"STUDIES ON CHARCOAL ROT OF MAIZE CAUSED BY *Macrophomina phaseolina* (Tassi) GOID"

by

Mr. CHARDE UTTAM MOHAN
(Reg.No. 018/ 226)

A thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

In partial fulfilment of the requirements for the degree
of

MASTER OF SCIENCE (AGRICULTURE)
In

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY

POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA
2021

"STUDIES ON CHARCOAL ROT OF MAIZE CAUSED BY *Macrophomina phaseolina* (Tassi) GOID"

by

Mr . CHARDE UTTAM MOHAN
(Reg.No. 018/ 226)

A thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

In partial fulfilment of the requirements for the degree
of

MASTER OF SCIENCE (AGRICULTURE)
in
PLANT PATHOLOGY

APPROVED BY

Dr. V. S. Shinde
(Chairman and Research Guide)

Dr. K.S. Raghuwanshi
(Committee Member)

Dr. S. B. Latake
(Committee Member)

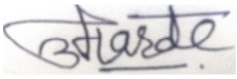
Dr. S. R. Dhonde
(Committee Member)

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA
2021

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
there of has not been submitted
by me or other person to any
other University or Institute
for a Degree or
Diploma

Place : M.P.K.V., Rahuri
Date :



(U. M. Charde)

Dr. V.S. Shinde
Assistant Plant Pathologist AICRP on Maize,
Pulse Improvement Project,
Mahatma Phule Krishi Vidyapeeth
Rahuri-413 722, Dist. Ahmednagar,
Maharashtra State (India)

CERTIFICATE

This is to certify that the thesis entitled. **"STUDIES ON CHARCOAL ROT OF MAIZE CAUSED BY *Macrophomina phaseolina* (Tassi) GOID."** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY,** embodies the result of a piece of bonafide research work carried out by **Mr. CHARDE UTTAM MOHAN** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

Place : M.P.K.V., Rahuri (V.S. Shinde)

Date : / /2021 Research Guide

Dr. T.K. Narute
Head,
Department, Plant Pathology and
Agricultural Microbiology,
Post Graduate Institute
Mahatma Phule Krishi Vidyapeeth
Rahuri-413 722, Dist. Ahmednagar (M.S)

CERTIFICATE

This is to certify that the thesis entitled. **"STUDIES ON CHARCOAL ROT OF MAIZE CAUSED BY *Macrophomina phaseolina* (Tassi) GOID."** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY,** embodies the result of a piece of bonafide research work carried out by **Mr. CHARDE UTTAM MOHAN** under guidance and supervision of **Dr. V.S. SHINDE,** Assistant Plant Pathologist, AICRP on Maize, Pulse Improvement Project, M.P.K.V., Rahuri and that no part of the thesis has been submitted for any other degree or diploma.

Place : M.P.K.V., Rahuri (T.K. Narute)

Date : / /2021

Dr. P.N. Rasal
Associate Dean,
Post Graduate Institute,
Mahatma Phule Krishi Vidyapeeth,
Rahuri - 413 722, Dist. Ahmednagar,
Maharashtra (INDIA)

CERTIFICATE

This is to certify that the thesis entitled. **"STUDIES ON CHARCOAL ROT OF MAIZE CAUSED BY *Macrophomina phaseolina* (Tassi) GOID."** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY,** embodies the result of a piece of bonafide research work carried out by **Mr. CHARDE UTTAM MOHAN** under guidance and supervision of **Dr. V.S. SHINDE,** Assistant Plant Pathologist, AICRP on Maize, Pulse Improvement Project, M.P.K.V., Rahuri and that no part of the thesis has been submitted to any other University for Degree or Diploma.

Place : M.P.K.V., Rahuri. (P.N. Rasal)

Date : / / 2021

ACKNOWLEDGEMENT

This thesis is the end of my journey in obtaining my master's degree. I have not travelled in a vacuum in this journey. This thesis has been kept on track and been seen through to completion with the support and encouragement of numerous people including my parents, my teachers, my friends, and well-wishers. At the end of my thesis I would like to thank all those people who made this thesis possible and an unforgettable experience for me. At the end of my thesis, it is a pleasant task to express my thanks to all those who contributed in many ways to the success of this study and made it an unforgettable experience for me.

At this moment of accomplishment, first of all I pay homage to my advisor and research guide ***Dr. V. S. Shinde****, Asstt. Plant Pathologist, AICRP on Maize, Mahatma Phule Krishi Vidyapeeth, Rahuri. This work would not have been possible without his guidance, support and encouragement. Under his guidance I successfully overcame many difficulties and learned a lot. I would like to express my deepest gratitude for his constant vigilance, stimulating suggestions and encouragement that he has bestowed upon me during all these works. I am thankful for all those countless moments that he shared with me from his busy schedule. His unflinching courage and conviction will always inspire me, and I hope to continue to work with his noble thoughts of living life to the fullest.*

I am extremely grateful to ***Dr. P. G. Patil****, Hon. Vice Chancellor, M. P. K. V., Rahuri,* ***Dr. A. L. Pharande****, Dean, Faculty of Agriculture, M. P. K. V., Rahuri. I am also extremely indebted to Head of Department* ***Dr. T.K. Narute****, Dept. of Plant Pathology and Agricultural Microbiology for providing necessary infrastructure and resources to accomplish my research work. I am very much thankful to him for all his comforting words and constant moral support at the critical stage of my M.Sc. degree. I take this golden opportunity to express my sincere thanks to* ***Dr. P. N. Rasal****, Associate Dean (PGI) providing necessary facilities for research work. I warmly thank members of my advisory committee Dr. K. S. Raghuwanshi, Dr. S. B. Latake and Dr. S. R. Dhonde for their valuable advice, constructive criticism and extensive discussions around my work.*

I take this opportunity to sincerely acknowledge Dr. A.M. Navale, Dr. V.A. Chavan, Dr. R.T. Gaikwad and Dr. S.R. Zanjare for their unconditional help, affection, concern, constructive criticism and repeated discussion over my work.

I extend my hearty thanks to my seniors Vipin sir, Chandrashekhar sir, Swachhand sir, Pramod sir, Shubham sir, Pratik sir, Vidyesh sir, Ravi sir, Vikas sir, Akshay sir, Sanganna sir, Dhananjay sir, Nilesh sir, Nagesh sir, Ashok sir, Ramkrushna sir and Monali mam, for their moral support, valuable advice and concern towards me.

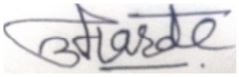
Friends who have witnessed all my rise, fall, joy and sorrow, who are the only cause to elevate myself from every hardship need to be unveiled. They are the source of inspiration in my life. I am thankful to Samrat, Akash, Onkar, Sushant, Krushna, Shreyash, Sushant, Sunil, Somnath, Girish, Dharmendra, Nikhita, Shivani, Archana, Kalyani, Yugpriya and Pallavi. My sincere thanks to the non-teaching staffs of Dept. of Plant Pathology Abhijeet sir, Nangare mam, Kamini mam, Lakshman bhaiya, Bhingarade Mama and Mavashi for all their help at official level.

I have no words to express my feelings in fathomable depths which springs in the very core of my heart for my Baba ***Shri. Mohan Tukaram Charde****, my Aai* ***Smt. Shema Mohan Charde****, Sisters Harsha, Mona & Shilpa, Brothers Moreshwar & Nikhil and Kishor Bhauji who always stood like a lighthouse for illumination in the pathway of every success and for their sacrifice in moulding me and in building up my educational career. I can't imagine if I could do anything without them. They remained the pillar of all sources for me since beginning of my life.*

I would like to express my sincere thanks to the ***Mahatma Phule Krishi Vidyapeeth, Rahuri.*** *Dist-Ahmednagar for providing me an opportunity to undertake my post graduate studies in this "Institute of Excellence".*

I am also deeply obliged to all those authors and scientists, past and present, whose contributions were of immense help to understand these research findings as their literature has been aptly cited to undertake the present investigation.

While travelling on the path of life and education, many hands pushed me forth, learned hearts put me on the right path, unlighted by their knowledge and experience, I ever remain thankful to them all.



Place: M.P.K.V., Rahuri. (Uttam Charde)

Date : / /2021

CONTENTS

Chapter No.	Title	Page No.
	CANDIDATES DECLARATION	ii
	CERTIFICATE OF RESEARCH GUIDE	iv
	CERTIFICATE OF HEAD OF THE DEPARTMENT	v
	CERTIFICATE OF ASSOCIATE DEAN	vi
	ACKNOWLEDGEMENT	vii
	CONTENTS	ix
	LIST OF TABLES	xii
	LIST OF FIGURES	xiii
	LIST OF PLATES	xiv
	LIST OF ABBREVIATIONS AND SYMBOLS	xv
	ABSTRACT	xvii
1.	**INTRODUCTION**	**1**
2.	**REVIEW OF LITERATURE**	**5**
	2.1 Occurrence, distribution and losses	6
	2.2 Pathogen	8
	2.3 Symptomatology	9
	2.4 Collection, isolation and pathogenicity	10
	2.5 Morphological characters of pathogen	11
	2.6 Cultural characters of the pathogen	13
	2.7 Management of disease	15
	2.7.1 Efficacy of fungicide	15
	2.7.2 Bio efficacy of bioagents	18
	2.7.3 Evaluation of fungicides and bioagents in pot culture	20
	2.8 Screening of genotypes against *M. phaseolina*	21

3	**MATERIALS AND METHODS**	**23**
	3.1 Materials	23
	3.1.1 Experimental site	23
	3.1.2 Disease samples	23
	3.1.3 Culture media	23
	3.1.4 Seeds	23
	3.1.5 Chemicals	23
	3.1.6 Glass ware	24
	3.1.7 Equipment's	24
	3.1.8 Miscellaneous	24
	3.1.9 Fungicides	24
	3.1.10 Biocontrol agents	25
	3.2 Methodology	25
	3.2.1 Sterilization of media and glassware's	25
	3.2.2 Preparation of culture media	25
	3.2.3 Symptomatology	25
	3.2.4 Isolation	26
	3.2.4.1 Isolation and Identification of the pathogen	26
	3.2.4.2 Purification and maintenance of culture	26
	3.2.4.3 Mass multiplication of *M. phaseolina* inoculum	26
	3.2.5.1 Pathogenicity test	27
	3.2.5.2 Re-isolation	28
	3.2.6 Effect of different culture media on growth and sclerotial production	28
	3.2.7 Disease management strategies	29
	3.2.7.1 *In vitro* evaluation of fungicides	29
	3.2.7.2 *In vitro* evaluation of bioagents	30
	3.2.7.3 Evaluation of fungicides and bioagents in pot culture	31
	3.2.8 Screening of maize genotypes	32

4	**RESULTS AND DISCUSSION**	**34**
	4.1 Collection of samples	34
	4.2 Isolation of the pathogen	34
	4.3 Identification of the pathogen	34
	4.3.1 Colony characters	35
	4.3.2 Symptomatology	35
	4.4 Pathogenicity test	35
	4.5 Morphological characteristics	36
	4.6 Cultural characteristics	37
	4.7 Disease management strategies	38
	4.7.1 *In vitro* evaluation of fungicides	38
	4.7.1.1 Mycelial growth	38
	4.7.1.2 Mycelial growth inhibition	40
	4.7.2 *In vitro* evaluation of bioagents / Antagonists	41
	4.7.2.1 Radial mycelial growth	41
	4.7.2.2 Mycelial growth inhibition	42
	4.7.3 Evaluation of fungicides and bioagents against *M. phaseolina* in pot culture	42
	4.8 Screening of genotypes against *M. phaseolina* under field conditions	43
5	**SUMMARY AND CONCLUSIONS**	**46**
6	**LITERATURE CITED**	**49**
7	**APPENDIX-I**	**61**
8	**VITAE**	**63**

