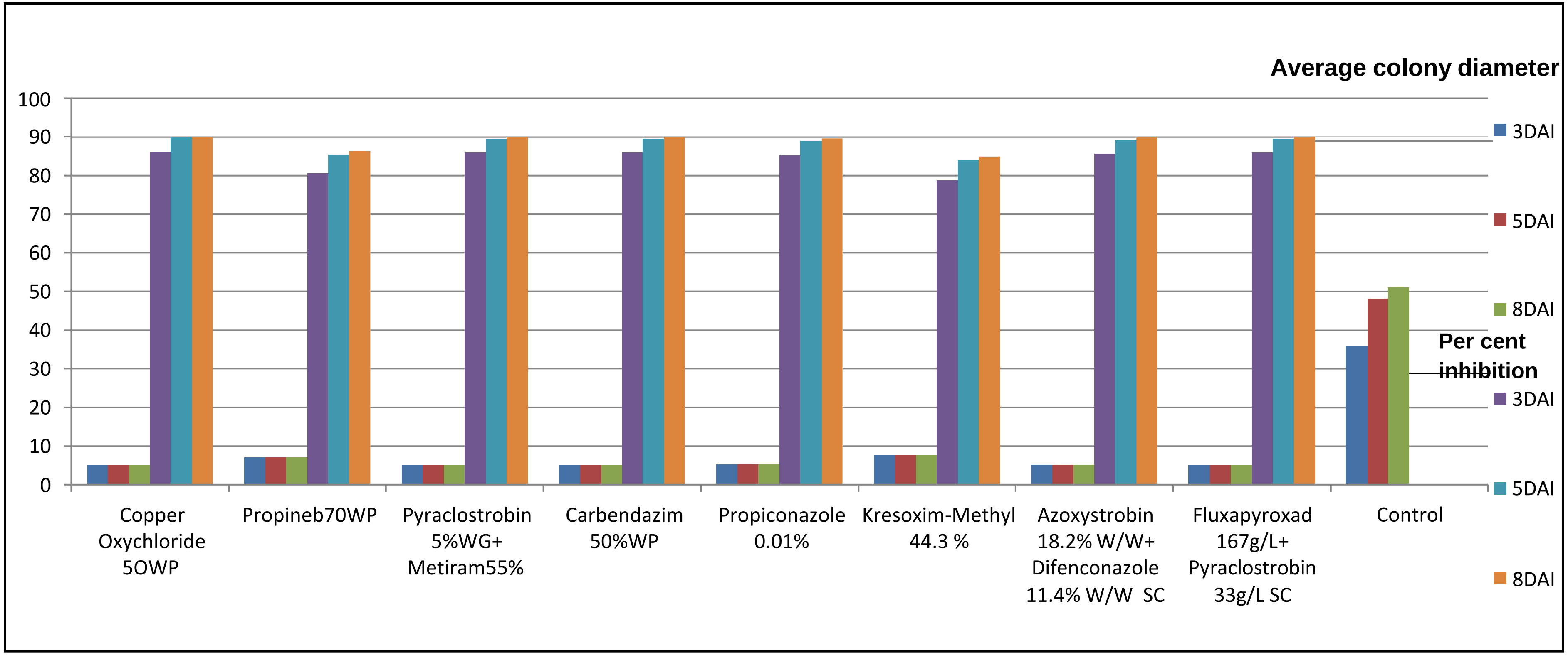


Figure 14. Effect of CIBRC label claim fungicides on average colony diameter and percent inhibition of *Paramyrothecium roridium in vitro*

Table 8c. Effect of CIBRC label claim fungicides on average colony diameter and per cent inhibition of *Rhizoctonia solani in vitro* causing root rot in cotton

Treatment	***Rhizoctonia solani***	**Conc. (%)**	**Average colony diameter (in mm)**			**Percent inhibition(%)**		
	CIBRC label claim fungicides		**3DAI**	**5DAI**	**8DAI**	**3DAI**	**5DAI**	**8DAI**
T1	Copper Oxychloride50WP	0.25	42.50	79.00	81.00	47.53	5.95	6.89
T2	Propineb70WP	0.25	0	6	12	100	92.85	86.20
T3	Pyraclostrobin 5%WG+ Metiram55%	0.25	5.00	6.33	6.33	93.82	92.46	92.72
T4	Carbendazim 50%WP	0.25	0	0	0	100	100	100
T5	Propiconazole 0.01%	0.1	6.34	6.67	7.33	92.17	92.05	91.57
T6	Kresoxim-Methyl 44.3 %	0.1	26.67	51.00	87.00	67.07	39.28	0
T7	Azoxystrobin 18.2% W/W+ Difenconazole 11.4% W/W SC	0.1	18.00	33.17	51.67	77.77	60.51	40.60
T8	Fluxapyroxad 167g/L+ Pyraclostrobin 33g/L SC	0.06	0	0	0	100	100	100
T9	Control		81.00	84.00	87.00			
	F Test		Sig	Sig	Sig			
	SE(m)±		0.54	0.34	0.33			
	CD@1%		2.20	1.41	1.35			

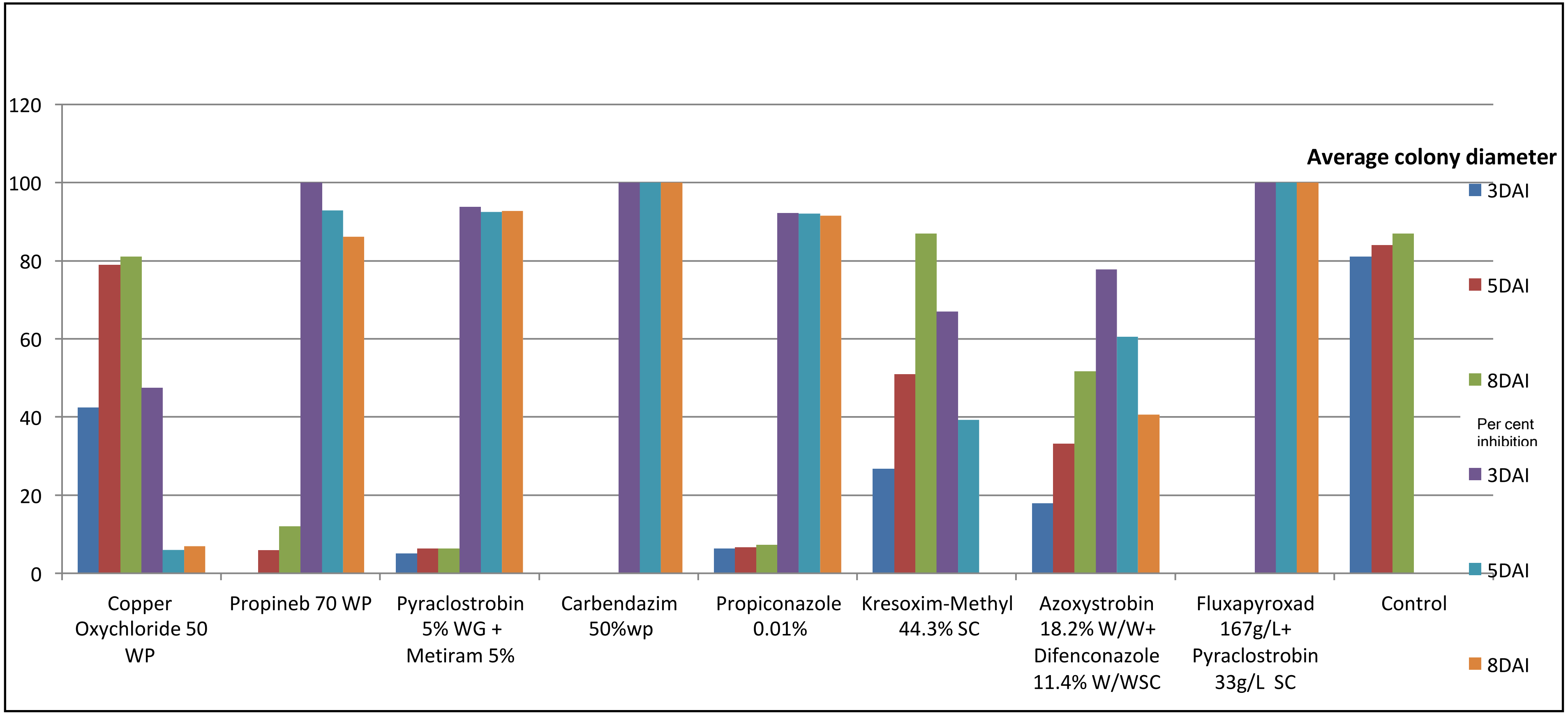


Figure 15. Effect of CIBRC label claim fungicides on average colony diameter and percent inhibition of *Rhizoctonia solani in vitro* causing root rot in cotton

Table 8d. Effect of CIBRC label claim fungicides on average colony diameter and per cent inhibition of *Macrophomina phaseolina* causing dry root rot in cotton *in vitro*

Treatment	***Macrophomina phaseolina***	**Conc.(%)**	**Average Colony Diameter (in mm)**			**Percent inhibition(%)**		
	CIBRC label claim fungicides		**3DAI**	**5DAI**	**8DAI**	**3DAI**	**5DAI**	**8DAI**
T1	Copper Oxychloride50WP	0.25	6.16	11.16	26.83	81.70	85.31	69.19
T2	Propineb70WP	0.25	0	0	7.50	100	100	91.37
T3	Pyraclostrobin 5%WG+ Metiram55%	0.25	6.16	6.16	7.50	81.70	91.89	91.37
T4	Carbendazim 50%WP	0.25	7.34	21.50	21.50	78.20	71.71	75.28
T5	Propiconazole 0.01%	0.1	6.67	10.50	10.67	80.19	86.18	87.73
T6	Kresoxim-Methyl 44.3 %	0.1	16.00	33.67	57.00	52.47	55.69	34.48
T7	Azoxystrobin 18.2% W/W+ Difenconazole 11.4% W/W SC	0.1	5.83	10.00	10.00	82.68	86.84	88.50
T8	Fluxapyroxad 167g/L+ Pyraclostrobin 33g/L SC	0.06	0	4.67	7.00	100	93.85	91.95
T9	Control		33.67	76.00	87.00			
	F Test		Sig	Sig	Sig			
	SE(m) ±		0.79	1.65	2.38			
	CD@1%		3.25	6.72	9.70			

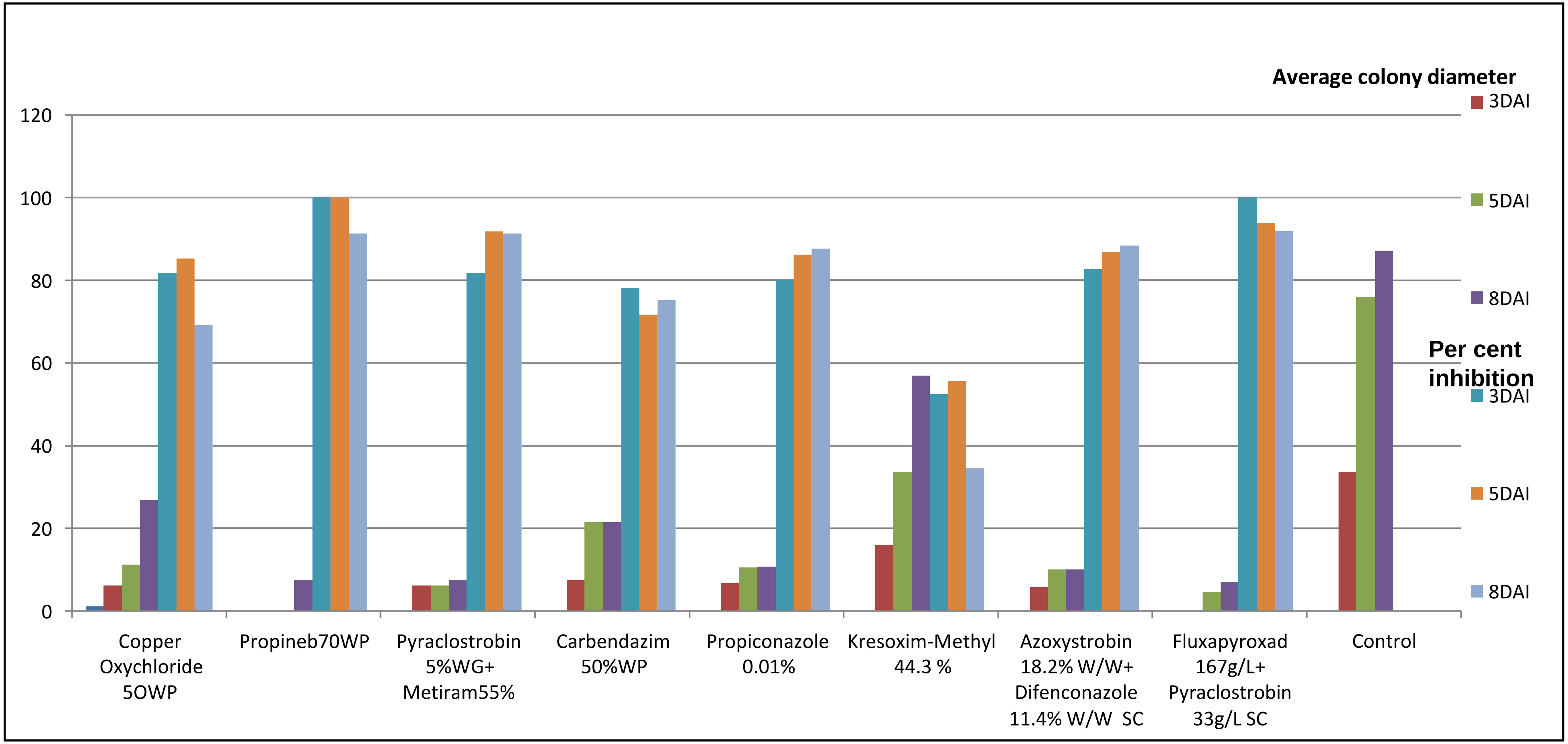


Figure 16. Effect of CIBRC label claim fungicides on average colony diameter and per cent inhibition of *Macrophomina phaseolina in vitro* causing dry root rot in cotton

Table 8e. Effect of CIBRC label claim fungicides on average colony diameter and per cent inhibition of *Nigrospora sphaerica in vitro*

Treatment	***Nigrospora sphaerica***	**Conc. (%)**	**Average colony diameter (in mm)**			**Per cent inhibition(%)**		
	CIBRC label claim fungicides		3DAI	5DAI	8DAI	3DAI	5DAI	8DAI
T1	Copper Oxychloride 50WP	0.25	23.83	24.33	36.16	52.02	70.92	58.43
T2	Propineb 70WP	0.25	0	0	0	100	100	100
T3	Pyraclostrobin 5%WG + Metiram 55%	0.25	0	6.67	6.67	100	92.02	92.33
T4	Carbendazim 50%WP	0.25	0	0	0	100	100	100
T5	Propiconazole 0.01%	0.1	0	8.83	9.17	100	89.44	89.45
T6	Kresoxim-Methyl 44.3 %	0.1	11.17	20.50	48.16	77.51	75.49	44.64
T7	Azoxystrobin 18.2% W/W+ Difenconazole 11.4% W/W SC	0.1	9.50	20.00	31.50	80.87	76.09	63.79
T8	Fluxapyroxad 167g/L+ Pyraclostrobin 33g/L SC	0.06	8.67	13.17	36.16	82.54	84.25	58.43
T9	Control		49.67	83.67	87.00			
	F Test		Sig	Sig	Sig			
	SE(m) ±		0.53	1.54	0.78			
	CD@1%		2.15	6.29	3.17			

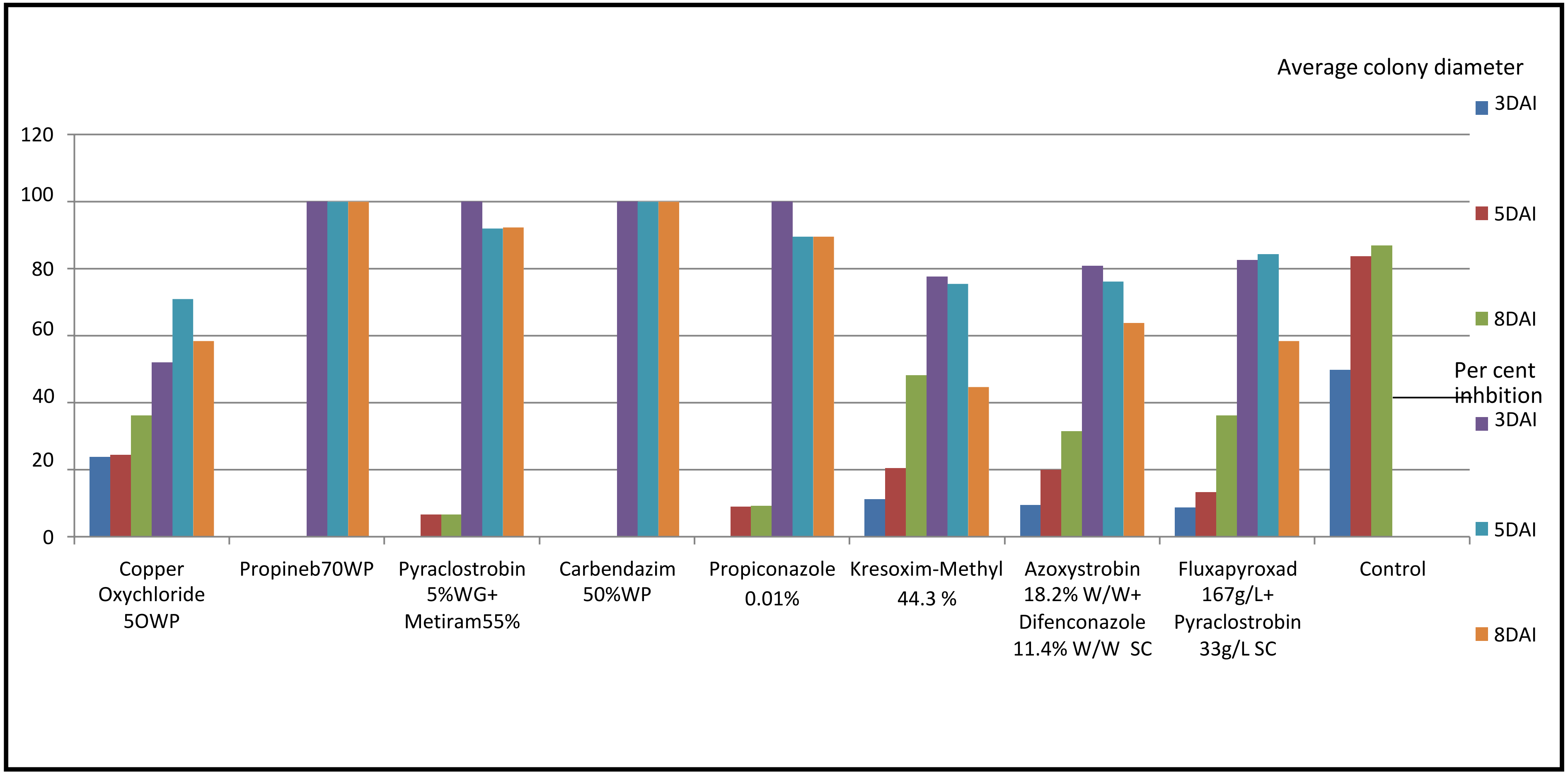


Figure 17. Effect of CIBRC label claim fungicides on average colony diameter and per cent inhibition of *Nigrospora sphaerica in vitro*

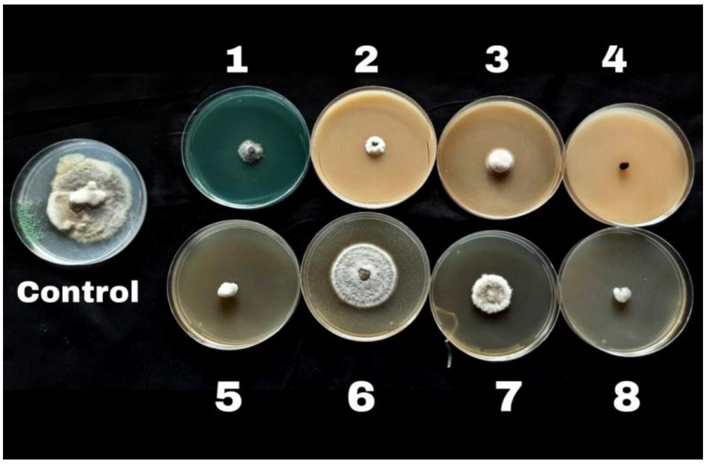


Plate 13a. ***In vitro*** **efficacy of CIBRC label claim fungicides against *Corynespora cassiicola* causing target spot in cotton**

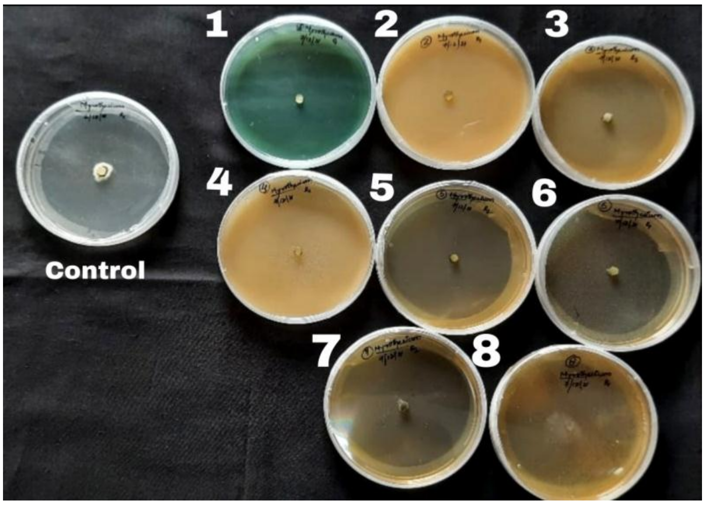


Plate 13b. ***In vitro*** **efficacy of CIBRC label claim fungicides against *Paramyrothecium roridium***

T1- Copper oxychloride 50 WP
T2- Propineb 70 WP
T3- Pyraclostrobin 5% WG + Metiram 55 %
T4- Carbendazim 50 % WP
T5- Propiconazole 0.01 %
T6- Kresoxim methyl 44.3 %
T7- Azoxystrobin 18.2 % W/W + Difenconazole 11.4 % W/W SC
T8- Fluxopyroxad 167 g/L + Pyraclostrobin 33 g / L