LIST OF TABLES

Table No.	Description	Page No.
3.1	List of fungicides used against *M. phaseolina.*	24
3.2	Sclerotial rating	29
3.3	Rating scale for screening of maize genotypes against charcoal rot disease.	33
4.1	Effect of different culture media on radial mycelial growth, cultural characteristics and sporulation of *M. phaseolina*	37
4.2	*In vitro* efficacy of different fungicides against mycelial growth and inhibition of *M. phaseolina*	39
4.3	Evaluation of bioagents against *M. phaseolina* under *in vitro* condition	41
4.4	Efficacy of fungicides and bioagents against *M. phaseolina* in pot culture	43
4.5	Reaction of maize genotypes against charcoal rot	44

LIST OF FIGURES

Figure No.	Description	Between pages
1.	Effect of different culture media on mycelial growth of *M. phaseolina*	37-38
2.	Efficacy of different fungicides against *M. phaseolina* under *in vitro* condition at half of concentration than recommended	39-40
3.	Efficacy of different fungicides against *M. phaseolina* under *in vitro* condition at recommended concentration	39-40
4.	Mean colony diameter of *M. phaseolina* against bioagents	41-42
5.	Evaluation of fungicides and bioagents against *M. phaseolina* in pot culture	43-44
6.	Screening of different genotypes against *M. phaseolina*	44-45

LIST OF PLATES

Plate No.	Description	Between page
1.	Infected maize samples showing typical charcoal rot symptom	34-35
2.	Pure culture and mass multiplication of *M. phaseolina*	35-36
3.	Pathogenicity test of *M. Phaseolina* on maize cv. G 25	35-36
4.	Mycelium and Sclerotia of *Macrophomina phaseolina*	36-37
5.	The effect of different culture media on growth and sclerotial production of *Macrophomina phaseolina*	37-38
6.	*In vitro* efficacy of fungicides against *M. phaseolina*	39-40
7.	*In vitro* efficacy of the bioagents against mycelial growth and inhibition of *M. phaseolina*	41-42
8.	Screening of maize genotypes against *M. phaseolina*	43-44

LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Per cent
/	:	Per
@	:	at the rate of
^{0}C	:	Degree celcius
Abst	:	Abstract
AICRP	:	All India Coordinated Research Project
CR	:	Charcoal Rot
cv.	:	Cultivar
DIST	:	District
e.g.	:	Exempli gratia, For example
et al.	:	et alia
etc.	:	et cetera
Fig.	:	Figure
FYM	:	Farmyard Manure
g	:	Gram (s)
h	:	Hour (s)
ha	:	Hectare (s)
i.e.	:	id est, that is
kg	:	Kilogram (s)
L	:	Litre (s)
mg	:	Milligram (s)
ml	:	Milliliter (s)
MPKV	:	Mahatma Phule Krishi Vidyapeeth
MR	:	Moderately Resistant
MS	:	Moderately Susceptible
MT	:	Metric tonne(s)
PDA	:	Potato Dextrose Agar
PDI	:	Per cent Disease Incidence
pv	:	Pathovar
R	:	Resistant
S	:	Susceptible
sp./spp.	:	Species (s)
viz.	:	videlicet (Namely)

ABSTRACT

"STUDIES ON CHARCOAL ROT OF MAIZE CAUSED BY *MACROPHOMINA PHASEOLINA* (TASSI) GOID."

by

Mr. CHARDE UTTAM MOHAN

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI-413 722
2020

Research Guide	:	Dr. V.S. Shinde
Department	:	Plant Pathology and Agril. Microbiology

Maize (*Zea mays* L.), also known as corn, is a cereal grain that was first grown by people in Central America. It is now the third most important cereal crop in the world and is called the 'Queen of Cereals'. Maize is a versatile crop grown over a range of agroclimatic zones. It is grown from 58^0N to 40^0S, to an altitude higher than 3000 M, and in areas with 250 mm to 5000 mm of rainfall per year.

In India major maize producing states are Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra. In Maharashtra, maize cultivation is concentrated in western Maharashtra and Marathwada region. The main districts in which maize cultivation is being undertaken includes Nashik, Aurangabad, Jalgaon, Dhule, Sangali, Jalna and Ahmednagar.

Diseased samples showing typical charcoal rot symptoms were collected from farmers field and brought to the laboratory for isolation. Fungal pathogens responsible for charcoal rot of maize was isolated on PDA medium.

The pathogenicity of isolated pathogen was proved on susceptible maize variety G-25 by soil inoculation method under glasshouse condition. After appearance of

disease symptoms, pathogen was reisolated from the artificially inoculated plants and resulting culture was compared with the original one to confirm the identity of the pathogen. The morphological characters of the pathogen causing charcoal rot of maize were carried out by studying the colony characters and microscopic observations. Mycelium was hyaline, slender, branched and septate, initially hyaline turning to a black colour. The vegetative mycelium formed monilid or barrel-shaped cells and the formation of septum near the branching of the mycelium. In 7-day-old cultures, the pathogen produced numerous black sclerotia on diseased plant parts, which were smooth to irregular and black. Microsclerotia ranged in size from 55 to 190 µm long by 45 to 120 µm wide (average 105×74 µm).

Based on the typical disease symptoms observed under field, pathogenicity test and morphological characters of the fungus responsible for causing charcoal rot in maize was identified as *Marophomina phaseolina*.

In cultural studies, it was observed that the test pathogen *Macrophomina phaseolina* showed maximum radial growth on Potato dextrose agar (PDA) and good sclerotial production was recorded on PDA, Corn meal agar, Oat meal agar, Standard nutrient agar and Malt extract agar media.

Under *in vitro* condition Fungicide, Carbendazim + Mancozeb at 0.1 and 0.2% concentrations and Carbendazim (0.1%) inhibited complete growth of the pathogen *Macrophomina phaseolina* on PDA medium. Copper hydroxide and Copper oxychloride were found least effective in inhibiting the growth of pathogen.

Among bio agents, *Trichoderma harzianum* was found most effective for inhibiting the mycelial growth of *Macrophomina phaseolina* (63.33%). It was followed by *Trichoderma viride* (59.26%), *Trichoderma hamatum* (42.22%), *Trichoderma koningii* (40.00%), *Bacillus subtilis* (32.22%) and *Psedomonas fluroscens* (28.88%) under *in vitro* condition.

Abstract contd… **Mr. Uttam M. Charde**

In pot culture studies, all the six treatments found effective to control charcoal rot disease incidence. However, among the fungicides Carbendazim + Mancozeb was most effective and among bioagents *Trichoderma harzianum* was most effective in controlling the charcoal rot incidence.

Fifty-three maize genotypes were screened for their reaction to charcoal rot disease. Two genotypes were resistant, twenty-four moderately resistant, twenty-three moderately susceptible whereas, four were found susceptible to charcoal rot disease.

Pages 1 to 63

1. INTRODUCTION

Maize (*Zea mays* L.), also known as corn, is a cereal grain belongs to the tribe Maydae of the family Poaceae. It is one of the oldest human-domesticated plants. Its origin is believed to be date back to at least 7000 years when it was grown in the form of a wild grass called *teosinte* in Central Mexico (Kumar, 2013). Cultivated maize *Zea mays* L. subsp. *mays* (Iltis and Doebley, 1980) are most likely the product of a single domestication event through human selection on annual teosinte *Zea mays* L. sub sp. *parviglumis* (Doebley *et al*., 1987, and Matsuoka *et al*., 2002). The number of chromosomes in *Zea mays* is 2n = 20 (Chopra, 2001). It is the most versatile crop with highest yield potential and wider adaptability among cereals. It is now the third most important cereal crop in the world and rightly called the 'Queen of Cereals'.

Maize has a very distinct growth form; a tall, determinate, annual C4 plant varying in height from 1 to 4 meters producing large, narrow, opposing leaves, borne alternately along the length of a solid stem. It is monoecious, annual grass having three types of roots; seminal roots, adventitious roots, and brace or prop roots, produced by lower two nodes. The stems are erect, conventionally 2-3 meters in height, with many nodes, casting off flag leaves at every node. The internodes are short and fairly thick at the base of the plant can reach up to 20-30 centimeters. The apex of the stem ends in the tassels, an inflorescence of male flowers and the female inflorescences (cobs or ears) are borne at the apex of condensed lateral branches known as shanks which protrudes from leaf axil. The male (staminate) inflorescence, a loose panicle, produces pairs of free spikelets each enclosed by a fertile and a sterile floret. The female (pistillate) inflorescence, a spike, produces pairs of spikelets on the surface of a highly condensed rachis (central axis, or "cob"). The silks are elongated stigmas that look like tuft of hairs, at first green and later red or yellow. Maize is wind pollinated and both self and cross pollination is usually possible.

Maize is one of the world's important food crop containing starch (71 – 72 %), protein (9 -10 %), fat (4 – 45 %), fiber (9 – 10 %), sugar (2 – 3 %) and minerals or ash (1.4 %) on dry matter basis. It is the major source of food, feed, fodder and industrial raw material and provides enormous opportunity for crop diversification, value addition

and employment generation. The wider adaptability and high yield potential of maize and its utility as food, feed and forage crop signifies the importance of maize. In addition to staple food for human being and quality feed for poultry and animals, it serves as a basic raw material for the industry for production of starch for textile, pharmaceutical, cosmetic industries, high quality corn oil, protein, alcoholic beverages, food sweeteners etc. It is also grown for many other special purposes *viz*. quality protein maize (QPM); for human nutrition, alleviation of malnutrition and quality feed for poultry and animals, sweet corn, baby corn; baby corn for vegetable, popcorn, waxy corn, high oil and high amylase corn and other table purposes including value added products like pickle, soup, corn *pakora, kheer etc.*, sweet corn for soup, and other recipes.

It is also a solution for various stresses like weed and lowering water table and abiotic stresses like drought, terminal heat, cold, *etc.* besides providing opportunity for farm mechanization and conservation agriculture and consequently increasing the resource-use efficiency and farm profitability. In the last 10 years, the uses of maize for fuel production significantly increased, accounting for approximately 40 per cent of the maize production in the United States. Low production cost along with the high consumption of maize flour and cornmeal, especially where micronutrient deficiencies are common public health problems, make this food staple an ideal food vehicle for fortification (Anon, 2017).

Maize is a versatile crop grown over a range of agroclimatic zones. In fact, the suitability of maize to diverse environments is unmatched by any other crop plant. It is grown from 58°N to 40°S, to an altitude higher than 3000 M, and in areas with 250 mm to 5000 mm of rainfall per year (Shaw, 1988; Dowswell *et al.,* 1996) and with a growing cycle ranging from 3 to 13 months (Anon, 2000). However, the major maize production areas are located in temperate regions of the globe.

The renowned Nobel laureate Dr. Norman E. Borlaug believes that "after the last two decades saw the revolution in rice and wheat, the next few decades will be known as maize era" (Patil *et al*., 2000).

Globally, it is cultivated on more than 160 m ha area across 166 countries having wider diversity of soil, climate, biodiversity and management practices. Among the maize growing countries, USA is the largest producer, contributing 35 per cent of the

total maize production, followed by China with more than 20 per cent production and near about same acreage as of USA. The United States produces more than 35 per cent of the world's maize harvest. Other top producing countries are China, Brazil, Mexico, Argentina and India. India produces about 2 per cent the world's maize produce (Anon, 2017).

Adaptability of maize crop to diverse environment and availability of high yielding hybrid seeds leads to increase area under maize cultivation in India as well as in Maharashtra. India is one of the top 10 maize producers in the world; it contributes around 2-3 per cent of the total maize produced globally and is one of the top 5 maize exporters in the world contributing almost 14 per cent to the total export. In India, maize is grown throughout the year. It is predominantly a *kharif* crop with 85 per cent of the area under cultivation in the season. It is the third most important cereal crop in India after rice and wheat. It accounts for around 10 per cent of total food grain production in the country. Presently maize is cultivated in India on an area of 9.2 m ha with production and productivity of 28.7 mt and 3.11 t/ha, respectively (Anon., 2020).

It is grown in many states, from Jammu and Kashmir in the North to Andhra Pradesh and Karnataka in the South while from Rajasthan in West to North-eastern states of the country. The major maize growing states that contribute to maize production are Karnataka (13.40 %), Madhya Pradesh (12.30 %), Maharashtra (10.61 %), Tamil Nadu (9.01 %), Telangana (8.88 %), Bihar (8.19 %). However, the productivity of maize is highest in Tamil Nadu (7.98 t ha-1) followed by Andhra Pradesh (6.91 t ha-1), West Bengal (4.80 t ha-1) and Punjab (3.70 t ha-1) (Anon., 2020).

Maharashtra is the fourth producer of maize in India producing around 10.61 per cent of India's total maize production. In Maharashtra, during the last ten years i.e. 2006-07 to 2016-17, the area under maize has increased from 5.81 lakh ha. to 10.03 lakh ha., whereas production increased from 11.50 lakh tons to 32.21 lakh tons. Similarly, the productivity has increased from 1.9 t/ha to 3.2 t/ha. The average per year increase in cultivated area of maize is near about 10 per cent and that of production and productivity to the tune of 12 per cent each in last 10 years.

In Maharashtra, maize is mainly grown in *kharif* season (9.13 lakh ha.) and summer season (0.35 lakh ha.). The major maize growing districts in Maharashtra are

Nashik (2.3 lakh ha.), Aurangabad (1.87 lakh ha.), Jalgaon (1.5 lakh ha.), Dhule (0.69 lakh ha.), Sangali (0.54 lakh ha.), Jalna (0.66 lakh ha.) and Ahmednagar (0.60 lakh ha.). In the state, crop is mostly utilized as source of human feed (24%), animal feed (11%), poultry feed (52%), starch (11%), brewery (1%) and seed (1%). Maize has a significant potential for doubling farmer's income as it generates better income and provides gainful employment.

Among the factors adversely affecting productivity, ubiquitous incidence of diseases is prominent. Maize diseases have been a major constraint in increasing productivity. Besides reduced production, heavy economic losses are incurred every year due to various diseases. In India, the crop is prone to a number of biotic stresses like foliar diseases, ear rot, and stalk rots caused by fungi, bacteria and viruses. These diseases are difficult to control because their populations are variable in time, space and genotype. Turcicum leaf blight, Maydis leaf blight, banded leaf and sheath blight, Post Flowering Stalk Rots (PFSR), common rust, Polysora rust, downy mildews, *Pythium* stalk rot, Fusarium stalk rot, charcoal rot and bacterial stalk rot are the major threat to the potential yield of maize (Kumar *et al*, 2013).

Corporality, losses due to maize diseases have been estimated to the tune of 9.4 per cent annually, for the countries of Asia it is 12 per cent, while for the African countries it is as high as 14 per cent (Cramer, 1967). Even for the developed countries like USA, 12 per cent of the produce is lost annually due to diseases. In India the total loss in economic products of the crop due to diseases has been estimated to the tune of Rs. 17,83,320 and in terms of percent losses is 13.2 per cent (Payak and Sharma, 1985). It has been estimated that about 13.2 per cent of the economic production of maize is lost annually due to diseases in India (Dhillon and Prasanna, 2001).

Charcoal rot, popularly known as Post flowering stalk rots is the most serious, destructive and widespread disease in maize. Most of the commercially grown cultivars have shown a high level of disease incidence during grain filling stage. This disease is prevalent in most of the maize growing areas of India particularly where there is scarcity of irrigation especially at post flowering stage of the crop growth. Even though significant improvements in management have been made, stalk rots continue to be a serious problem (Kaiser and Mukherjee, 1979; White, 1999). The major pathogen

responsible for the disease is *Macrophomina phaseolina* (Murali *et al.,* 2013*)*. The extent of loss in grain yield ranged from 25-32.2 per cent and along with decrease in fodder quality (Mukesh Kumar *et al*, 1996).

Presently, maize production is 28.7 million tons and projected demand of maize to be 45 million ton by 2030. So, there is a scope of manipulating production technologies in respect of crop diversification, resource conservation, insect-pest control for improving crop yields on sustainable basis. To make farming sustainable and economically viable, there is a need for rethinking, planning and management in order to face the emerging challenges.

Keeping all these points in view, the following objectives were chosen for the investigation in the present study:

1. To collect charcoal rot infected maize plant samples, isolate the pathogen and prove its pathogenicity.
2. To study morphological and cultural characteristics of pathogen.
3. To evaluate the fungicides and biocontrol agents against *Macrophomina phaseolina* in *vitro* and in pot culture.
4. To screen maize genotype against *Macrophomina phaseolina.*

2. REVIEW OF LITERATURE

2.1 Occurrence, Distribution and Losses

Koehler (1960) reported that charcoal rot attacks many crops in various parts of the world. In the United States it appears to be particularly damaging to corn and sorghum in the states from Texas to Nebraska during hot and moderately dry weather. This disease is also damaging to corn in Illinois when similar weather prevails in late summer. The disease was unusually prevalent in the southern half of Illinois in 1953 and 1955.

Cramer (1967) reported that reduction in world maize grain production owing to the diseases is estimated to an average of 9.4 per cent, for USA a figure of 12 per cent has been computed.

Kaiser *et al. (*1988) reported that the *rabi* maize in eastern parts of the country suffer from charcoal rot disease [*Rhizoctonia bataticola* Taub. Butl. (*Macrophomina phaseolina* (Tassi) Goid.)] if the pathogen gets timely entry into the host. It was further reported that the spread of the pathogen within the maize stalk is influenced by high temperature, the optimum being 38°C.

Desai *et al*. (1991) reported that the charcoal rot disease affects the stalk of the plant at post-flowering stages. The disease caused losses to the tune of 10 to 42.9% in Karnataka.

Harlapur *et al.* (2000) reported 16.50 per cent charcoal rot incidence and 15-20 per cent yield loss in the local cultivars of maize.

Shekhar *et al.* (2006) reported that the charcoal rot of maize is one of the economically important disease of maize all over the world. It occurs in areas where drought conditions generally prevail at or after flowering. The disease is favored by high soil temperature ranging from 30 to 42^0C and low soil moistures.

Mukerji and Manoharachary (2010) reported that fungus (*M. phaseolina*) is worldwide in distribution and causes many plant diseases including seed rots, root rot, leaf blight, and stalk rot. It affects a wide variety of plants including corn, sorghum, oilseeds and pulses. It causes charcoal rot on corn, sorghum, soybean and other economical crop plants.

Shekhar and Kumar (2012) reported that charcoal rot of maize is caused by *Macrophomina phaseolina* (Tassi) Goid. This disease in India is capable of causing appreciable change in standing crops and may attack all types of corn. The loss in terms of harvested grain per annum has been determined to be the order of 13.2%, amounting to 1459.6 million rupees.

Kumar *et al*. (2013) reported that in India charcoal rot of maize is distributed in states of Jammu and Kashmir, West Bengal, Punjab, Haryana, Rajasthan, Delhi, Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Karnataka, and Tamil Nadu. It is a common stalk rot disease in warm and dry areas. It occurs in areas where drought conditions generally prevail. The disease is favored by soil temperature ranging from 30^0C to 42^0C and low soil moistures.

Krishna *et al. (*2013) reported that post flowering stalk rots, popularly known as charcoal rot is the most serious, destructive and widespread disease in maize. This disease is prevalent in most of the maize growing areas of India particularly where there is scarcity of irrigation at post flowering stage of the crop growth and causes reduction in grain yield and decreases fodder quality. The pathogen responsible for the disease is *Macrophomina phaseolina.* The extent of loss in grain yield ranged from 25-32.2 per cent and this disease also caused a decrease in fodder quality.

Khokar *et al.* (2014) reported that the charcoal rot of maize, caused by *Macrophomina phaseolina* (Tassi) Goid. is an important disease of this crop. The pathogen is reported to infect nearly 500 species of plants in tropical and subtropical countries. In India, the charcoal rot disease was observed in an epidemic form during 1960 *kharif* season in Kashmir valley. Later it was noticed at Hyderabad (Andhra Pradesh) during 1965-66 *rabi* season and at Pantnagar (Uttar Pradesh) in 1966 *kharif.*

Khaire (2016) reported incidence of *Macrophomina* blight of mungbean in Jalna district of Maharashtra was 28.83 percent. The incidence was recorded varied from 7.34 to 40.00 per cent.

Hooda *et al.* (2018) reported that *Macrophomina phaseolina* causes charcoal rot disease on more than 500 plant species throughout the world. Yield losses as high as 70 per cent have been documented in Africa. The disease is particularly prevalent

in drought years and in arid regions where maize is regularly cultivated in rotation with other host crops.

2.2 Pathogen

Goidanich (1947) followed the work of Tassi and compared with *Macrophomina phaseoli, M. corchori, M. cajani, M. sesami, M. philippinesis*, *Dothorela cajani* and *D. phaseolina* and found that all of them identical. He correlated the mistake made by Ashby and according to the International code of Botanical Nomenclature, *Macrophomina phaseolina* is the valid name for pycnidial stage of *R. bataticola*.

Reichert and Hellinger (1947) reported that *M. phaseolina* (Tassi) Goid (synonyms *Tiarosporella phaseolina, Macrophoma phaseolina* and *Rhizoctonia lamellifera*) is an anamorphic ascomycete of the family Botryosphaeriaceae and causes the disease charcoal rot on a broad range of plants in many areas of the world.

Dhingra and Sinclair (1977) reported that colour of *Macrophomina* varied in culture from black to brown or gray and became dark in colour with age. Abundant aerial mycelium is produced in the culture plate with sclerotia imbedded within the hyphae or engrossed in the agar or on the agar surface with smooth precincts. Hyphae are septate, initially hyaline turning to a honey or black colour. Numerous dark browns to black coloured sclerotia can be seen on the reverse side of the culture plate. The vegetative mycelium is characterized by the formation of monilid or barrel-shaped cells and the formation of septum near the branching of the mycelium. Branching occurs at right angle to parent hyphae but branching at acute angles is also common.

Malcom (1980) reported that charcoal rot of maize, caused by *Macrophomina phaseolina* (Tassi) Goid., is an important disease of crop. *Rhizoctonia bataticola* is considered to be the sclerotial and mycelial stage of *M. phaseolina*.

Shekhar (2004) reported that the charcoal rot of maize is caused by the pathogen *Macrophomina phaseolina* (Tassi) Goidanich [=*M. phaseoli* (Maubl.) S. Ashby = *Botryodiplodia phaseoli* (Maubl.) Thirumalachar = *Sclerotium bataticola* Taubenhaus]. Maize isolate of the pathogen, *M. phaseolina* are sterile and do not form conidia.

Crous *et al*. (2006) reported that lack of a known teleomorph has stalled its taxonomy over the years. However, thorough phylogenetic study of 113 members of the

family Botryosphaeriaceae using ribosomal DNA sequences was able to separate the genera *Macrophomina* and *Tiarosporella.*

Tetali and Karpagavalli (2016) reported that *Macrophomina phaseolina* a soil-inhabiting fungus is an important root pathogen and causes dry root rot, stem canker, stalk rot and charcoal rot diseases.

2.3 Symptomatology

Livingston (1945) observed that the charcoal rot organism caused both root rot and stalk rot of sorghum and corn when grown in inoculated soil in the greenhouse. Blighted seedlings, drying of the tips of the lower leaves. This drying progressed, but the entire plant usually turned yellow and rotted off at or just below the ground line before any but the lowest leaves became completely dry and the mesocotyl was black, shriveled throughout most of its length.