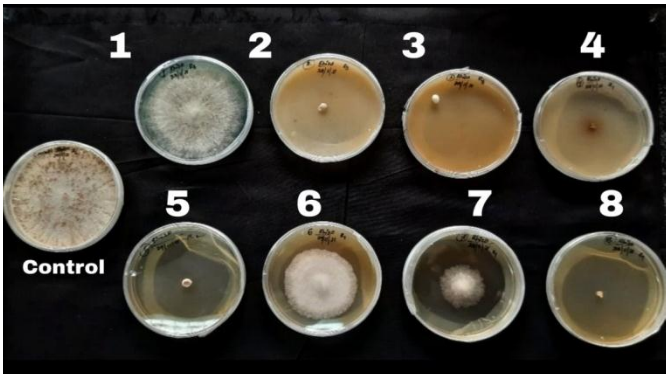


Plate 13c. ***In vitro*** **efficacy of CIBRC label claim fungicides against *Rhizoctonia solani* causing root rot in cotton**

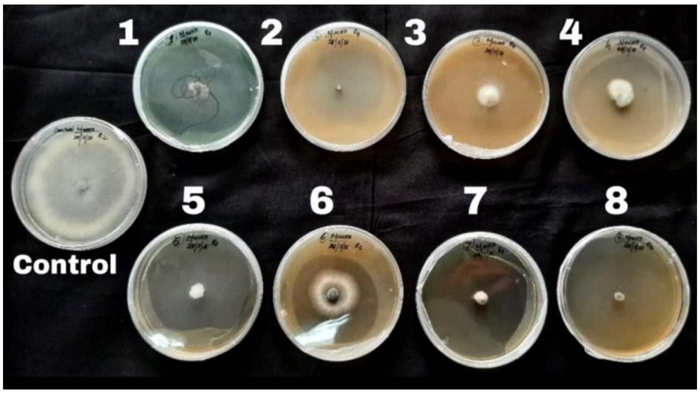


Plate 13d. ***In vitro*** **efficacy of CIBRC label claim fungicides against *Macrophomina phaseolina* causing dry root rot in cotton**

Plate 13e. ***In vitro*** **efficacy of CIBRC label claim fungicides against *Nigrospora sphaerica***

T1- Copper oxychloride 50 WP
T2- Propineb 70 WP
T3- Pyraclostrobin 5% WG + Metiram 55 %
T4- Carbendazim 50 % WP
T5- Propiconazole 0.01 %
T6- Kresoxim methyl 44.3 %
T7- Azoxystrobin 18.2 % W/W + Difenconazole 11.4 % W/W SC
T8- Fluxopyroxad 167 g/L + Pyraclostrobin 33 g / L

Table 8f. Effect of CIBRC label claim fungicides on pathogens associated with leaf spot of cotton *in vitro*

Sr. No.	**Fungal Pathogen**		***Corynespora cassiicola***		***Paramyrothecium roridium***		***Rhizoctonia solani***		***Macrophomina phaseolina***		***Nigrospora sphaerica***	
	Fungicide	Conc.	Average colony diameter (in mm)	Per cent inhibition %	Average colony diameter (in mm)	Per cent inhibition %	Average colony diameter (in mm)	Per cent inhibition %	Average colony diameter (in mm)	Per cent inhibition %	Average colony diameter (in mm)	Per cent inhibition %
1	Copper Oxychloride 50 WP	0.25	17.67	69.00	5.05	90.09	81.00	6.89	26.83	69.19	36.16	58.43
2	Propineb 70 WP	0.25	11.00	80.70	7	86.27	12	86.20	7.50	91.37	0	100
3	Pyraclostrobin 5% WG + Metiram 5%	0.2	16.50	71.05	5.06	90.07	6.33	92.72	7.50	91.37	6.67	92.33
4	Carbendazim 50%wp	0.25	0	100	5.06	90.07	0	100	21.50	75.28	0	100
5	Propiconazole0.01%	0.1	10.00	82.45	5.3	89.60	7.33	91.57	10.67	87.73	9.17	89.45
6	Kresoxim-Methyl 44.3% SC	0.1	44.50	21.92	7.67	84.96	87.00	0	57.00	34.48	48.16	44.64
7	Azoxystrobin 18.2% W/W+ Difenconazole 11.4% W/WSC	0.1	23.17	59.36	5.16	89.88	51.67	40.60	10.00	88.50	31.50	63.79
8	Fluxapyroxad 167g/L+ Pyraclostrobin 33g/L SC	0.06	3.67	93.56	5.06	90.07	0	100	7.00	91.95	36.16	58.43
9	Control		57.00		51.00		87.00		87.00		87.00	

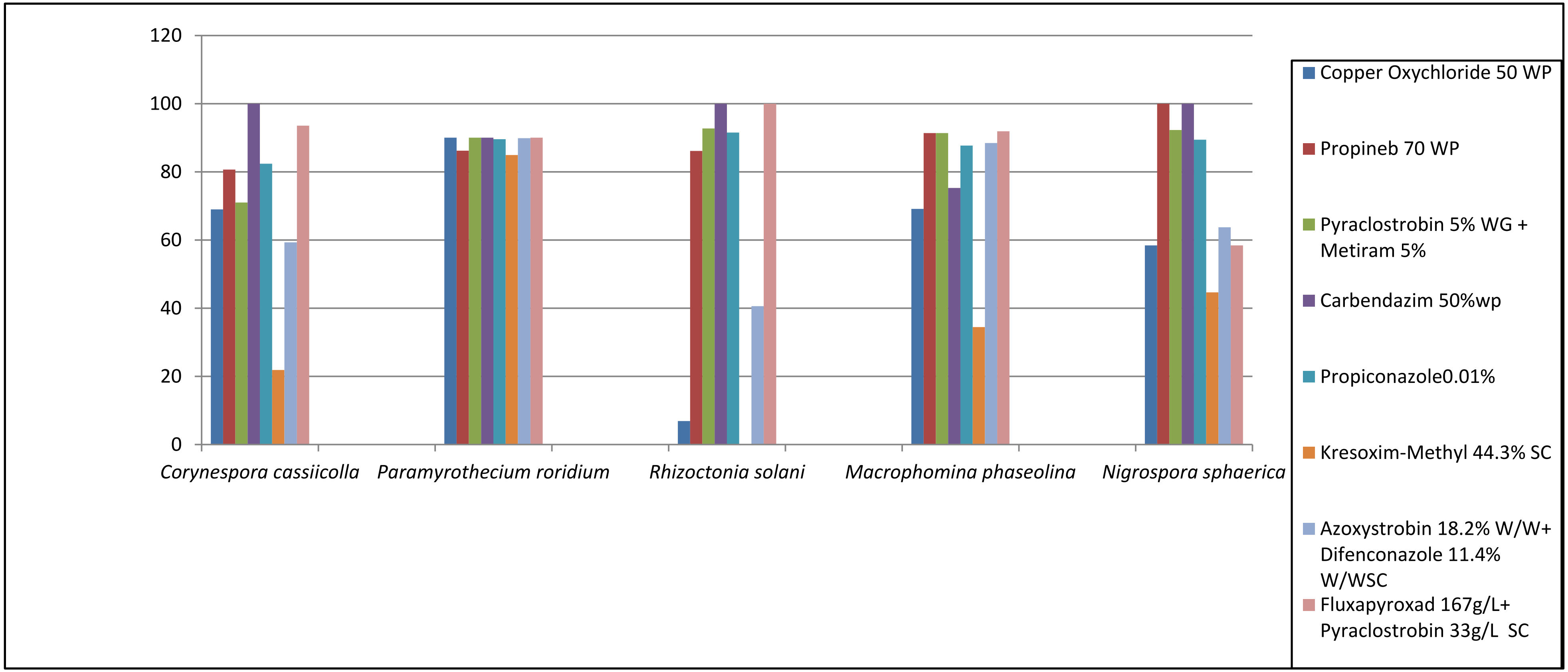


Figure 18. Effect of CIBRC claim fungicides on pathogens associated with leaf spot of cotton *in vitro*

Chapter V

SUMMARY AND CONCLUSIONS

Cotton (*Gossypium* spp.) is also called the king of fibres or white gold. It dominates the Indian textile industry. Both domestic and foreign economic landscape is clad by the natural fibre. Cotton is one of the most economically important crops, and diseases are responsible for significant losses in the crop production. Leaf spot diseases involve a wide range of disease symptoms and damage types from the time cotton is planted in the field till the end of the harvesting season. Several fungi and bacteria are known to be associated with leaf spot symptoms which ultimately makes it difficult for managing the diseases. The investigations on the leaf spot complex of cotton were carried out as the interests that permeate this sector command a privileged status, provided the fact, it has a significant impact on yield and fibre quality of the cotton crop. In the present investigations attempts were made to investigate the prevalence of pathogens associated with leaf spot and its management under *in vitro conditions.* This chapter summarises the experimental results obtained in the present investigation.

Cotton leaves showing showing typical symptoms of leaf spot were collected from experimental fields of ICAR-Central Institute for Cotton Research, Nagpur. The isolations were carried out from the collected leaf samples and pure cultures were obtained and maintained for further studies.

The major pathogens associated with leaf spot were identified as *Corynespora cassiicola, Paramyrothecium roridium, Rhizoctonia solani*, *Macrophomina phaseolina and Nigrospora sphaerica.*

The pathogenicity test using Koch's postulates were carried out by attached leaf inoculation (Francis *et al.,* 2010) method and also by detached leaf assay (Yin *et al.,* 2011) under *in vitro* condition.

The leaves developed symptoms and confirmed that *Corynespora cassiicola (*CICR- F2), *Paramyrothecium roridium* (F10), *Rhizoctonia solani*, (CICR-F6), *Macrophomina phaseolina* (CICR-F4) *and Nigrospora sphaerica* (F9) were pathogenic by reisolating from the inoculated leaves.

Further investigation for morphological characterisation, molecular Identification, *in vitro* efficacy of bioagents and fungicides were done.

The mycelia of *Corynespora cassiicola* were white to dark grey with profuse and fuzzy growth. Conidiophores were pale brown, simple, cylindrical and septate with intermittent branching. Conidia were subhyaline to olivaceous, solitary or in chains, straight to slightly curved, obclavate to cylindrical in shape and measured 40-128 × 10-12 µm.

Morphology of *Paramyrothecium roridium* were characterised as abundant and white aerial mycelium seen as white colonies with green concentric masses of sporodochia that contained dark green to black conidial masses on PDA medium and sporodochia was nonseptate with a cylindrical to ellipsoidal shape, one celled, both ends rounded with hyaline to pale green colour and measured 6×2µm.

The fungal colonies of *Rhizoctonia solani* were light brown in colour and exhibited vigorous growth. Hyphae were branched with right-angle and a slight septal constriction at their bases. The fungus does not produce any conidial structure and is mostly found in its vegetative stage, but certain ellipsoid to globose, barrel-shaped cells, produced in chains give rise to sclerotia. Sclerotia were dark brown, irregularly shaped, and produced after 5 days on PDA and measured 8-10µm.

The mycelial growth of *Macrophomina phaseolina* were dark olive black colonies that ultimately turn black, embedded with microsclerotia. Morphologically microsclerotia were black, smooth, round to oblong, Pycnidia were dark to greyish, globose, conidia were single celled, hyaline, aseptate, obovoid and measured 83-124µm.

The fungal colonies of *Nigrospora sphaerica* were fast growing, initially white, becoming light to dark grey with the onset of sporulation with black, spherical to subsphaerical conidia borne on a hyaline vesicle at the tip of each conidiophores. A light orange colour secretion could be seen in the fungal cultures at seven days of incubation. Conidia were spherical to sub spherical, single-celled, black, and were borne on a hyaline vesicle at the tip of each conidiophores and measured 19-21µm.

The molecular identification of all the sixteen fungal cultures were carried out by using ITS-rDNA PCR method. All the isolates produced the expected amplicon products ranged from 500-600 bp using ITS1-F/ITS4-R primers after successful polymerase chain reaction. Amplified products were resolved by electrophoresis on 1.2% agarose gel and photographed under gel documentation system. The selected pathogenic isolates were sent to the Barcode Biosciences Pvt. Ltd., Hyderabad, India for sequencing. The result of DNA sequencing was searched online BLAST-nucleotide against other sequences deposited in the NCBI Genbank database and were identified with 100 per cent sequence identity. The phylogenetic tree was generated by using MEGA X software.

In the study for efficacy of bioagents *Bacillus aryabhattai* showed 63.86% fungal growth inhibition followed by *Pseudomonas fluoroscens* exhibiting 48.73% fungal growth inhibition over control. *Bacillus tequilensis* inhibited 4.20% (least) of fungal growth over control against *Corynespora casiicola*. Under *in vitro* evaluation of fungal bioagents *Trichoderma viride, Trichoderma longibracheatum*, *Talaromyces purpureogeaus* were found most effective among all the strains fungal bioagents against *Corynespora cassiicola* and exhibited complete (100%) fungal growth inhibition over control. *Talaromyces pinophulus* (Deoli) strain of *Trichoderma* performed least fungal growth inhibition of 6.53% over control.

Pseudomonas fluoroscens showed 47.29% inhibition of fungal growth over control followed by *Bacillus aryabhatttai* showing 42.97% fungal growth inhibition over control against *Paramyrothecium roridium*. *Bacillus tequilensis* inhibited 3.51% (least) of fungal growth over control. *Trichoderma harzianum* (TH-CICR) isolated from CICR was found most effective against *Paramyrothecium roridium* and showed fungal growth inhibition of 66.01% over control. *Talaromyces purepureogenus* showed least fungal growth inhibition of 2.91% over control.

Bacillus aryabhatttai inhibited fungal growth upto 45.11% over control where as *Pseudomonas fluorescens* showed 30.17% of fungal growth inhibition over control against *Rhizoctonia solani*. *Bacillus tequilensis* was ineffective (0%) in inhibiting fungal growth over control. *Trichoderma harzianum* (TH-CICR) isolated from CICR was found most effective against *Rhizoctonia solani* exhibiting highest fungal growth inhibition of 33.90% over control and *Talaromyces purpurogenus* showed least fungal growth inhibition of 10.34% over control.

Pseudomonas fluoroscens showed maximum of 59.51% inhibition of fungal growth over control followed by *Bacillus aryabhatttai* showing 9.31% inhibition of fungal growth over control in inhibiting fungal growth over control against *Macrophomina phaseolina* and *Bacillus tequilensis* was ineffective (3.64%) in inhibiting fungal growth over control. *Talaromyces pinophilus* 2 (neem strain) was found most effective against *Macrophomina phaseolina* inhibiting fungal growth upto 58.98% over control and *Talaromyces purpurogenus* showed least 12.26% fungal growth inhibition over control

Pseudomonas fluoroscens completely (100%) inhibited the fungal growth over control followed by *Bacillus aryabhattai* showing 53.59% inhibition of fungal growth over control against *Nigrospora* *sphaerica* where as *Bacillus tequilensis* was ineffective (2.76%) in inhibiting fungal growth over control *Talaromyces pinophilus* 2 (neem strain) showed highest fungal growth inhibition upto 41.36% over control and *Talaromyces purpurogenus* showed least 17.56% fungal growth inhibition over control against *Nigrospora sphaerica.*

Under *in vitro* evaluation of fungicides, all the test fungicides significantly reduced radial growth of *Corynespora cassiicola* compared to control (57 mm). Carbendazim 50% WP at 0.25% (2500ppm) was found most effective against *Corynespora cassiicola* showing complete (100%) fungal growth inhibition over control and hence it was recorded as significantly superior to other treatments. Fluxapyroxad 167g/L + pyraclostrobin 33g/L (T8) at 0.06% (600ppm) showed only upto 93.56% fungal growth inhibition over control. Other fungicides such as propineb 70WP at 0.25% (2500ppm), pyraclostrobin 5% WG +metiram 5% at 0.25% (2500ppm) and propiconazole 0.1% (1000ppm), recorded over 70% inhibition of fungal growth over control. The least effective fungicide was kresoxim-methyl 44.3% at 0.1% (1000ppm) exhibiting fungal growth inhibition upto only 21.92% over control.

All the test fungicides copper oxychloride 50WP at 0.25% (2500ppm), propineb 70WP at 0.25% (2500ppm), pyraclostrobin 5% WG + metiram 5% at 0.25% (2500ppm), carbendazim 50% WP at 0.25% (2500ppm), propiconazole 0.1% (1000ppm), kresoxim-methyl 44.3%, azoxystrobin 18.2% W/W + difenconazole 11.4% W/W SC at 0.1% (1000ppm), fluxapyroxad 167g/L + pyraclostrobin 33g/L (T8) at 0.06% (600ppm) were effective in controlling the fungal growth of *Paramyrothecium roridium* due to slow growing nature of the fungus and showed more than 80% inhibition over control.

The test fungicides effectively reduced the fungal growth over control (87mm). carbendazim 50% WP (T4) at 0.25% (2500ppm) and fluxapyroxad 167g/L + pyraclostrobin 33g/L at 0.06% (600ppm) showed complete (100%) fungal growth inhibition over control against *Rhizoctonia solani.* Fungicides such as pyraclostrobin 5% WG +

metiram 5% at 0.25% (2500ppm), propiconazole 0.1% (1000ppm), and propineb 70WP at 0.25% (2500ppm), showed above 85% inhibition of fungal growth over control. In contrast, kresoxim-methyl 44.3% at 0.1% (1000ppm) exhibiting 0% fungal growth inhibition over control was recorded as the least effective fungicide against *Rhizoctonia solani.*

The test fungicides were effective in reducing the fungal growth over control *Macrophomina phaseolina* (84mm). fluxapyroxad 167g/L + pyraclostrobin 33g/L at 0.06% (600ppm) was found most effective against *Macrophomina phaseolina* showing 91.95% fungal growth inhibition over control and therefore recorded as significantly superior over other treatments.

All the test fungicides effectively reduced the fungal growth over control (84mm). Carbendazim 50% WP at 0.25% (2500ppm) and propineb 70WP at 0.25% (2500ppm) were most effective fungicide against *Nigrospora sphaerica* showing complete inhibition (100%) of fungal growth over control. Kresoxim-methyl 44.3% at 0.1% (1000ppm) showing 44.64% fungal growth inhibition was recorded as the least effective fungicide against *Nigrospora sphaerica.*

Conclusions

1. The fungal pathogens associated with leaf spot of cotton were identified as *Corynespora cassiicola* (CICR- F1, CICR-F2, CICR-F5, CICR-F7-CICR-F15), *Paramyrothecium roridium* (F10), *Rhizoctonia solani* (CICR-F6), *Macrophomina phaseolina*, (CICR-F4) *and Nigrospora sphaerica* (F9).

2. *Corynespora cassiicola* was found to be most prevalent fungal leaf spot pathogen in the study. Out of 16 samples, 12 samples were associated with *Corynespora cassiicola* infection.

3. Fungus such as *Rhizoctonia solani*, *Macrophomina phaseolina* responsible for causing root rot in cotton were found to be associated as a leaf spot causing pathogen of cotton.

4. *Paramyrothecium roridium* infection was also found to be associated with leaf spot of cotton.

5. The infection of *Nigrospora sphaerica* as a fungal leaf spot of cotton is the first report of its kind in the cotton crop in India.

6. *Bacillus aryabhattai* was most effective in controlling *Corynespora cassicola* and *Rhizoctonia solani. Pseudomonas fluorescens* was most effective in controlling *Paramyrothecium roridium*, *Macrophomina phaseolina* and *Nigrospora sphaerica*.

7. *Trichoderma harzianum* and *Talaromyces pinophilus* 2 (neem strain) were most effective against the test fungal pathogen *Paramyrothecium roridium* (F10)*, Rhizoctonia solani* CICR-F6 and *Macrophomina phaseolina* CICR-F4*, Nigrospora sphaerica* F9 respectively. *Trichoderma viride*, *Trichoderma longibracheatum*, *Talaromyces purpurogenus* were found most effective against *Corynespora cassiicola* (CICR-F2).

8. Carbendazim 50% WP at 0.25% (2500ppm) was found most effective against *Corynespora cassiicola* (CICR-F2), *Paramyrothecium roridium* (F10), *Rhizoctonia solani* (CICR-F6) and *Nigrospora sphaerica* (F9) showing complete inhibition of the fungal growth over control. On the other hand, fluxapyroxad 167g/L + Pyraclostrobin 33g/L at 0.06% (600ppm), was found most effective against *Macrophomina phaseolina* (CICR-F4).

9. In general, carbendazim 50% WP at 0.25% (2500ppm), propineb 70WP at 0.25% (2500ppm), pyraclostrobin 5% WG + Metiram 5% at 0.25% (2500ppm), propiconazole 0.1% (1000ppm), fluxapyroxad 167g/L + pyraclostrobin 33g/L at 0.06% (600ppm), were found most effective and kresoxim-methyl 44.3% at 0.1% (1000ppm) was recorded as the least effective fungicide against the fungal leaf spot disease complex of cotton.

Chapter VI

LITERATURE CITED

Aamir, S., S. Sutar, S. K. Singh and A. Baghela, 2015. A rapid and efficient method of fungal genomic DNA extraction, suitable for PCR based molecular methods. *Pl. Pathol. Quar*. **5**(2):74-81.

Abass, M. H. and H. M. Najlaa, 2014. Morphological, molecular and pathological study on *Nigrospora oryzae* and *Nigrospora sphaerica*, the leaf spot fungi of date palm. *Basra J. for Date Palm Res* **13**(1):26-38.

Ahmed, M. F., P. P. Shete, P. Dhaval and D. Dholu, 2021. Integrated management of dry root rot of green gram caused by *Macrophomina phaseolina* by using bioagents, botanicals and fungicides: A review.

Al-Fadhal, A. Fadhal, N. Aqeel, AL-Abedy and D. A. Alkhafije, 2019. Isolation and molecular identification of *Rhizoctonia solani* and *Fusarium solani* isolated from cucumber (*Cucumis sativus* L.) and their control feasibility by *Pseudomonas fluorescens* and *Bacillus subtilis. Egyptian J. of Bio.Pest Cont.* **29**(1):1-11.