Malcom (1980) reported that the charcoal rot produces a variety of symptoms, which ranged from seedling blight, rotting of stalk, roots and kernels in maize crop. It also produces brown, water-soaked lesions on the roots that later turns black. As the plants mature the fungus spreads into the lower inter nodes of the stalk, causing premature ripening, shredding and breaking at the crown. Numerous black sclerotia on the vascular strands give the interior stalks a charred appearance.

Shekhar *et al*. (2006) reported that a characteristic sign of charcoal rot pathogen is the presence of numerous, minute black sclerotia, particularly on the vascular bundles and outside the rind of the stalk. In the diseased plants, the outer rind and pith tissues are rotten, whereas the vascular bundles remain intact.

Iqbal *et al.* (2010) studied the charcoal rot infected plants and reported that dark lesions appear on the epicotyls and hypocotyls followed by seedling death due to obstruction of xylem vessels.

Kumar *et al.* (2013) reported that the charcoal rot affected plant dry prematurely, the affected internodes becomes disintegrated and the presence of small pinhead like black sclerotia on the rind of the stalks.

Groves and Smith (2013) observed that the charcoal rot infected plants may display a premature yellowing of the top leaves and premature leaf drop. Infected plants wilt in the midday heat and recover at night until permanent wilt point is reached.

Freije and Wisen (2016) reported that the fungus invades the stalk through roots and lower stems. It then progresses into the stalk and disintegrates the pith tissue. In the stalk *M. phaseolina* creates many tiny structures (called microsclerotia) that give the inside of the stalk a speckled appearance, similar to a silvery black charcoal dust.

Hooda *et al.* (2018) stated that the after flowering, initial symptoms are the abnormal drying of upper leaf tissue, stem lodging and premature death. At maturity, the lower stem internodes (usually limited to the first 5 nodes) show a typical charcoal, grey-black discoloration.

2.4 Collection, Isolation and Pathogenicity

Dhingra and Sinclair (1978) isolated *M. phaseolina* by hyphal tip method separately from roots, stem, petiole, pods and seeds of three field grown soybean plants.

Hooda and Grover (1990) isolated and proved the pathogenicity of *M. phaseolina* infecting mungbean and reported maximum mycelial growth and virulence at 30-35°C temperature.

Su *et al.* (2001) investigated the host specialization in *Macrophomina phaseolina*. The fungus was isolated from soybean, corn, sorghum, and cotton root tissue and soil from fields cropped continuously by these species for 15 years.

Jana *et al*. (2005) isolated *Macrophomina phaseolina* from soybean and cotton. The samples were obtained from India and North America. Fungal cultures were grown in Potato dextrose broth (PDB) for 10 days at 25–28^{0}C.

Iqbal *et al.* (2010) collected the stem bark tissues of urd bean bearing fungal sclerotia and characteristics charcoal rot symptoms for isolation of the pathogen. The tissues were cut into small pieces of 5-10 mm length and 2-3 mm thickness, surface sterilized with 1 per cent sodium hypochlorite for 2 minutes and then rinsed thrice in sterile distilled water. The Petri dishes containing infected tissue were incubated in dark at 26 ± 2°C for 6 days and purified the pathogen was identified as *Macrophomina phaseolina.*

Singh *et al*. (2012) isolated *M. phaseolina* the causal organism of the charcoal rot of maize. Isolations were made by plating surface sterilized (4% sodium hypochlorite) pieces of infected tissues. Temperature for the growth of the fungus was maintained at 27 ± 2°C, in Biological Oxygen Demand (BOD) incubator for seven days

on Potato dextrose agar (PDA) medium. Purification of cultures was made by single spore / sclerotia method.

Iqbal and Mukhtar (2014) collected 65 isolates of *Macrophomina phaseolina* from 14 major mungbean producing districts, located in six different agroecological zones of Pakistan.

Ashraf *et al*. (2015) reported that stem samples bearing microsclerotia of the fungus and characteristic symptoms of charcoal rot were collected from the farmers' fields. Isolated *Macrophomina phaseolina* on Rose Bengal Medium.

Srinivas *et al*. (2016) isolated the pathogen *M. phaseolina* from the basal portion of the charcoal rot infected sorghum stem collected from the fields of National Research Centre for Sorghum (NRCS) Rajendranagar, Hyderabad. Small bits of sclerotia bearing strands were surface sterilized by immersing in 0.1 per cent mercury chloride for two minutes. The surface sterilized strands washed in three changes of sterile distilled water. They were planted on PDA under aseptic conditions and incubated at 25^0C in SEW, BOD incubator.

Emayavarman *et al.* (2019) collected the maize plants with charcoal rot disease from the field of Department of Millets, TNAU, Coimbatore and isolated the pathogen *Macrophomina phaseolina* from the basal stem portion of the charcoal rot infected maize plants and prove its pathogenicity using sand maize medium.

2.5 Morphological Characters of Pathogen

Pearson *et al*. (1987) reported that *M. phaseolina* typically showed dense growth on media. Maize isolate of the pathogen, *Macrophomina phaseolina* are sterile and do not form conidia. Sclerotia are generally smooth to irregular, black and 0.05-0.22 mm.

Shekhar *et al.* (2006) conducted studies on morphological characters of *M. phaseolina* incitant of the charcoal rot of maize in India and based on colony colour assigned, the cultures to four groups i.e. grayish white (Bangalore and Arabhavi); blackish gray (Udaipur and Hyderabad); dark black in center; periphery creamish (Ludhiana, Delhi); cottony white color (Coimbatore). Isolates were also assigned four groups, on the basis of mycelial growth and colony texture.

Kumar and Gaur (2010) studied sclerotial size of 14 *Macrophomina* isolates and reported that sclerotia were oval, spherical in shape, black to dark brown in colour, size ranging between 62.4 - 76.8 µm. Sclerotia were produced in all the cultures of *Macrophomina phaseolina*.

Mukerji and Manoharachary (2010) reported that colonies of *Macrophomina phaseolina* on the oat agar were variable but generally grayish brown to black, floccose with abundant aerial mycelium and sclerotia nestling amongst the hyphae or immersed in the agar. The mycelium is inter- and intra-cellular. Sclerotia abundant, dark brown to black in colour smooth, up to 1 mm in diameter (in culture 50-300 µm). Pycnidia sometimes develops in culture subjected to a regime of 12 h near UV-radiation and 12 h darkness and alternating cycle of 20-24^{0}C. It is dark brown to black, sub globose to lageniform, up to 300 µm in diameter, ostilate. Conidiophores simple, septate or branched.Conidia hyaline, obvoid, base truncate but eventually becoming rounded, apex rounded. 16-24 × 5-9 µm, surrounded by a thin outer sheath.

Almomoni *et al.* (2013) studied the morphological characteristics of the isolates on synthetic cultural media. There was a significant variation of their mycelial linear growth rate on PDA medium. Morphological appearance of the microsclerotia distribution on PDA medium was found in three forms: scattered irregular, scattered round and uniform.

Nagamma *et al*. (2015) studied the morphological variability of the pathogen and concluded that the mycelium was pale white in colour in the initial stages of the growth but later turned to dark brown to black as and when sclerotial formation started. Mycelia showed right angled branching. The sclerotia varied in shape and size with growth pattern of scattered to cluster.

Mahadevkumar and Janardhana (2016) isolated the fungus from charcoal rot infected maize samples and observed that the fungal colonies formed many dark sclerotia (65- 152 × 35-98 µm) after 10-12 days of incubation. Pycnidia that developed on necrotic tissues measured 182-210 µm and contained single-celled conidia (15-19 × 5-7 µm). Based on these traits, the fungal pathogen was identified as *Macrophomina phaseolina.*

Gavali *et al.* (2017) determined the morphology of 11 isolates of *Macrophomina phaseolina* incitant of charcoal rot of *Sorghum bicolor* L. obtained from different agro-ecological areas of Solapur districts in Maharashtra. On the basis of colony colour, isolates were divided into four groups i.e. blackish grey, grey, blackish in center periphery creamish and grayish white.

Hooda *et al.* (2018) stated that *Macrophomina phaseolina* causal agent of charcoal rot of maize showed black and homogenous growth on agar. Mycelium dark grey-green, size varies from 2.5-7.5 µm. Sclerotia 60-120 µm in diameter. Microsclerotia are black and homogenous in size. Pycnidium black and globose and ostiolate apically. Size ranges from 130-230 µm in diameter while ostiole size found 13-23 x 3-6 µm in diameter. Conidiophores were hyaline, simple, cylindrical, narrowing epically and in size 13-23 x 3-6 µm. Conidia found hyaline, cylindrical, 1-celled and in size 14-35 x 6-11.5 µm.

2.6 Cultural Characteristics of the Pathogen

Knox-Devis (1966) reported that a number of isolates of *Macrophomina phaseoli* (Maubl.) Ashby sporulated on filter paper treated with peanut meal-ether extract on a basal agar medium containing $MgSO_4.7H_2O$, KH_2PO_4, glucose and peptone. High glucose concentrations favored the formation of sclerotia, high peptone concentrations favored pycnidium production. Sporulation also occurred when DL-asparagine replaced peptone in the medium.

Suriachandraselven and Seetharaman (2003) tested five culture media for mycelial growth and sclerotial production of 25 geographical isolates of *Macrophomina phaseolina* causing charcoal rot of sunflower. Potato dextrose agar supported the best growth and sclerotial production of the isolates of the pathogen requiring the lowest period for the latter.

Priya Santosh (2006) studied the cultural characters of the *Macrophomina phaseolina* on ten different synthetic, semi synthetic and natural media and reported that Potato dextrose agar, Oat meal agar and Czapek's dox agar medium showed maximum colony diameter (90 mm) of the test fungus. Asthana and Hawker's and Martin's Rose Bengal showed good growth and were at par followed by Richard's and Malt extract

agar. Coon's medium showed moderate growth (60.6 mm), while the least growth (21.6 mm) was recorded in host leaf extract medium.

Csöndes (2012) investigated the colony morphology of fifty *Macrophomina phaseolina* isolates. The fungus exhibited most intensive growth on Maize-flour agar, followed by the Sabouraud-glucose, Malt extract, Potato dextrose, Czapek-dox and Watery agar media.

Suryawanshi *et al*. (2014) reported that ten different culture media encouraged better growth and varied population of microsclerotia of *M. phaseolina*. The mean colony diameter / mycelial growth recorded with the test media ranged from 60.50 mm (Czapek's dox agar) to 88.90 mm (Potato dextrose agar). However, significantly highest mean mycelial growth was recorded on Potato dextrose agar (88.90 mm).

Sayyad *et al.* (2015) observed the excellent sclerotial production in PDA and host leaf extract agar, fair sclerotial production was observed in Czpek's dox agar and Richards's agar, good sclerotial production was observed in Asthana hawker's media and among all poor sclerotial production was observed in oat meal agar medium.

Parmar *et al.* (2018) evaluated the effects of different solid and liquid media for their effect on mycelial growth and sclerotial formation of test fungus. Among the solid media, potato dextrose agar (90.00 mm) and Richard's agar (87.71 cm) were the best for fungus growth as well as for sclerotial formation followed by Elliott's medium (80.08 cm). Whereas in liquid media, Richard's agar (862.00 mg) was found excellent followed by potato dextrose agar (824.33 mg) for mycelial growth and sclerotial formation of the test fungus.

Nagamma *et al*. (2015) tested eight media and observed that there was a significant variation in radial growth of *M. phaseolina* among eight media. Significantly maximum radial growth was recorded on PDA and Czapek's agar with mean colony diameter of 90 mm and these two media at par with each other compared to other media tested.

Tetali and Karpagavalli (2016) reported that mean radial mycelial growth of *M. phaseolina* on different solid media ranged between 41.50 mm and 86.43 mm. Among the seven solid media, Potato dextrose agar medium was significantly superior and recorded the highest mycelial growth of 86.43 mm and it was followed by Maize

meal agar (78.36 mm), Czapek's agar (71.66 mm), Richard's agar (67.66 mm), Carrot dextrose agar (51.50 mm), Oat meal agar (47.20 mm) and Rose Bengal agar (41.50 mm).