Almomani, F., M. Alhawatema and K. Hameed, 2013. Detection, identification and morphological characteristic of *Macrophomina phaseolina*: the charcoal rot disease pathogens isolated from infected plants in Northern Jordan. *Arch. of Phytopath. and Pl. Protec* **46**(9):1005-1014.

American Phytopathological Society, 1940. *Phytopath*.**30**:361-368.

Bashi, E., Y. Sachs and J. Rotem. 1983. Relationships between disease and yield in cotton fields affected by *Alternaria macrospora*. *Phytoparasi*. **11**(2):89-97.

Basu, A. K. 2001. Impact of WTO on Indian Cotton and Textiles. *Nat. J. of Pl. Improv*. **3**:6-10.

Behere, G. T., V. S. Tajane, V. E. Aage and E. Burgoni, 2003. Management of root rot of cotton, caused by *Rhizoctonia solani* through bioagents. World cotton res Confer- 3, Cape Town-South Africa.

Bessey, E. A., (1950). Morphology and taxonomy of fungi. Morphology and taxonomy of fungi.

Bhattiprolu, S. L., M. Kumar, V. Divyamani, A. K. Patibanda, K. Jayalalitha and S. R. K. DV, (2022). First report of *Corynespora cassiicola* (Berk. and Curt.) Wei. causing target spot on cotton (*Gossypium hirsutum* L.) in South India. *Pl. Dis.*

Blank, L. M., 1953. The leaf spots of cotton plants.Yearbook of agriculture: 316.

Bowen, K. L., A. K. Hagan, M. Jones and H. B. Miller, 2018. Epidemics and yield losses due to *Corynespora cassiicola* on cotton. *Pl.Dis*. **102**:2494– 2499.

Butler, S., H. Young-Kelly, T. Raper, M. A. Cochran and J. Jordan, 2016. First report of target spot caused by *Corynespora cassiicola* on cotton in Tennessee. *Pl. Dis*. **100**:535.

Chakrabarty, P. K., 2007. Development of sensitive molecular diagnostic tools for detection of economically important fungal pathogens of cotton.World Cotton Res Confer–4, Lubbock, Texas, USA.

Chandra, S., K. Singh, P. Kumar and N. Yadav, 2016. Screening of rice (Oryza sativa L.) genotypes for sheath blight (*Rhizoctonia solani*) in changing climate scenario. *J AgriSear*. **3**(2):130-132.

Chauhan, M. S., D. Suryanarayana and D. P Singh, 1976. Myrothecium leaf spot-a disease of cotton in Haryana State. Cotton Dev.

Chavhan, R. L., V. R. Hinge, U. S. Kadam, B. B. Kalbande and P. K. Chakraborty, 2018. Real-time PCR assay for rapid, efficient and accurate detection of *Paramyrothecium roridum* a leaf spot pathogen of *Gossypium* species. *J. of Pl. Biochem. and Biotech.* **27**(2):199-207.

Chen, Y., X. Yang, A. F. Zhang, Y. H. Zang, C. Y. Gu, U. Hameed and Y. L. Xu, 2016. First report of leaf spot caused by *Nigrospora sphaerica* on kiwi fruit in China. *Pl. Dis*. **100**(11):2326.

Ciccarone, A., 1953. New records of tomato diseases in Italy. FAO. *Pl. Prot. Bull.* **3**:11-12.

Cotton Corporation of India. 2022. https://cotcorp.org.in/shares.aspx-ICAC *J. Cott. th. Mon*.

Crop Life International, 2014. Integrated Pest Management.Crop Life International.

Dadwal, V. S. and Savitri B., 2012. New report of a leaf spot disease of *Chlorophytum borivillianum* caused by *Macrophomina phaseolina* from India. *J. of Mycol. and Plant Patho*. **42**(3):397-398.

Dennis, C. and J. Webster, (1971). Antagonistic properties of species groups of. *Trichoderma.Trans. Br. Mycol. Soc.* **57**:363-369.

Dhingra, O. D. and J. B. Sinclair, 1985. Basic Plant Pathology Methods. C R C Florida. USA. 418-419.

Dixon, L. J., R. L. Schulb, K. Pernezny and L. E. Datnoff, 2009. Host Specialization and Phylogenetic Diversity of *Corynespora cassiicola. Phytopath*, **99**:1015-1027.

Donk, M. A. 1956. Notes on resupinate fungi II. The tullasneloid fungi. *Reinwardtia*. **3**:363-379.

Dragana, B., J. Barry, G. Eva and J. Stevan, 2007. Identification of *Rhizoctonia solani* isolates from sugar beet roots by analyzing the ITS region of ribosomal DNA.

Dutta, J. 2015. First report of Nigrospora leaf blight on tea caused by *Nigrospora sphaerica* in India. *Pl. Dis.* **99**(3):417.

Ekhuemelo, C. and M. N. Chikwado, 2020. Pathogenicity of fungi associated with leaf spot disease of sweet potato (*Ipomea batatas* L. (Lam) in Makurdi, Benue State, Nigeria. *GSC Bio. and Pharmaceut. Sci.* **11**(2):250-256.

Ellis, M. B., 1957. Some Species of Corynespora Commonwealth Mycological Institute, Kew, UK: 65.

Elliott, P. E., R. S. Lewis, H. D. Shew, W. A. Gutierrez and J. S. Nicholson, 2008. Evaluation of tobacco germplasm for seedling resistance to stem rot and target spot caused by *Thanatephorus cucumeris*. *Pl. Dis.* **92**:425-430.

Erlich, H. A., 1989. PCR technology New York: Stockton press. 246.

Farr, D. F. and A. Y. Rossman, 2013. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA- http://nt. ars-grin.gov/fungaldatabases/.

Farr, D. F. and A. Y. Rossman, 2018. Fungal Databases, U.S. National Fungus Collections, U.S. Department of Agriculture Agricultural Research Service. https://nt.ars-grin.gov/fungaldatabases/.

Faske, T. R., 2017. Target spot of Soybean: What do we know? Arkansas Row. http://www.arkansascrops.com/2016/11/02/ target-spot-of Soyabean- what- do- we- know.

FRAC, 2019. Pathogen risk list, 2019. Crop Life International.

Francis, M. I., Pena A., Graham J. H., 2010. Detached leaf inoculation of germplasm for rapid screening of resistance to citrus canker and citrus bacterial spot. *Europ. J. Pl. Path*. **127**(4):571-578.

Fuhlbohm, M. J., M. J. Ryley and E. A. B. Aitken, 1996. *Macrophomina phaseolina* causing leaf spot of mungbean. *Australas. Pl. Patho.* **25**(4):247-248.

Ghosh, T. 2018. A review on characterization, therapeutic approaches and pathogenesis of *Macrophomina phaseolina*. *Pl. Cell Biotechnol. Mol. Biol* **19**:72-84.

Gilman, J. 1957. A manual of soil fungi. *Soil Sci*. **84**(2):183.

Godoy, C. V., G. L. Hatman, J. C. Rupe, E. J. Sikora, L. L. Domier, J. A. Davis and K. L. Steffey (Eds.), 2015. Target spot in compendium of soybean diseases and pests. *Am Phytopatho Soc*. :62-63.

González-García, V., M. A. Portal-Onco and V. Rubio, 2006. Biology and systematics of the form genus *Rhizoctonia*. *Span. J. Agric. Res.* **4**:55-79.

Gonzalez, Marleny., 2011. Tobacco leaf spot and root rot caused by *Rhizoctonia solani* Kühn. *Mol pl. patho.* **12**(3):209-216.

Grover, R. K. and J. D. Moore, 1962. Assessment of antifungal property in vitro by poison-food technique. *J. Phytopatho*. **52**: 876.

Hagan, A. K., 2012. First report of *Corynespora cassiicola*-Incited target spot on cotton in Alabama. *The Amer. Phytopatho. Soc* (APS). **97**(10):1379.

Hevner, A. R., S. T. March, J. Park and S. Ram, 2004. Design Science in IS Research, Management Information, **28**:75-105.

Hiremani, N. S., 2020. Antagonistic potential and phylogeny of culturable endophytic fungi isolated from desi cotton (*Gossypium arboreum* L.). *South African J. of Bot.* **134**:329-335.

Hieu, N. D., N. A. Nghia, V. T. Q. Chi and P. Dung, 2014. Genetic diversity and pathogenicity of *Corynespora cassiicola* isolates from rubber trees and other hosts in Vietnam. *J Rubber Res* **17**(3):187-203.

Hosagoudar, G. N., S. N. Chattannavar and S. Kulkarni, 2008. Survey for foliar diseases of Bt Cotton Karnataka. *J. Agric. Sci*. **21**:139-40.

Houghton, I. J. T., G. J. Jenkins and J. J. Ephraums (eds.), 1990. Report prepared for Intergovernmental Panel on Climate Change by Working Group.

Huang, X. and A. Madan, (1999). CAP3: A DNA sequence assembly program. *Geno. res*. **9**(9):868-877.

Huda-Shakirah and A. Rahim, 2019. Identification and characterization of *Macrophomina phaseolina* causing leaf blight on white spider Lilies (*Crinum asiaticum* and *Hymenocallis littoralis*) in Malaysia. *Mycobiol.* **47**(4):408-414.

ICAR - AICRP. Annual Report 2020-2021. ICAR-All India Coordinated Research Project on Cotton, Coimbatore, Tamil Nadu, India. :7-10.

Ingole, O. V. and R. W. Ingle, 2011. Occurrence of leaf spot and efficacy of fungicides against *Myrothecium Roridum* (tode ex fr.) causing leaf spot in bt cotton. *J. of Pl. Dis Sci.* **6**(1):25-26.

Ishwari, G. H., N. H. Shalini and R. H. Yashoda, 2020. Efficacy of Fungicides against *Corynespora cassiicola* (Berk. and Curt.) wei causing target leaf spot of soybean. *Int. J. Curr. Microbiol. App. Sci.* **9**(6):2536-2541.

Ismail, S. I. and Nur F., 2021. First report of *Nigrospora sphaerica* causing leaf spot on watermelon (*Citrullus lanatus*) in Malaysia. *Pl. Dis*. **105**(2):488.

Iqbal, U. and T. Mukhtar, 2020. Inhibitory effects of some fungicides against *Macrophomina phaseolina* causing charcoal rot. *Pak. J. of Zool.* **52**(2):709.

Jacob, C. K. 2006. Corynespora Leaf Disease of *Hevea brasiliensis*: A threat to natural rubber production in Corynespora leaf disease of *Hevea brasiliensis* :Strategies for Management, Kuruvilla Jacob *(ed.), Rubb Res Inst Ind* :9-16.

Johnson, L. F., E. A. Curl, J. H. Bond and H. A. Fribourg, 1959. Methods for studying soil micro-flora plant disease relationships. BurgessPublication Company, Minneapolis, *Minn.* **66**:111-119.

Jones, J. P. 1961. A leaf spot of cotton caused by *Corynespora cassiicola*. *Phytopath*. **51**(5):307-308.

Kaur, S., G. S. Dhillon, S. K. Brar, G. E. Vallad, R. Chand and V. B. Chauhan, 2012, Emerging phytopathogen *Macrophomina phaseolina*: biology, economic importance and current diagnostic trends. *Crit. Rev in Microbiol*. **38**(2):136- 151.

Keijer, J. B., S. Sneh, Jabaji-Hare, S. Neate and G. Dijst, 1996. The initial steps of the infection process in *Rhizoctonia solani*. In: *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology,* Pathology and Disease Control. *Spring.*:149-162.