Gaikwad and Rajurkar (2018) studied cultural variations of *Macrophomina phaseolina* by using different six solid media and five liquid media. The maximum radial growth was observed on Potato dextrose agar (8.1 cm) followed by Malt extract agar (7.4 cm), Czapek dox agar (6.6 cm), Nutrient agar (5.1 cm), Corn meal agar (4.9 cm) and least growth was observed on Glucose nitrate agar (4.1 cm). There were three different types of colony texture of *Macrophomina phaseolina*; fluffy cottony growth was seen on Czapek dox agar, Potato dextrose agar and Malt extract agar media. On Glucose nitrate agar a slightly flat cottony growth was recorded and a flat cottony growth on Corn meal agar, however sparse cottony growth was obtained on Nutrient agar. Among all media, maximum sclerotia formation was observed on Potato dextrose agar medium.

Satpathi and Gohel (2018) concluded that out of three media tested, potato dextrose agar was found an excellent media for growth and sclerotial formation of isolates of *M. phaseolina*.

2.7 Management of Disease

2.7.1 Efficacy of Fungicide

Devi and Singh (1997) assessed six fungicides *viz*., Carbendazim (50% Bavistin), Thiophanate-methyl (Topsin-M), Mancozeb (Dithane M-45), Thiram (75% Thiram), Copper oxychloride (Blitox 50 WP) and Chlorothalonil (Kavach) against three representative at 0.1, 0.15 and 0.2% concentration in agar medium. These isolates showed great variation in their sensitivity in mycelial growth to different fungicides. Carbendazim and Thiophanate-methyl treatments completely inhibited the growth of all three isolates.

Malathi and Doraisamy (2003) tested efficacy of fungicides, *viz*. Captan, Thiram, Carbendazim under *in vitro* condition against *M. Phaseolina* and found that fungicides Carbendazim and Thiram inhibited *M. phaseolina* completely.

Jha and Sharma (2006) investigated fungicides *in vitro* at three concentrations (10, 50 and 100 ppm) against isolates of *R. bataticola* [*Macrophomina phaseolina*] by employing poisoned food technique to determine their effect

on growth and sclerotial morphology. Bavistin was the most effective at all the concentrations, while Thiram controlled the growth at 50 and 100 ppm concentrations. Dithane M-45 and Captan were less effective at 10 and 50 ppm but caused considerable reduction in growth and sclerotial size at 100 ppm.

Verma and Ram (2006) tested different fungicides *in vitro*, Bavistin and Cabendazim + Mancozeb against the paddy sheath blight pathogen *Rhizoctonia solani* and found that, both the fungicides Carbendazim and Carbendazim + Mancozeb inhibited 100 % mycelial growth and sclerotial development at all (0.01, 0.02 and 0.03% conc.).

Zote *et al.* (2006) evaluated different fungicides *in vitro* against *M. phaseolina* and reported that all the fungicides tested under laboratory condition significantly inhibited mycelial growth of the test pathogen and percent inhibition ranged from 71.90 to 94.18. However, fungicides carbendazim (@ 0.05 and 0.1%), Mancozeb (@ 0.2%) and Thiram (0.3%) were reported most effective and significantly inhibited mycelial growth of the test pathogen.

Mondhe *et al*. (2008) evaluated two fungicides (Mancozeb and Zineb) to determine their activities against green gram leaf blight (*Macrophomina phaseolina*). Mancozeb at 0.25% showed the highest mycelial growth inhibition (98.16%) followed by Zineb at 0.25% .

Surywanshi *et al.* (2008) tested fungicides *in vitro* against *Macrophomina phaseolina* and reported that all the tested fungicides significantly inhibited mycelial growth of the pathogen and per cent inhibition was ranged from 71.90 to 94.18. However, fungicides Carbendazim (0.05 and 0.1%), Mancozeb (0.2%) and Thiram (0.3%) were found most effective and at par with each other.

Devlash *et al.* (2011) reported Bavistin as a highly effective seed dressing fungicide with 48.7 per cent disease control and highest yield of 64.7 q/ha over control in maize.

Khalikar *et al.* (2011) tested seven fungicides against *Macrophomina phaseolina in vitro*. The highest inhibition (100%) of test pathogenwas observed due to Carbendazim (500 ppm), Chlorothalonil (500 ppm), Hexaconazole (500 ppm) and Captan (2500 ppm) followed by Mancozeb (2500 ppm) (94.39%) and Benomyl (1000 ppm) 93.4% and rest of the treatments significantly inhibited colony growth over control.

Gowdra *et al.* (2012) tested different fungicides *in vitro* against *Macrophomina phaseolina* which causes dry root rot in chickpea. Among the systemic fungicides, complete inhibition was recorded in Hexaconazole, Carbendazim, Difenoconazole and Propiconazole at 50 ppm. while, in Thiophanate methyl at 500 ppm. In contact fungicides and combi-products cent per cent inhibition was observed in Mancozeb, Carboxin 37.5 per cent + Thiram 37.5 per cent and Carbendazim 12 per cent + Mancozeb 63 per cent WP at 250 ppm.

Thori *et al.* (2012) evaluated five fungicides *in vitro* at different concentrations against post flowering stalk rot (PFSR) of maize caused by *Fusarium moniliforme.* Systemic fungicides, Bavistin and Tebuconazole were found most effective in inhibiting 100% mycelial growth of at 250 ppm.

Parmar *et al*. (2017) evaluated different systemic and non-systemic fungicides at three different concentrations *in vitro* against castor root rot pathogen *Macrophomina phaseolina*. Among the different contact (non-systemic) fungicides evaluated, maximum mean mycelial growth inhibition was observed in Propineb and Mancozeb (99.97%) followed by Captan (85.63%). Out of six systemic fungicides tested, Carbendazim was found best with 95.23 per cent mycelial growth inhibition. Carbendazim showed maximum inhibition of mycelial growth (99.97%) of the test fungus at 250 ppm and 500 ppm concentration.

Chaudhary *et al.* (2017) tested ten fungicides *in vitro* against the pathogen *Macrophomina phaseolina*. The highest inhibition (100%) of the test pathogen was observed due to Carbendazim 50% WP at different concentration (250, 500, 1000 ppm), Mancozeb 75% WP (1500, 2000, 2500 ppm), ridomil-MZ 72% WP (1000, 1500, 2000 ppm) and Carbendazim 12% + Mancozeb 63% (1500, 2000, 2500 ppm).

Thombare and Kohire (2018) tested eight systemic and twelve non systemic and contact fungicides *in vitro* against *Macrophomina Phaseolina*. All the fungicides tested caused significant inhibition at all six concentrations tested over untreated control. Highest average mycelial inhibition was recorded with the fungicide Carbendazim + Mancozeb (100%). This was followed by Tebuconazole + Trifoxysrobin (95.35%), Metalaxyl + Mancozeb (93.14%), Cymoxanil + Mancozeb (91.75%) and fungicides Copper oxychloride, Propineb and Copper hydroxide were found

comparatively less effective with maximum mycelial inhibition of 12.05, 17.12 and 23.03 per cent, respectively.

Bashir (2018) evaluated six fungicides i.e. Carbendazim, Tebuconazole, Propiconazole, Hexaconazole, Mancozeb and Cheshunt with five concentrations of 50, 100, 250, 500 and 1000 ppm against *Macrophomina phasiolina* by poison food technique and diminished that Carbendazim, Tebuconazole and Propiconazole completely inhibited the mycelial growth of the pathogen even at 50 ppm as compared to all other concentrations.

Khan and Sahi (2020) reported that Bavistin was the most effective fungicide against the mycelial growth, sclerotial production and germination of *M. phaseolina*, Calixin and Rubigon reduced mycelial growth significantly but decrease in sclerotial production and germination was lowered as compared to Panoram.

2.7.2 Bio efficacy of Bioagents

Elad *et al.* (1986) tested four isolates of *T. harzianum* and reported that the fungus inhibited linear growth and microsclerotia production in *Macrophomina phaseolina*. The antagonist proliferated in dual liquid culture with *M. phaseolina* and significantly decreased the number of its viable propagules.

Sankar and Sharma (2001) tested species of *Trichoderma* isolated from rhizosphere of healthy maize plants against charcoal rot of maize pathogen by using dual plate technique and reported that all the nine isolates of *T. viride* were found to produce inhibitory volatile substances *in vitro* as well as all the isolates were found to reduce the radial growth of the pathogen over control.

Pal *et al.* (2001) reported that the fluorescent *Pseudomonas* sp. EM85 strongly inhibited the fungi, *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum*, both on PDA and NA.

Indra and Subbiah (2003) evaluated bio agents against *Macrophomina phaseolina* (Tassi.) Goid. infecting black gram under glass house and field conditions. The antagonistic organisms used were *Trichoderma viride* and *T. harzianum* in biomass and in different carriers like talc and gypsum and along with *Rhizobium*. The incidence of root rot in black gram was significantly reduced by 50% when treated with *Trichoderma*

spp. alone or in combination with biofertilizer both under glass house and field conditions.

Ramezani (2008) tested the efficacy of four fungal bio agents *viz*., *Trichoderma hamatum, T. harzianum, T. polysporum* and *T. viride* under *in vitro* condition against the eggplant root - rot pathogen, *Macrophomina phaseolina*. Among the bio agents, *T. harzianum* produced the maximum inhibition zone of 18.20 per cent compared to the minimum of 7.30 per cent by *T. hamatum.*

Singh *et al.* (2008) studied biological control of root rot fungus *M. phaseolina* and reported that *B. subtilis* BN1 produced lytic enzymes, chitinase and β-1, 3-glucanase, which are known to cause hyphal degradation and digestion of the cell wall component of *M. phaseolina.* Positive root colonization capacity of *B. subtilis* BN1 proved it as a potent bio control agent.

Kulkarni and Anahosur (2011) conducted an experiment for integrated management of charcoal rot of maize and reported that the plant stand of maize was maximum (97.33%) with treatment of *T. harzianum* + FYM and FYM + Neem cake + *T. harzianum* + *T. viride.* They also reported that the pre-sowing application of FYM, Neem cake and *T. harzianum* was most effective in avoiding the infection and it reduces the stalk rot at later stage.

Gowdra *et al.* (2012) tested bio agents against *M. phaseolina* and reported that maximum inhibition was recorded in *Trichoderma harzianum* (Th-55) (81.48%) followed by *Bacillus subtilis* (75.85) and *T. viride* (TV-27) (74.07) compared to control.

Arora and Dhurwe (2013) evaluated *Trichoderma spp.* to determine suitable integrated control measures and reported that *Trichoderma viride* as potential antagonist against *Macrophomina phaseolina. In vivo* data also revealed that the introduction of *T. viride* reduced the disease incidence to an appropriate level.

Karthikeyan *et al.* (2015) tested bio agents efficacy against *M. phaseolina* and reported that the maximum reduction of the root rot incidence (59.54%) was recorded in *Trichoderma viride.* It was followed by *Trichoderma harzianum* (49.71%) and *Trichoderma hamatum* (32.13%) percent reduction over control.

Meena and Pandey (2015) tested total four bio agents *viz*., *T. virens, T. harzianum, T virens* and *Pseudomonas fluorescens* for their antagonistic property against

M. phaseolina by dual culture method. All the biological agents were significantly superior over control in checking the growth of *M. phaseolina*. All the antagonists suppressed the formation of sclerotia. Among all the biological agents *T. viride* was significantly superior bio control agent in inhibiting the growth of the pathogens.