Khaerati, K., Y. Ferry and R. Rusli, 2018. Selection of phylloplane and endophyte microbes as biocontrol for rubber leaf fall disease (*Corynespora cassiicola*). *J. Tan. Industri dan Pen*. **5**(3):113-122.

Khalili, E., M. A. Javed, F. Huyop, S. Rayatpanah, S. Jamshidi, and R. A Wahab, 2016. Evaluation of Trichoderma isolates as potential biological control agent against soybean charcoal rot disease caused by *Macrophomina phaseolina*. *Biotech. and Biotechnol. Equip.* **30**(3):479-488.

Khan, M. R. and J. Gupta, 1998. Antagonistic efficacy of *Trichoderma* Species against *Macrophomina Phaseolina* on Eggplant. *J. of Pl. Dis. and Protec.* **105**(4):387-93.

Kumar, S. and H. K. Singh, 2021. First report of *Myrothecium roridum* causing leaf spot on bael (*Aegle marmelos* Correa.) from India.

Kumar, S., G. Stecher, M. Li, C. MKnyaz and K. Tamura, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. biol. and evol*. **35**(6):1547.

Kwon, H. W., J. Y. Kim, M. A. Choi, S. Y. Son and S. H. Kim, 2014. Characterization of *Myrothecium roridum* isolated from imported anthurium plant culture medium. *Mycobio*. **42**(1):82-85.

Lakhran, Lalita., 2018. Isolation, purification, identification and pathogenicity of *Macrophomina phaseolina* (Tassi) goid caused dry root rot of chickpea. *J. of Pharmaco.and Phytochem.* **7**(3): 3314-3317.

Lakshmanan, P., S. Mohan and R. Jeyarajan, 1988. Formation of perfect stage of *rhizoctonia solani* kuhn causing leaf spot and boll rot of cotton. *Curr. Sci*. **57**(23):1295-1297.

Li, B. J., H. Y. Ben, Y. X. Shi, X. W. Xie and A. L. Chai, (2014). First report of *Myrothecium roridum* causing leaf spot on *Zantedeschia aethiopica* in China. *Pl. Dis*. **98**(6):854.

Lombard, L., J. Houbraken, C. Decock, R. A. Samson, M. Meijer, M. Reblov, J. Z. Groenewald and P. W. Crous, 2016. Generic hyper-diversity in Stachybotriaceae. *Persoonia-Mol. Phyl. and Evol. of Fung.* ***36***(1):156-246.

Lopez, D., S. Ribeiro, P. Label, B. Fumanal, J. S. Venisse, A. Kohler, R.R. de Oliveira, K. Labutti, A. Lipzen, K. Lail, D. Bauer, R. A. Ohm, K.W. Barry, J. Spatafora, I. V. Grigoriev, F. M. Martin and V. Pujade-Renaud, 2018.Genome-wide analysis of *Corynespora cassiicola* leaf fall disease putative effectors. *Front. Microbio*. **9**:1-21.

Mane, S., R. W. Ingle, P. Shinde and A. Kumar, 2018. *In vitro* evaluation of fungicides and bio- agents against *Drechslera tetramera*, *Bipolaris sacchari*, *Nigrospora sphaerica* and *Alternaria alternata* causal agent of leaf spot of medicinal and aromatic grasses. *Int. J. Chem. Stud* **6**(6):1688-1692.

Mane, S. S., 2020. Occurrence and morphological studies on foliar fungal pathogens of medicinal and aromatic grasses.

Manju, M. J., S. Mushrif, H. M. Santosh, R. S. Patil, T. H. Shankarrapa, V. I. Benagi and S. P. Idicula, 2019. Evaluation of different fungi toxicants against *Corynespora cassiicola* causing Corynespora leaf fall (CLF) disease of rubber. *Int. J. Curr.Microbiol. App. Sci*. **8**(2):1640-1647.

Matic S., G. Gilardi, M. L. Gulino and A. Garibaldi, 2019. Emergence of leaf spot disease on leafy vegetable and ornamental crops caused by *Paramyrothecium* and *Albifimbria* species. *Phytopatho* **109**(6):1053-1061.

Mayee, C. D. and P. M. Mukewar, 2007. Loss inducing diseases of cotton and their management with special reference to Andhra Pradesh. In N.G.P. Rao, A. Rao and E.A Siddiq, (eds)-Cotton in Andhra Pradesh, Farm and Rural Science Foundation and ANGRAU, Hyderabad.197-199.

Meena, R. P. and Vasudha A. K., 2022. Characterization of *Macrophomina phaseolina* associated with leaf blight disease on *Chlorophytum borivilianum* meeting of committee on COCPC.

Monga D., K. Kranthi, N. Gopalakrishnan, C. Mayee, 2011. Changing scenario of cotton diseases in India-the challenge ahead. World Cotton Res Conf-5, Mumbai, India.

Monga, D., 2012. Avoidable losses due to cotton diseases in India. Global Cotton Production Technologies vis-a-vis Climate Change. Cotton Res and Dev Assoc., CCS Haryana Agricultural University, Hisar-125. **4**:62.

Munjal, R. L., 1960. A commonly occurring leaf spot disease caused by *Myrothecium roridum* Tode ex Fr. *Ind. Phytopath*. **13**:150-155.

Naito, S. and T. Sugimoto, 1978. Basidiospore infection and lesion development on sugar beet leaves by *Thanatephorus cucumeris* (Frank) Donk. *Ann. Phytopathol. Soc. Jpn.* **44**:426-431.

Nagrale, D. T., S. P. Gawande, and V. Shah, 2022. Biocontrol potential of volatile organic compounds (VOCs) produced by cotton endophytic rhizobacteria against *Macrophomina phaseolina*. *Eur J Plant Pathol*. **163**:467-482.

Naz, Sumera., 2017.Geographical distribution of Myrothecium leaf spot disease of *Momordica charantia* L. caused by *Myrothecium roridum* Tode in agro-ecological zones of Punjab, Pakistan. *Pak. J. Bot* **49**(4):1599-1604.

Nene, Y. L. and P. N. Thapliyal. 1993. Poison food technique. Fungicides in Plant Disease Control. Oxford and IBH publication, New Delhi, India. **2**:413-415.

Ning, Y., Y. Wang, Z. Y. Fang, M. Zhuang, Y. Y. Zhang, H. H. Lv and L. M. Yang, 2018. First report of *Myrothecium roridum* causing leaf spot on *Brassica oleracea* in China. *Pl. Dis.* **102**(12):2658.

Onofre, R. B., J. C. Mertely, F. M. Aguiar, S. Timilsina, P. Harmon, G. E. Vallad, and N. A. Peres, 2016. First report of target spot caused by *Corynespora cassiicola* on blueberry in North America. *Pl. Dis.* **100**(2):528.

Pandey, A. K., R. R. Burlakoti, A. Rathore and R. M. Nair, 2020. Morphological and molecular characterization of *Macrophomina phaseolina* isolated from three legume crops and evaluation of mungbean genotypes for resistance to dry root rot. *Crop Protec.* **127**:104962.

Preston, N. C., 1943. Observations on the genus Myrothecium Tode: I. The three classic species. *Trans. Brit. Mycolog. Soc.* **26** (3c.4):158-I56.

Poltronieri, L.S., M. L. R. Duarte, A. C. Alfenas, D. R. Trindade and F.C. Albuquerque, 2003.Three new pathogens infecting Antilles Cherry in the state of para. *Fitopatologia brasileira*, **28**:424-426.

Qi, Y. X., X. Zhang, J. J. Pu, X. M. Lieu, Y. Lu, H. Zhang and X. Y. Xie, 2011. Morphological and molecular analysis of genetic variability within isolates of *Corynespora cassiicola* from different hosts. *Euro. J. of Pl. Patho.* **130**(1):83-95.

Qiu, C. 2022. *Nigrospora sphaerica* causing leaf spot in a new host, *Eclipta prostrata* (False Daisy), in China. *J. of Phytopatho*. **170**(4):242-246.

Ranjini, A. P. and R. Naika, 2019. Efficacy of biocontrol agents on *Myrothecium roridum*, the stem necrosis and leaf spot pathogen of coffee seedlings*. J. of Biopest.* **12**(1):109-113.

Rattan, G. S., Parminder S. and R. N. V. V. Chowdary, 2012. Studies on *Myrothecium roridum*-an emerging problem of tree species in the nurseries of Punjab. *Pl. Dis. Res.* **27**(1):60-65.

Raut, J. G., N. R. Holy and P. G. Moghe, 1980. Occurrence of Myrothecium leaf spot on cotton in Vidarbha. *Ind. Phytopatho*. **33**:510-511.

Roberts, P., 1999. *Rhizoctonia-Forming Fungi: A Taxonomic Guide*. Kew: Royal Botanical Gardens. :239.

Ruggiero, M. A, D. P. Gordon, T. M. Orrel, N. Bailly, T. Bourgoin, R. C. Brusca, T. Cavalier-Smith, M. D. Guiry and P. M. Kirk, 2015. A higher level classification of all living organisms. *PloS one*, **10**(4):0119248.

Saccardo, P. A., 1886*. italici autographice delineati:(additis nonnullis extra-italicis, Asterisco notatis)*.(1).Sumpt. Auctoris (Lithogr. P. Fracanzani).

Salunkhe, V. N., S. P. Gawande, D. T. Nagrale, N. S. Hiremani, N. Gokte-Narkhedkar and V. N. Waghmare, 2019. First report of Corynespora leaf spot of cotton caused by *Corynespora cassiicola* in central India. *Pl. Dis*. **103**(7):1785.

Salvatore, M. M., C. Félix, F. Lima, V. Ferreira, D. Naviglio, F. Salvatore and A. C. Esteves, 2020. Secondary metabolites produced by *Macrophomina phaseolina* isolated from *Eucalyptus globulus*. *Agricul*. **10**(3):72.

Sandipan, P. B., G. R. Bhanderi, R. D. Patel and B. G. Solanki, 2017. Survey and status of different diseases of cotton under south Gujarat region, India. *Int J. of Curr Microbio and Applied Sci*. **6**(9):1362-1367.

Sarbhoy, A. K., J. L. Varshney and D. K. Agarwal, 1971. Fungi of India, Navyug Trades.

Savaliya, V. A., L. Akbari, J. Talaviya and S. lathiya, 2020. Effect of different fungicides on growth and sclerotial formation of *Macrophomina phaseolina* (tassi) goid causing root rot of sesame. *Int. ReS. J. of chem.* (32):1-13.

Schlub, R. L., L. J. Smith, L. E. Datnoff and K. Pernezny, 2007. An overview of target spot of tomato caused by *Corynespora cassiicola. Int. Symp. on Tom. Dis.* **808**:25-28.

Silva, W. P. K., B. J. Deverall and B. R. Lyon, 1998. Molecular, physiological and pathological characterization of Corynespora leaf spot fungi from rubber plantations in Sri Lanka. *Pl. Patho.* **47**(3):267-277.

Singh, P. N. and P. Shukla, 1986. Screening of cowpea germplasm against *Myrothecium roridum* Tode ex Fr. *Indian J. of Myco. and Pl. Patho.* **163**(3):351.