Deshmukh *et al*. (2016) revealed that the use of bio agents provided significant inhibition of *M. phaseolina*. *Trichoderma harzianum* (80.5%) was found most promising in inhibiting the growth of *M. phaseolina* at 7 days after inoculation followed by *P. fluorescens.*

2.7.3 Evaluation of Fungicides and Bioagents in Pot culture

Elad *et al.* (1986) evaluated four isolates of *T. harzianum* in the field against *M. phaseolina* and reported that *T. harzianum* reduced charcoal root rot of melons and corn by 22 and 28 per cent, respectively.

Singh and Kaiser (1994) evaluated different fungicides against charcoal rot pathogen of maize and reported that among systemic fungicides, seed treatment with Carbendazim and Tops in were reported to reduce the disease significantly over other fungicides.

Murthy *et al.* (2003) tested efficacy of fungicides and bioagent as seed treatment against *Macrophomina phaseolina* in Green gram and found that Carbendazim (0.2 %) and *T. harzianum* was most effective in controlling the pathogen.

Tandel *et al*. (2010) tested *in vivo* efficacy of seven fungicides against Green gram leaf blight caused by *Macrophomina phaseolina* and reported that Carbendazim + Mancozeb was found significantly superior (8.13%) to reduce disease intensity. The next best treatment was Carbendazim (11.06%) followed by Mancozeb (19.56%). The maximum disease control (84.16%) was recorded in the plot where Carbendazim + Mancozeb was sprayed followed by Carbendazim (78.45%), Mancozeb (61.89%), Thiophanate methyl (48.81%), Chlorothalonil (34.12%), Propiconazole (29.80%) and Propineb (19.05%).

Arora and Dhurwe (2013) evaluated bioagents against *Macrophomina phaseolina* in pot culture and reported that the introduction of *Trichoderma viride* reduced the disease to a appreciate level in comparison and it checked with *T. viride*. The per cent of disease control was 70 per cent.

Karthikeyan *et al.* (2015) tested *in vivo* efficacy of *Trichoderma spp* against the charcoal rot of Mung caused by *Macrophomina phaseolina* and reported that seed treatment of *Trichoderma viride* recorded the maximum root rot incidences (21.4%) followed by *Trichoderma harzianum* (26.6%). The maximum seed germination (75%) shoot length (43.2 cm) and root length (16.0 cm) was recorded in the same seed treatment of *Trichoderma viride* followed by *Trichoderma harzianum*.

2.8 Screening of Genotypes against *M. phaseolina*

Anwar and Nashir (1994) screened maize cultivars against *M. phaseolina* and reported that among six commercially grown cultivars, Akbar was highly susceptible, Sultan was moderately resistant and remain were susceptible.

Shekhar *et al.* (2010) reported that under systematic breeding program on resistance to post flowering stalk rot, germplasm screening was carried out at four 'hot spot' locations in India for different diseases: Hyderabad (*Cephalosporium maydis*), Udaipur (*Fusarium moniliforme*), Ludhiana and Delhi (*Macrophomina phaseolina*). Across the locations, promising maize genotypes were artificially inoculated using the toothpick method, year after year and resistant plants were selfed to derive resistant inbred.

Iqbal *et al*. (2010) screened the mash bean genotypes and recorded the data by using 1-9 disease rating scale. Where, 1 = No symptoms on plants (highly resistant); 3 = Lesions are limited to cotyledonary tissues (resistant); 5 = Lesions have progressed from cotyledons to about 2 cm of stem tissues (tolerant); 7 = Lesions are extensive on stem and branches (susceptible) and 9 = Most of the stem and growing points are infected.

Kaur *et al.* (2010) screened twenty maize inbred lines against (*Drechslera maydis*) charcoal rot (*Macrophomina phaseolina*) under artificial epiphytotic conditions during monsoon 2005 and 2006. The severity of charcoal rot was recorded following 1-9 scale and the genotypes screened were grouped into three categories *viz*. resistant, moderately resistant and susceptible based on their disease reaction.

Anis *et al.* (2011) screened six sunflower varieties against *Macrophomina phaseolina* (Tassi) Goid. All six varieties showed significantly improved plant growth and vigor in non-inoculated autoclaved soil whereas all varieties showed incidence of root rot by *M. phaseolina* in naturally infested soil.

Subedi *et al.* (2016) screened 30 genotypes of maize against charcoal rot (*Macrophomina phaseolina*) that appeared in the post-flowering phase.

Coser *et al.* (2017) conducted field as well as greenhouse screening of 465 soybean genotypes by using the growing margin of a four-day old culture of *M. phaseolina* on PDA, a mycelial plug was obtained using a 200 µL pipette tip. The pipette tip with mycelial plug was immediately placed over the stem cut by the razor blade and ensuring the agar was in embedded in the stem. Ratings were based on the recorded measurements of lesion length.

Kumar *et al.* (2017) screened maize genotypes for the first time with multi-environments screening of 137 inbred at three and 48 maize hybrids at six environments under artificially created epiphytotic at hot-spot locations to identify stable sources of charcoal rot resistance in Indian maize germplasm. Additive Main Effects and Multiplicative Interactions (AMMI) analysis could identify, DQL1020, DML339, DML1, DQL1019, CM117-1-1 in inbred and A-7501, CMH08-287, CMH08-292, BIO-562, and CMH08-350 in hybrids as stable sources of charcoal rot resistance.

Hooda *et al.* (2018) reported that germplasm screening in sick plot is most accepted approach for disease screening against soil borne diseases irrespective of any crop and therefore, screening for resistance against charcoal rot can easily be done in sick plots (SP). However, artificial inoculation is necessary where sick plots are not available. For this purpose, the fungal material should be isolated from the infected stalks, cultured and multiplied in the laboratory. Among various methods of field inoculation, the toothpick inoculation is followed for this disease.

Khaire *et al*. (2018) screened 35 germplasm lines of Mung bean against *Macrophomina* blight along with JL-781 as susceptible check in sick plot. Twenty nine cultivars found resistant, two moderately resistant, one cultivar moderately susceptible, one susceptible while JL-781 was found susceptible.

Shoaib *et al.* (2019) assessed the resistance in maize (*Zea mays* L.) cultivars against *Macrophomina phaseolina* which causes charcoal rot in variety of plants. Maize cultivars were sown in artificially inoculated potting soil and the pot experiment was laid in a completely randomized designed for 60 days.

3. MATERIALS AND METHODS

During the present investigations on charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. of maize (*Zea mays* L.) various experiments were conducted at the Department of Plant Pathology, Post Graduate Institute, and AICRP on Maize, Mahatma Phule Krishi Vidyapeeth, Rahuri during 2018-2020 to fulfill the objectives defined. The details of the materials used, and methods followed for various experiments are described herein the following paragraphs.

3.1 Materials

3.1.1 Experimental site

All the experiment (plate culture and pot culture) was conducted at the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, and AICRP on Maize, MPKV, Rahuri.

3.1.2 Disease samples

Maize plants exhibiting typical symptoms of charcoal rot (*Macrophomina phaseolina*) disease were collected in the paper bags from the local farmers' maize fields and resorted to tissue isolation on Potato dextrose agar medium.

3.1.3 Culture media

Potato Dextrose Agar (PDA) was used as basal culture medium for isolation, purification and maintenance of the pure culture of *M. phaseolina* and biocontrol agents. Sand: sorghum (3:1) medium was used for mass multiplication of the pathogen. For studying cultural characteristics of *M. phaseolina,* synthetic readymade (make: Hi media) and non-synthetic (prepared) media were used.

3.1.4 Seeds

Seeds of maize varieties, cultivars, hybrids and germplasm lines were obtained from the AICRP on Maize, MPKV, Rahuri and were used for various pot culture experiments.

3.1.5 Chemicals

Standard chemicals, reagents, fungicides, culture media etc. required for the experimentation were obtained from the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri.

3.1.6 Glass ware

The common glass-wares (Borosil, J-sil and Corning make) *viz.,* Petri dishes, test tubes, conical flasks, volumetric flasks, measuring cylinder, glass rods, beakers, funnel, pipette, etc. were obtained from the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri.

3.1.7 Equipment's

The laboratory equipment's *viz.,* autoclave, hot air oven, laminar airflow cabinet, BOD Incubator, refrigerator, binocular research microscope, electronic balance, pH meter etc. available at the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri were utilized as and when required.

3.1.8 Miscellaneous

Earthen pots (30 cm. dia.), plant protection appliances, inoculation needle, corkborer, forceps, knife, blotter paper, paper bags, polythene bags, spirit lamp, mercuric chloride, labels, scales, sand, soil, FYM, screen house etc. were used during the course of present investigations.

3.1.9 Fungicides

The following 9 fungicides (systemic and non-systemic) were used during the present studies.

Table 3.1 List of fungicides used against *M. phaseolina*

Sr. No.	Common Name	Trade Name	Active Ingredients	Manufacturer
1.	Carbendazim	Bavistin	50 WP	CRYSTAL
2.	Thiram	Thiram	75 WP	Amba chem Industries
3.	Thiophanate Methyl	ROKO	75 WP	BIOSTAD
4.	Mancozeb	M-45	70 WP	INDOFIL
5.	Copper Oxychloride	Blitox	50 WP	RALLIS
6.	Captan	Captaf	50 WP	RALLIS
7.	Propineb	Antracol	70 WP	BAYER
8.	Copper hydroxide	Kocide	77 WP	DUPONT
9.	Carbendazim 12 % + Mancozeb 63 %	SAAF	75 WP	UPL

3.1.10 Biocontrol agents

Pure cultures and talc based formulation of biocontrol agents *viz., Trichoderma viride, T. harzianum, T. hamatum, T. koningii, Bacillus subtilis* and *Pseudomonas fluorescens* were obtained from the Biofertilizer Production Unit, Post Graduate Institute, MPKV, Rahuri multiplied and maintained on appropriate culture media and used for further studies.

3.2 Methodology

3.2.1 Sterilization of media and glassware's

Glassware was sterilized in hot air oven at 160^0 to 180^0C for two hours. Culture media, soil and distilled water were sterilized in an autoclave at 1.05 kg/cm^2 for 15 minutes. For surface sterilization of plant tissues, 0.1 per cent mercuric chloride ($HgCl_2$) was used and rectified spirit for other material like inoculation needles, forceps, inoculation chamber and hands.

3.2.2 Preparation of culture media

Potato dextrose agar (PDA) was used for isolation and maintenance of fungal isolates. Sliced pieces of peeled potatoes (200 g) were first boiled in 500 ml distilled water till cooked. Potato broth thus obtained was strained through muslin cloth and measured. In 500 ml of water, agar (20 g) and dextrose (20 g) were dissolved and then the potato broth was mixed. Finally, the volume of medium was adjusted to one liter by adding distilled water. The medium was poured in flasks and tubes to one third capacities, plugged with cotton and sterilized in autoclave at 1.05 kg/cm^2 for 15 minutes. To prepare slants, PDA tubes were kept in slanting position till the medium was solidified.

3.2.3 Symptomatology

Visual observations were recorded for manifestation of the symptoms induced by *M. phaseolina* in maize under *in vitro* and *in vivo* conditions, in pathogenicity test and various pot culture experiments. Temporary mounts of the pure culture of *M. phaseolina* and charcoal rot affected maize plant parts were prepared on clean glass slide in lactophenol, covered with cover slip and observed under compound microscope.

3.2.4 Isolation

3.2.4.1 Isolation and Identification of the pathogen

Isolation of pathogen responsible for charcoal rot of maize was done by standard tissue isolation method on PDA media.

Naturally infected maize plants showing typical symptoms of charcoal rot disease were collected from the fields, brought to laboratory, washed thoroughly with distilled water, blot dried and cut with sharp sterilized blade into small bits (5 mm). These bits were then surface sterilized with 0.1 per cent aqueous solution of mercuric chloride ($HgCl_2$) for two minutes, washed by giving three successive changes with sterile distilled water in glass Petri plates to remove traces of mercuric chloride and blot dried. These surface sterilized bits were inoculated aseptically on autoclaved and cooled PDA medium in sterilized glass Petri plates under aseptic conditions of Laminar-air-flow cabinet and incubated in BOD incubator at 27±2°C temperature. Within 3-4 days of incubation, blackish mycelial mat was developed and within next 10-12 days, micro sclerotia were developed in the plates.

Applying hyphal tip isolation technique, the test pathogen was transferred aseptically on the PDA slant in test tubes. Through frequent sub-culturing, the test pathogen was purified, and the pure culture was maintained on PDA slant in test tubes and maintained in refrigerator for further studies.

Identification of the pathogen causing charcoal rot of maize was carried out by studying the symptomatology, cultural and morphological characters, microscopic observations and pathogenicity test. The morphological character *viz*., mycelial colour and micro-sclerotial formation was studied under low power magnification (10X) from 10 to 15 days old culture.

3.2.4.2 Purification and maintenance of culture

Culture of the pathogen obtained as above was purified and maintained on PDA at room temperature by giving subsequent sub-culturing at periodic regular intervals. 7 days old culture was used for present studies.

3.2.4.3 Mass multiplication of *M. phaseolina* inoculum

Sand sorghum medium was used for mass multiplication of *M. phaseolina.* Sand:sorghum medium (1 part partially broken sorghum grains + 3 part sand + distilled

water to moisten the medium) was prepared, filled into the polypropylene bags (9 x 12 cm) and autoclaved at 20 lbs pressure for 30 min, for two consecutive days. After cooling at room temperature, the sterilized sand sorghum medium in bags was inoculated with 8-10 mycelial discs (5 mm dia.) of the test pathogen obtained from a week-old culture and incubated at 27 ± 1^0C for 10 days. This mass multiplied inoculum was added (@ 50 g/kg soil or potting mixture) to the upper 4-5 cm layer, mixed thoroughly, watered adequately and these pots (30 cm dia.) were incubated in the screen house for two weeks to proliferate the test pathogen in pots.

3.2.5.1 Pathogenicity test

Pathogenicity of the test pathogen was carried out by sick soil *(M. phaseolina)* method in plastic pots under screen house condition.

Potting mixture, soil + sand + FYM (2:1:1) was prepared and autoclaved at 30 lbs pressure for 30 minutes for two consecutive days, after cooling at room temperature filled into plastic pots (30 cm dia.) disinfected with 5 per cent Copper sulphate solution. The test pathogen *M. phaseolina* multiplied on sand:sorghum medium was inoculated (@50g/kg potting mixture) in these pots, mixed thoroughly, watered adequately and incubated in screen house for two weeks. Surface sterilized (0.1% $HgCl_2$) healthy seeds of maize genotype G-25 were sown (10 seeds / pot) in these pots containing sick soil (*M. phaseolina*), watered regularly and maintained in the screen house. Plastic pots containing autoclaved soil / potting mixture without any inoculum and sown with surface sterilized healthy seeds (10 seeds / pot) of maize genotype G-25 were maintained as untreated (without inoculum) control. Both (inoculated and uninoculated) pots were watered regularly and maintained in the screen house for further studies.

Observations on incidence of charcoal rot were recorded, respectively at 30^{th}, 45^{th} and 60^{th} days after sowing. The test pathogen was re-isolated aseptically on PDA plates from artificially charcoal rot affected maize seedlings, studied and compared its cultural and morphological characteristics with the original culture of *M. phaseolina* obtained from naturally charcoal rot diseased maize plants. Further, infectivity of the pure culture (*M. phaseolina*) obtained from artificially charcoal rot diseased maize seedling was proved by sick soil method, using susceptible maize cv. G-25.

3.2.5.2 Re-isolation

Reisolation of the organism was made under aseptic condition from the charcoal rot infected stem of maize plants. The culture obtained was compared with original culture and found identical with original culture. The reisolated culture was maintained on PDA slants for further studies.

3.2.6 Effect of different culture media on growth and sclerotial production

A total of eight solid culture media, *viz.,* Corn meal agar, Oat meal agar, Czapek's-dox agar, Richards agar, Malt extract agar, Rose bengal medium, Standard nutrient agar and Potato dextrose agar were used to study their effect on growth and microsclerotia of *M. phaseolina.* The media was sterilized in autoclave at 1.05 kg/cm^2 pressure for 15 min.

Autoclaved and cooled media were poured (@20 ml / plate) in sterilized glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. On solidification of the media, plates of each culture medium (three plates / medium / replication) were inoculated by placing at the center a 5 mm mycelial disc of actively growing 7 days old pure culture of *M. phaseolina,* plates were incubated at 28±2°C for a week.

Experimental details

Design : CRD

Replication : Three

Treatments : Eight (Culture media)

Treatment details

T_1 : Potato Dextrose Agar

T_2 : Corn Meal Agar

T_3 : Oat Meal Agar

T_4 : Czapek's-Dox Agar

T_5 : Richards Agar Medium

T_6 : Malt Extract Agar

T_7 : Rose Bengal Medium

T_8 : Standard Nutrient Agar

The observations on radial mycelial growth /colony diameter (mm), colony color and colony morphology were recorded at week and microsclerotia (stereo binocular microscope) at two weeks of incubation. Sclerotial production was determined on the basis of microscopic observations and rated as below (Das, 1988).

Table 3.2 Sclerotial rating

Sr. No.	Grade	Description	Average No. of Sclerotia / microscopic field
1.	-	No sclerotial formation	-
2.	+	Poor sclerotial formation	10-20
3.	++	Fair sclerotial formation	21-30
4.	+++	Good sclerotial formation	31-50
5.	++++	Excellent sclerotial formation	51 and above

3.2.7 Disease management strategies

3.2.7.1 *In vitro* evaluation of fungicides

Efficacy of nine fungicides was evaluated (at half of concentration than recommended and at recommended concentration) *in vitro* against *M. phaseolina,* by poisoned food technique (Nene and Thapliyal, 1993) using PDA as basal culture medium. Based on active ingredient, the requisite quantity of each test fungicide was calculated and mixed thoroughly with autoclaved and cooled (40^0C) PDA medium separately in conical flasks to obtain desired concentrations of fungicides. Fungicide amended PDA medium was then poured (20 ml/plate) aseptically in Petri plates (90 mm dia.) and allowed to solidify at room temperature.

For each test fungicide and its test concentration, three plates / treatment / replication were maintained and replicated thrice. After solidification of the medium all the plates were inoculated aseptically with a 5 mm culture disc obtained from a week old actively growing pure culture of *M. phaseolina.* The culture disc was placed on PDA in inverted position in the center of the Petri plate and plates were incubated at 28 ± 2°C. Petri plates filled with plain PDA (without any fungicide) and inoculated with the culture disc of the test pathogen were maintained as control (untreated).

Experimental details

Design : CRD

Replication : Three

Treatments : Ten

Treatment details

Treatment	Fungicides	Trade name	Formulation	Recommended Concentration (%)
T_1	Carbendazim	Bavistin	50 WP	0.1
T_2	Thiram	Thiram	75 WP	0.2
T_3	Thiophanate Methyl	ROKO	75 WP	0.1
T_4	Mancozeb	M-45	70 WP	0.25
T_5	Copper oxychloride	Blitox	50 WP	0.3
T_6	Captan	Captaf	50 WP	0.25
T_7	Propineb	Antracol	70 WP	0.25
T_8	Copper hydroxide	Kocide	77 WP	0.25
T_9	Carbendazim 12 % + Mancozeb 63%	SAAF	75 WP	0.2
T_{10}	Control (Untreated)	-	-	-

Observations on radial mycelial growth/colony diameter of the pathogen were recorded at 24 hrs. interval and continued till the untreated control plate was fully covered with mycelial growth of the test pathogen. Per cent mycelial growth inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying the following formula (Vincent, 1927).

$$\text{Per cent Inhibition (I)} = \frac{C - T}{T} \times 100$$

Where,

C = Growth (mm) of test fungus in untreated control plate

T = Growth (mm) of test fungus in treated plates

3.2.7.2 *In vitro* evaluation of bioagents

Four fungal antagonists *viz., Trichoderma viride, T. harzianum, T. hamatum, T koningii* and two bacterial antagonists *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro* against *M. phaseolina,* applying dual culture technique (Dennis and Webster, 1971).

Seven days old culture of the test bioagents and the test pathogen (*M. phaseolina)* grown on agar media were used for the study. The culture discs (5 mm) of the test pathogen and bioagent were cut out with sterilized corkborer from a weak old culture. Then two culture discs, one each of the test pathogen and bioagent were placed aseptically at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates and plates were incubated at 28 ± 2°C. Three plates/ treatment/ replication were maintained. PDA plates inoculated only with culture disc of test pathogen were maintained as untreated control.

Experimental details

Design : CRD

Replication : Three

Treatments : Seven

Treatment details

Tr. No.	Treatment	Tr. No.	Treatment
T_1	*Trichoderma viride*	T_2	*T harzianum*
T_3	*T. hamatum*	T_4	*T. koningii*
T_5	*Bacillus subtilis*	T_6	*Pseudomonas fluorescens*
T_7	Control (untreated)		

Observations on linear mycelial growth of the test pathogen and bioagent were recorded at an interval of 24 hours and continued till untreated control plate was fully covered with mycelial growth of the test pathogen. Per cent inhibition of the test pathogen over untreated control was calculated by applying the following formula (Arora and Upadhyay, 1978).

$$\text{Per cent growth inhibition (I)} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

3.2.7.3 Evaluation of fungicides and bioagents in pot culture

A pot culture experiment on effect of fungicides and biocontrol agents on *M. phaseolina* was conducted under glass house condition by using variety G-25 susceptible to charcoal rot.

The inoculum of the test pathogen *M. phaseolina* was mass cultured on crushed cotton seeds and added to soil @ 100 g per kg of soil. Prior to use, plastic pots were disinfected with 5 per cent Copper sulphate. The *Macrophomina* culture was also added to the sterilized soil for fungicide trial. The seeds of G-25 were sown in the pots containing *Macrophomina* sick soil. In this trial, the seeds were treated with Carbendazim + Mancozeb, Carbendazim, Mancozeb, Thiram (dry seed treatment) and *T. harzianum* and *T. viride* (wet seed treatment).

The treated seed were sown in pots containing *Macrophomina* sick soil. Six seeds were sown in each pot. The pots were watered lightly and kept in glass house. The observations of the rot were recorded at 30, 45, 60 and 90 days after sowing.

Experimental details

Design : CRD

Replication : Three

Treatments : Seven

Treatment details:

T_1: Carbendazim + Mancozeb	T_2: Carbendazim
T_3:Thiram	T_4: Mancozeb
T_5: *Trichoderma harzianum*	T_6: *Trichoderma viride*
T_7: Untreated (Control)	

3.2.8 Screening of maize genotypes

Seed of different maize genotypes were obtained from AICRP on maize, MPKV, Rahuri. Fifty-three maize genotypes were screened against charcoal rot during *Rabi* 2018-19 at AICRP on Maize, MPKV, Rahuri. The susceptible check G-25 was sown after every ten test entries.

Toothpick inoculation was followed for inoculation of maize genotypes. Round bamboo toothpicks about 6.5 cm long were boiled three times (about 1 hour each time) in tap water to remove toxic substances. After each boiling these toothpicks were thoroughly washed in fresh water and dried in the sun. After thorough drying, were loosely packed in bundles and put into the autoclavable jars and enough Potato dextrose broth (one- third length of toothpicks) was added to thoroughly moisten the toothpicks

plus some quantity in the bottom of the jars. The jars with the toothpicks were autoclaved immediately after the broth added. Later the sterilized toothpicks were inoculated with the culture of the pathogen aseptically. The growth of the fungus covered the toothpicks and inoculum is ready for use after 10 days of inoculations. The inoculation was made just after flowering stage of plants (45-50 days old). For inoculating plants, the lower internode (second / third) above soil level is opened with a jabber and the toothpick is inserted into the hole. The jabber is made by driving a nail of the diameter of the toothpick into a wooden handle. The head of the nail is ground off to a point and to the desired length (2cm). The round toothpicks effectively seal the hole in the stalk and prevent drying. Symptoms may appear in inoculated plants 15-20 days after inoculation.