Singh, V. K. and U. Narain, 2008. Bio-efficacy of certain fungicides against Myrothecium leaf spot of grapevine (*Myrothecium roridum* Tode ex Fr.). *Crop Res. (Hisar).* **35**(1/2): 128-130.

Smith, G. S. and O. N. Carvil, 1997. Field screening of commercial and experimental soybean cultivars for their reaction to *Macrophomina phaseolina*. *Pl. Dis.* **81**:363-368.

Sneh, B., S. Jajabi-Hare, S. Neate and G. Dijst, 1996. *Rhizoctonia Species:*Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht:Kluwer Academic Publishers :578.

Srinivasan, K. V., 1994. Cotton diseases. *Ind. Soc. for cotton improv, Bombay*, 168-172.

Subramaniam, C. V., 1971. Hyphomycetes: An Account of Indian Species, Except Cercosporae. Indian Council of Agricultural Research.

Sushmita, V. T., M. Arya, P. P. Jambhulkar, N. manjunatha, A. Singh, and S. K. Chaturvedi, 2021. Identification of Donors and Molecular Characterization of *Corynespora cassiicola* causing fungal leaf spot of mungbean and urdbean. *Int. J. of Agri.Env.andBiotech*. **14**(2): 265-276.

Sultana, N. and A. Ghaffar, 2009. Pathogenesis and control of *Myrothecium* spp. the cause of leaf spot on bitter gourd (*Momordica charantia* L.). *Pak. J. Bot*. **41**(1):429-433.

Tarazi, R., J. L. S. Jimenez and M. F. S. Vaslin, 2019. Biotechnological solutions for major cotton (*Gossypium hirsutum*) pathogens and pests. *Biotech. Res. and Inno*. **3**:19-26.

Tomar, D. S., 2005. Studies on the Myrothecium leaf blight of cotton. Ph.D. Thesis submitted to Dr. B. R. Ambedkar University, Agra. :116.

Tripathy, S. K., M. Maharana, D. M. Ithape, D. Lenka, D. Mishra, A. Prusti, D. R. Mohanty and K. R. R. Raj, 2017. Exploring rapid and efficient protocol for isolation of fungal DNA. *Int.J. of Curr. Microbiol. and Applied Sci.* **6**(3):951-960.

Usendi, P. N., G. K. Giri and S. H. Kabade, 2020. Evaluation of Fungicides, Bioagents and botanicals against *Rhizoctonia* spp. incitant of sheath blight of Rice. *Int. J. Curr. Microbiol. App. Sci* **9**(8):3039-3046.

Vairavan, R., 2021. Survey on fungal leaf spot complex in cotton at different locations of Tamil Nadu. *The Pharm. Inno. J.* **10**(11): 1165-1167.

Vincent, J. M., 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 59:850.

Vishwakarma, S. P., H. K. Singh, M. K. Maurya, V. K. Yadav, K. Kumar, S. B. Gautam and R. Prashad, 2021. *In vitro* efficacy of fungicides against *Myrothecium roridum* causing leaf spot of Bael.

Wallon, T., A. Sauvageau and H. V. der Heyden, 2020. Detection and quantification of *Rhizoctonia solani* and *Rhizoctonia solani* AG1-IB causing the bottom rot of lettuce in tissues and soils by multiplex qPCR. *Plants* **10**(1):57.

White, T. J., T. Bruns, S. J. W. T. Lee, J. Taylor, M. A. Innis, D. H. Gelfand and J. J. Sninsky, 1990. PCR protocols: a guide to methods and applications. 315-322.

Wright, E. R., M. Folgado, M. C. Rivera, A. Crelier, P. Vasquez and S. E. Lopez, 2008. *Nigrospora sphaerica* causing leaf spot and twig and shoot blight on blueberry: a new host of the pathogen. *Pl. Dis*. **92**(1):171-171.

Xavier, S. A., M. G. Canteri, D. Barros and C. V. Godoy, 2013. Sensitivity of *Corynespora cassiicola* from soybean to carbendazim and prothioconazole *Trop. Pl. Patho.* **38**(5):431-435.

Xie, Y., L. Fang, L. Wang and H. Wang, 2018. First report of *Rhizoctonia solani* AG-1-IB causing leaf spot on *Peucedanum praeruptorum* in China. *Pl. Dis*. **102**(08):1668.

Xu, J., X. Qi, X. Zheng, Y. Cui, X. Chang and G. Gong, 2016. First report of Corynespora leaf spot on sweet potato caused by *Corynespora cassiicola* in China. *Pl. Dis*. **100**(10):2163.

Yadav, Ramniwas., 2020. Cultural and pathogenic variability of the different isolates of *Rhizoctonia solani* causing root rot of cotton collected from Rajasthan. *Int. J. Curr. Microbiol. App. Sci* **9**(2): 2829-2838.

Yamuna C., S. L. Bhattiprolu, Prasanna Kumari, V. and C. Chiranjeevi, 2020. *In vitro* evaluation of fungicides against fungal foliar pathogens of cotton. *Int J Chem Stud*. **8**(2):2829-2835.

Yin W, Xing-Zeng Fu, Ji-Hong Liu and Ni Hong, 2011. Differential structure and physiological response to canker challenge between ‘Meiwa’ kumquat and ‘Newhall’ navel orange with contrasting resistance. *Sci. Hort*. **128**:115-123.

Zhao, Y. J., B. J. Li, Y. X. Shi and X. W. Xie, 2010. First report of Myrothecium leaf spot of common bean in China caused by *Myrothecium roridum*. *Pl. Dis.* **94**(1):127.

VITA

1. Name of Student : Priyanka Priyadarshini

2. Date of Birth : 29/08/1997

3. Name of college : College of Agriculture, Nagpur

4. Residential Address :
Plot-3364, Prachivihar, Palasuni
Dist.-Khurdha, Odisha
Pincode: 751025
priyurainy1@gmail.com
Mob. No. 8767123539

5. Academic qualification:

Sr. No.	Name of degree Awarded	Year in which obtained	Division / Class	Name of awarding University/ Board	Subjects
1.	B.Sc. (Horti)	2020	First	Odisha University Of Agriculture and Technology (O.U.A.T)	Agriculture and Horticulture
2.	HSC	2016	First	Council Of Higher Secondary Education (C.H.S.E)	Science
3.	SSC	2014	First	Central Board Of Secondary Education (C.B.S.E)	General

6. Field of Interest: Research in Agriculture

Place: Nagpur
Date: / /2022

Signature of the student
(Priyanka Priyadarshini)

APPENDIX I

List of bioagents used in the study.

Sl.no.	**Bacterial bioagents**
1.	*Bacillus aryabhattai*
2.	*Bacillus tequillensis*
3.	*Pseudomonas fluorescens*

Sl.no.	**Fungal bioagents**
1.	*Trichoderma viride*
2.	*Trichoderma harzianum* TH-CICR
3.	*Talaromyces pinophilus* 1(Deoli strain)
4.	*Talaromyces pinophilus* 2 (Neem strain)
5.	*Acrimonium cellulolyticus*
6.	*Trichoderma longibracheatum*
7.	*Talaromyces purpurogenus*

List of CIBRC Label claim fungicides

1. Copper oxychloride(Blitox-50)

Chemical name : Copper oxychloride

Active ingredient : 50% WP

Concentration : 0.25%

Manufacturer : Crystal crop protection Ltd., Delhi,

2. Propineb

Chemical name : Antracol
Active ingredient : 70% WP
Concentration : 0.25%
Manufacturer : Bayer India Ltd., Mumbai

3. Pyraclostrobin + Metiram

Chemical name : Cabrio Top
Active ingredient : Metiram 55% + Pyraclostrobin 5% WG
Concentration : 0.2%
Manufacturer : BASF India Ltd.

4. Carbendazim

Chemical name : Bavistin
Active ingredient : 50% WP
Concentration : 0.1%
Manufacturer : Crystal crop protection Ltd., Delhi, India

5. Propiconazole

Chemical name : Tilt
Active ingredient : 25% EC
Concentration : 0.1%
Manufacturer : Syngenta India Ltd., Pune.
Chemical name : Ergon,

6. Kresoxim-methyl

Active ingredient : 44.3% SC

Concentration : 0.1%

Manufacturer : Rallis India Ltd.

7. Azoxystrobin + Difenoconazole

Chemical name : Amistar Top

Active ingredient : Azoxystrobin 18.2% w/w

Difenoconazole 11.4 % w/w SC

Concentration : 0.1%

Manufacturer : Syngenta India Ltd., Pune.

8. Fluxapyroxad + Pyraclostrobin

Chemical name : Priaxor

Active ingredient : Fluxapyroxad 167 g/L +

Pyraclostrobin 333 g/L SC

Concentration : 0.6%

Manufacturer : BASF India Ltd., Mumbai.

APPENDIX II

I ANOVA TABLES for Bacterial Biological control agent

Isolate: *Corynespora cassiicola* CICR-F2

4DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	3	541.84	180.61	91.74	6.59@5% 16.69@1%	S
ERROR	4	7.87	1.96			
TOTAL	7	549.71				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	0.99	1.40	6.460@1%	21.06	6.66

Isolate: *Paramyrothecium roridium* F10
4DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	3	129.13	43.04	212.56	6.59@5% 16.69@1%	S
ERROR	4	0.81	0.20			
TOTAL	7	129.94				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	0.31	0.45	2.071@1%	14.15	3.18

Isolate*: Rhizoctonia solani* CICR-F6
4DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	3	2314.12	771.37	98.73	6.59@5% 16.69@1%	S
ERROR	4	31.25	7.81			
TOTAL	7	2345.37				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	1.98	2.79	12.870@1%	70.62	3.960

Macrophomina phaseolina CICR-F4
4DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	3	1732.84	577.61	198.74	6.59@5% 16.69@1%	S
ERROR	4	11.62	2.90			
TOTAL	7	1744.46				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	1.20	1.70	7.849@1%	50.31	3.38

Nigrospora sphaerica F9
4DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	3	2909.09	969.69	52.69	6.59@5% 16.69@1%	S
ERROR	4	46.12	11.53			
TOTAL	7	2955.21				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	2.96	4.18	19.278@1%	27.56	15.19

II ANOVA TABLES for Biological control through fungal bioagent

Isolate: *Corynespora cassiicola* CICR-F2

3DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	2922.18	417.45	173.48	3.50@5% 6.18@1%	S
ERROR	8	19.25	2.40			
TOTAL	15	2941.43				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	1.097	1.55	5.20@1%	17.18	9.02

5DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	6277.10	896.73	58.98	3.50@5% 6.18@1%	S
ERROR	8	121.62	15.20			
TOTAL	15	6398.73				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	2.75	3.89	13.08@1%	20.37	16.26

8DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	9973.48	1424.78	48.52	3.50@5% 6.18@1%	S
ERROR	8	234.87	29.35			
TOTAL	15	10208.35				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	3.831	5.41	18.18@1%	28.84	18.78

Isolate : *Paramyrothecium roridium* F10
3DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	52.75	7.53	6.02	3.50@5% 6.18@1%	S
ERROR	8	10.00	1.25			
TOTAL	15	62.75				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	0.791	1.11	3.75@1%	13.87	8.05

5DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	88.98	12.71	3.96	3.50@5% 6.18@1%	S
ERROR	8	25.62	3.20			
TOTAL	15	114.60				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	1.26	1.79	6.00@1%	17.40	10.28

8DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	1798.50	256.92	97.87	3.50@5% 6.18@1%	S
ERROR	8	21	2.62			
TOTAL	15	1819.50				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	1.14	1.62	5.43@1%	24.25	6.68

Isolate :*Rhizoctonia solani* CICR-F6
3DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	347.75	49.67	4.81	3.50@5% 6.18@1%	S
ERROR	8	82.50	10.31			
TOTAL	15	430.25				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	2.27	3.21	10.77@1%	64.12	5.00

5DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	1238.85	176.98	4.44	3.50@5% 6.18@1%	S
ERROR	8	318.37	39.79			
TOTAL	15	1557.23				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	4.461	6.30	21.16@1%	68.46	9.21

8DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	1302.35	186.051	5.24	3.50@5% 6.18@1%	S
ERROR	8	283.87	35.484			
TOTAL	15	1586.23				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	6.23	8.81	19.98@1%	72.53	8.21

Isolate*: Macrophomina phaseolina* CICR-F4
3DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	47.006	6.715	4.792	3.50@5% 6.18@1%	S
ERROR	8	11.210	1.401			
TOTAL	15	58.217				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	0.83	1.18	3.97@1%	33.12	3.54

5DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	2607.93	372.56	8.40	3.50@5% 6.18@1%	S
ERROR	8	354.50	44.31			
TOTAL	15	2962.43				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	4.707	6.65	22.33@1%	46.31	14.374

8DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	4423.24	631.89	68.44	3.50@5% 6.18@1%	S
ERROR	8	73.86	9.23			
TOTAL	15	4497.10				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	2.149	3.03	10.19@1%	51.22	5.93

Isolate:*Nigrospora sphaerica* F9
3DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	640.60	91.51	7.01	3.50@5% 6.18@1%	S
ERROR	8	104.37	13.04			
TOTAL	15	744.98				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	2.55	3.61	12.11@1%	51.71	6.98

5DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	1124.93	160.70	11.13	3.50@5% 6.18@1%	S
ERROR	8	115.50	14.43			
TOTAL	15	1240.43				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	2.68	3.80	12.74@1%	61.68	6.16

8DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	1624.85	232.12	18.50	3.50@5% 6.18@1%	S
ERROR	8	100.37	212.54			
TOTAL	15	1725.23				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	2.50	3.54	15.02@1%	62.53	5.66

III ANOVA TABLES for *In vitro* evaluation of fungicides

Isolate: *Corynespora cassiicola* CICR-F2

3 DAI

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	7	640.60	91.51	7.01	3.50@5% 6.18@1%	S
ERROR	8	104.37	13.04			
TOTAL	15	744.98				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	2.55	3.61	12.11@1%	51.71	6.98

5DAI

<table>
<tr><th colspan="7">ANOVA table for CRD with 9 treatments and 3 replications</th></tr>
<tr><th>C.V.</th><th>D.F</th><th>S.S</th><th>M.S.S</th><th>CAL.F</th><th>TAB. F</th><th>F. TEST</th></tr>
<tr><td>TREAT</td><td>8</td><td>48.358</td><td>6.045</td><td>94.751</td><td>2.51@5%
3.71@1%</td><td>S</td></tr>
<tr><td>ERROR</td><td>18</td><td>1.148</td><td>0.064</td><td></td><td></td><td></td></tr>
<tr><td>TOTAL</td><td>26</td><td>49.506</td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="7"></td></tr>
<tr><th>S.V.</th><th>S.E(m)</th><th>S.E(d)</th><th>C.D</th><th>G.M</th><th colspan="2">C.V</th></tr>
<tr><td>TREAT</td><td>0.146</td><td>0.206</td><td>0.594@1%</td><td>1.370</td><td colspan="2">18.431</td></tr>
</table>

8DAI

<table>
<tr><th colspan="7">ANOVA table for CRD with 9 treatments and 3 replications</th></tr>
<tr><th>C.V.</th><th>D.F</th><th>S.S</th><th>M.S.S</th><th>CAL.F</th><th>TAB. F</th><th>F. TEST</th></tr>
<tr><td>TREAT</td><td>8</td><td>85.302</td><td>10.663</td><td>63.870</td><td>2.51@5%
3.71@1%</td><td>S</td></tr>
<tr><td>ERROR</td><td>18</td><td>3.005</td><td>0.167</td><td></td><td></td><td></td></tr>
<tr><td>TOTAL</td><td>26</td><td>88.307</td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="7"></td></tr>
<tr><th>S.V.</th><th>S.E(m)</th><th>S.E(d)</th><th>C.D</th><th>G.M</th><th colspan="2">C.V</th></tr>
<tr><td>TREAT</td><td>0.236</td><td>0.334</td><td>0.960@1%</td><td>2.039</td><td colspan="2">20.040</td></tr>
</table>

Isolate: *Paramyrothecium roridium* F10

3DAI

<table>
<tr><th colspan="7">ANOVA table for CRD with 9 treatments and 3 replications</th></tr>
<tr><th>C.V.</th><th>D.F</th><th>S.S</th><th>M.S.S</th><th>CAL.F</th><th>TAB. F</th><th>F. TEST</th></tr>
<tr><td>TREAT</td><td>8</td><td>2475.48</td><td>309.43</td><td>1979.80</td><td>2.51@5%
3.71@1%</td><td>S</td></tr>
<tr><td>ERROR</td><td>18</td><td>2.81</td><td>0.15</td><td></td><td></td><td></td></tr>
<tr><td>TOTAL</td><td>26</td><td>2478.29</td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="7"></td></tr>
<tr><th>S.V.</th><th>S.E(m)</th><th>S.E(d)</th><th>C.D</th><th>G.M</th><th colspan="2">C.V</th></tr>
<tr><td>TREAT</td><td>0.22</td><td>0.32</td><td>0.92@1%</td><td>9.04</td><td colspan="2">4.37</td></tr>
</table>

5DAI

ANOVA table for CRD with 9 treatments and 3 replications						
C.V.	**D.F**	**S.S**	**M.S.S**	**CAL.F**	**TAB. F**	**F. TEST**
TREAT	8	4800.41	600.05	3839.19	2.51@5% 3.71@1%	S
ERROR	18	2.81	0.15			
TOTAL	26	4803.23				

S.V.	**S.E(m)**	**S.E(d)**	**C.D**	**G.M**	**C.V**
TREAT	0.22	0.32	0.92@1%	10.37	3.81

8DAI

ANOVA table for CRD with 9 treatments and 3 replications						
C.V.	**D.F**	**S.S**	**M.S.S**	**CAL.F**	**TAB. F**	**F. TEST**
TREAT	8	5501.65	687.70	4400.01	2.51@5% 3.71@1%	S
ERROR	18	2.81	0.15			
TOTAL	26	5504.46				

S.V.	**S.E(m)**	**S.E(d)**	**C.D**	**G.M**	**C.V**
TREAT	0.22	0.32	0.92@1%	10.70	3.69

Isolate: *Rhizoctonia solani* CICR-F6

3DAI

ANOVA table for CRD with 9 treatments and 3 replications						
C.V.	**D.F**	**S.S**	**M.S.S**	**CAL.F**	**TAB. F**	**F. TEST**
TREAT	8	17662.63	2207.79	2509.91	2.51@5% 3.71@1%	S
ERROR	18	15.83	0.87			
TOTAL	26	17678.17				

S.V.	**S.E(m)**	**S.E(d)**	**C.D**	**G.M**	**C.V**
TREAT	0.54	0.75	2.74@1%	19.94	4.70

5DAI

ANOVA table for CRD with 9 treatments and 3 replications

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	8	27740.85	3467.60	9602.60	2.51@5% 3.71@1%	S
ERROR	18	6.50	0.36			
TOTAL	26	27747.35				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	0.34	0.49	1.41@1%	29.24	2.05

8DAI

ANOVA table for CRD with 9 treatments and 3 replications

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	8	37003.85	4625.48	13876.44	2.51@5% 3.71@1%	S
ERROR	18	6.00	0.033			
TOTAL	26	37009.85				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	0.33	0.51	1.35@1%	36.92	1.56

Isolate: *Macrophomina phaseolina* CICR-F4

3DAI

ANOVA table for CRD with 9 treatments and 3 replications

C.V.	D.F	S.S	M.S.S	CAL.F	TAB. F	F. TEST
TREAT	8	2561.01	320.127	167.02	2.51@5% 3.71@1%	S
ERROR	18	34.50	1.917			S
TOTAL	26	2595.51				

S.V.	S.E(m)	S.E(d)	C.D	G.M	C.V
TREAT	0.79	1.13	0.669@1%	9.09	15.22

5DAI

ANOVA table for CRD with 9 treatments and 3 replications						
C.V.	**D.F**	**S.S**	**M.S.S**	**CAL.F**	**TAB. F**	**F. TEST**
TREAT	8	13570.66	1696.33	168.23	2.51@5% 3.71@1%	S
ERROR	18	181.50	10.083			S
TOTAL	26	13752.16				

S.V.	**S.E(m)**	**S.E(d)**	**C.D**	**G.M**	**C.V**
TREAT	1.83	2.45	0.746@1%	18.88	16.81

8DAI

ANOVA table for CRD with 9 treatments and 3 replications						
C.V.	**D.F**	**S.S**	**M.S.S**	**CAL.F**	**TAB. F**	**F. TEST**
TREAT	8	18718.33	2339.79	137.26	2.51@5% 3.71@1%	S
ERROR	18	306.83	17.046			S
TOTAL	26	19025.16				

S.V.	**S.E(m)**	**S.E(d)**	**C.D**	**G.M**	**C.V**
TREAT	2.38	3.37	9.703@1%	26.11	15.81

Isolate: *Nigrospora sphaerica* F9

3DAI

ANOVA table for CRD with 9 treatments and 3 replications						
C.V.	**D.F**	**S.S**	**M.S.S**	**CAL.F**	**TAB. F**	**F. TEST**
TREAT	8	6427.21	803.40	953.49	2.51@5% 3.71@1%	S
ERROR	18	15.16	0.84			
TOTAL	26	6442.40				

S.V.	**S.E(m)**	**S.E(d)**	**C.D**	**G.M**	**C.V**
TREAT	0.53	0.74	0.539@1%	11.64	7.88

5DAI

ANOVA table for CRD with 9 treatments and 3 replications						
C.V.	**D.F**	**S.S**	**M.S.S**	**CAL.F**	**TAB. F**	**F. TEST**
TREAT	8	15662.24	1957.78	272.47	2.51@5% 3.71@1%	S
ERROR	18	129.33	7.18			
TOTAL	26	15791.57				

S.V.	**S.E(m)**	**S.E(d)**	**C.D**	**G.M**	**C.V**
TREAT	1.54	2.18	0.703@1%	19.68	13.61

8DAI

ANOVA table for CRD with 9 treatments and 3 replications						
C.V.	**D.F**	**S.S**	**M.S.S**	**CAL.F**	**TAB. F**	**F. TEST**
TREAT	8	19230.74	2403.84	1317.84	2.51@5% 3.71@1%	S
ERROR	18	32.83	1.82			
TOTAL	26	19263.57				

S.V.	**S.E(m)**	**S.E(d)**	**C.D**	**G.M**	**C.V**
TREAT	0.78	1.10	1.233@1%	28.31	4.77