The observations were recorded based on the proportion of disease present in the inoculated internodes and its subsequent spread. For scoring disease severity of charcoal rot, 1-9 rating scale (AICMIP, 1983) was used and grouped into respective categories as follows.

Table 3.2. Rating scale for screening of maize genotypes against charcoal rot disease

Rating Scale	PDI	Reaction
1	≤11.11	Resistant (R) (Score : ≤ 3.0) (PDI : ≤ 33.33)
2	22.22	
3	33.33	
4	44.44	Moderately Resistant (MR) (Score :3.1-5.0) (PDI : 33.34-55.55)
5	55.55	
6	66.66	Moderately Susceptible (S) (Score : 5.1-7.0) (PDI : 55.56-77.77)
7	77.77	
8	88.88	Susceptible (S) (Score : ≥ 7.0) (PDI : 77.77)
9	99.99	

4. RESULTS AND DISCUSSION

Present studies on charcoal rot of maize caused by *Macrophomina phaseolina* (Tassi.) Goid. of maize (*Zea mays* L.) was undertaken during 2018-20 at Department of Plant Pathology, Post Graduate institute, MPKV, Rahuri on the aspects *viz*., isolation and identification, pathogenicity test, effect of different culture media on growth, *in vitro* and in pot culture evaluation fungicides and bio-agents and screening of genotypes. The results obtained are being narrated and discussed under following paragraphs.

4.1 Collection of Samples

Charcoal rot infected samples were collected from AICRP on Maize, MPKV, Rahuri and farmers field and brought to laboratory for isolation (Plate I).

Ashraf *et al*. (2015) reported that samples of stems bearing microsclerotia of the fungus and characteristic symptoms of charcoal rot were collected from the farmers' fields.

4.2 Isolation of the Pathogen

Applying tissue isolation technique, the test pathogen was isolated aseptically from the naturally charcoal rot affected maize stem basal portion on Potato dextrose agar (PDA) medium. After 2-3 days of incubation, black mycelial mat was developed on the PDA plates (Plate II) and after 7-8 days of incubation microsclerotia were developed in the plates. Test pathogen was aseptically sub-cultured purified and maintained on agar slant tube (Plate II) in refrigerator for further studies.

The present results are also consonance with results of Srinivas *et al*. (2016) and Emayavarman *et al.* (2019), who isolated the pathogen from charcoal rot infected samples.

4.3 Identification of the Pathogen

Test pathogen was identified on the basis of symptomatology, morphological characteristics (Plate III) and pathogenicity test and it was confirmed that *Macrophomina phaseolina* (Tassi.) Goid. is the cause of charcoal rot of maize.

4.3.1 Colony Characters

The pathogen isolated in pure form was subculture in Petri plates on PDA and incubated at 28 ± 2ºC and observed daily for appearance and growth of the mycelium. Aerial mycelium of *M. phaseolina* showed variabiliy on the PDA. Colonies of the tested isolate was dense to light dense and the colour was mostly grayish to black, floccose with abundant aerial mycelium.

Similarly, Dhingra and Sinclair (1978) studied colony colour of *Macrophomina* varies in culture from gray black and becomes dark in colour with abundant aerial mycelium.

4.3.2 Symptomatology

Under field conditions, typical symptoms of charcoal rot disease were first noticed when maize was in the tassel stage and later. Upper leaves of the maize were dry out. Infected maize plants had shredded stalks with completely rotted pith, leaving only stringy vascular strands intact. The fungus produced small, black and spherical bodies inside the vascular strands (microsclerotia), numerous enough to give the internal stalk tissue a grey coloring. Translocation of water and nutrients disrupted due to hyphae of the fungi growing intercellularly through the xylem and into the surrounding vascular tissue which resulted in to lodging of crop.

The fungus grown into the lower internode of the stalk as the plant matures, causing plants to dry prematurely and weaken their stalks, causing breakage and lodging. Under severe condition the whole field showed the burning appearance.

These findings were similar to earlier workers, (Shekhar *et al*., 2006; Kumar *et al.,* 2013, and Hooda *et al.* 2018).

It was reported that at maturity, the lower stem internodes show a typical charcoal, grey-black discoloration. Stem is cut open numerous minute black specks (microsclerotia) are visible. Lower internode of the stalk as the plant matures, causing plants to ripen prematurely and weaken their stalks, causing breakage and lodging.

4.4 Pathogenicity test

Pathogenicity of *M. phaseolina* was proved by soil inoculation (sick soil) with pure culture of *M. phaseolina* (Plate IV (A and B) on charcoal rot susceptible maize cv. G 25, in pot under screen house conditions.

Results revealed that in the pots containing sick soil, infection and symptoms were recorded. After flowering (55 Days after sowing), the leaves of inoculated plants initially started drying, the internode near the collar regions became blakish in colour. The drying of inoculated plant was observed. When the diseased plant spit open, the shredded pith with abundant minute blackish sclerotia was observed (Plate IV). From these, artificially diseased maize basal portion, the pathogen was re-isolated and incubated at 28 ± 2^0C. After 2-3 days of incubation, dark colored mycelial mat developed and about 7-8 days of incubation black colored microsclerotia were developed. Morphological and cultural characteristics were similar to that of the original test pathogens culture obtained from naturally diseased maize samples.

Thus, applying Koch's postulates pathogenicity of *M. phaseolina*, the incident of charcoal rot of maize was proved.

Results of the present study on pathogenicity of *M. phaseolina* are in confirmity with Emayavarman *et al.* (2019).

4.5 Morphological Characteristics

The pure culture of the fungus obtained in petri plates after incubation at 28 ± 2 ºC was examined under the microscope for morphological characteristics *viz*., size, shape and septation.

The microscopic observation made by the slides prepared directly from active cultures. Observed under low (10X) and higher (40X) power magnification the fungus revealed that mycelium was hyaline, slender, branched and septate, initially hyaline turning to a black colour. The vegetative mycelium formed monilid or barrel-shaped cells and the formation of septum near the branching of the mycelium.

In seven days old cultures, the pathogen produced numerous black sclerotia on diseased plant parts, which are smooth to irregular and black. Microsclerotia ranged in size from 55 to 190 µm long by 45 to 120 µm wide (average 105 × 74 µm) (Plate III).

The present results are in consonance with the earlier reports given by (Malcom, 1980; Pearson *et al*., 1987; Kumar and Gaur, 2010; Mukerji and Manoharachary, 2010).

4.6 Cultural Characteristics

Cultural characteristics *viz*., mycelial growth, colony characteristics and sporulation of *M. phaseolina* were studied *in vitro* using ten different semi-solid culture media. All the media tested encouraged better growth of the test pathogen (Plate VI). The mean colony diameter varies from 37.00 mm to 88.33 mm after seven days of inoculation.

The data presented in Table 4.1 revealed that, among eight culture media tested, Potato dextrose agar was found most suitable and encouraged maximum radial mycelial growth (88.33 mm). The second best culture medium found was Standard nutrient media (70.00 mm). This was followed by Oat meal agar (68.66 mm), Malt extract agar (65.00 mm), Richards medium (63.00 mm), Czapek's dox medium (50.66 mm), Rose bengal medium (45.66 mm). Corn meal agar was found least suitable which recorded minimum mycelial growth (37.00 mm) of the test pathogen (Fig 1).

Table 4.1 Effect of different culture media on radial mycelial growth, cultural characteristics and sporulation of *M. phaseolina*

Treatment	Medium	Mean colony diameter in mm (*)	Cultural Characteristic	Sclerotial formation
T_1	Potato dextrose agar	88.33	Colony dark black to light olive grey, raised, aerial.	++++
T_2	Corn meal agar	37.00	Dark white to slight brown with black center, thin mycelial mat	+++
T_3	Oat meal agar	68.66	Dark black with brown edges of colony, dense mycelial mat	+++
T_4	Czapek's dox agar	49.00	Colony jet black to slightly whitish, thin cottony growth	+
T_5	Richards medium	63.00	Colony black to dirty white with white edges, thin and sparse.	+
T_6	Standard nutrient agar	70.00	Colony whitish to slight black, raised, dense mycelium.	+++
T_7	Malt extract agar	65.00	Colony whitish to black with smooth margins, sparse growth	+++
T_8	Rose Bengal medium	45.66	Colony blackish to white, dense with uneven edges	++
	S.E. ±	0.85	-	-
	CD at 5%	2.59	-	-

+ = Poor, ++ = Fair, +++ = Good, ++++ = Excellent
(*) = Average colony diameter of three replication DAI = Days after inoculation

All the culture media exhibited a variable range of sporulation from poor (+) to excellent (++++). However, only Potato dextrose agar recorded excellent (++++) sporulation. while good (+++) sporulation was recorded on Corn meal agar, Oat meal agar and Standard nutrient agar. Rose Bengal medium exhibited fair (++) sporulation; whereas poor (+) sporulation was recorded on Richards medium and czapek's dox medium.

Results of the present study on the effect of various culture media on colony characteristics and sporulation in *M. phaseolina* are in consonance with those reported earlier by several workers, (Suriachandraselven and Seetharaman, 2003; Priya Santosh, 2006; Suryawanshi *et al*., 2014; Sayyad *et al.,* 2015; Gaikwad and Rajurkar, 2018; Satpathi and Gohel, 2018; Tetali and Karpagavalli, 2016).

4.7 Disease management strategies

4.7.1 *In vitro* evaluation of fungicides

All the nine fungicides (systemic, non-systemic and combi) at different conc. i.e., at half of concentration than recommended (at lower concentration) and at recommended concentration (higher concentration) evaluated *in vitro* against *M. phaseolina* exhibited a wide range of mycelial growth and inhibition of the test pathogen. The results obtained are presented in Table 4.2.

4.7.1.1 Mycelial growth

A total of nine fungicides evaluated *in vitro* against *M. phaseolina* exhibited a wide range of mycelial growth and inhibition of the test pathogen.

Results (Table 4.2) revealed that at lower concentration, radial mycelial growth of the test pathogen ranged from 00.00 mm (Carbendazim + Mancozeb) to 88.66 mm (Copper hydroxide). However, significantly least mycelial growth was recorded with the fungicide Carbendazim + Mancozeb (00.00 mm). It was followed by fungicides *viz*., Carbendazim (9.33 mm), Thiram (11.33 mm), Captan (32.00 mm), Mancozeb (41.33 mm), Copper oxychloride (76.33mm) and Thiophanate methyl (86.66 mm). Fungicides Propineb and Copper hydroxide were found comparatively less effective with maximum mycelial growth of 87.66 mm and 88.66 mm, respectively (Plate VII A and Fig. 2).

Table 4.2 ***In vitro*** **efficacy of different fungicides against mycelial growth and inhibition of** ***M. phaseolina***

Tr. No.	Treatments	Concentration (ppm)	Mean Col. Dia. (mm) after 7 DAI*	Mean inhibition zone (mm)	Percent inhibition over control
T_1	Carbendazim	500	9.33	80.67	89.63
		1000	00.00	90.00	100.00
T_2	Thiram	1000	11.33	78.67	87.41
		2000	11.00	79.00	87.77
T_3	Thiophanate methyl	500	41.33	48.67	54.07
		1000	40.00	50.00	55.55
T_4	Mancozeb	1250	32.00	58.00	64.44
		2500	13.66	76.34	84.82
T_5	Copper oxychloride	1500	86.66	3.34	3.71
		3000	82.66	7.34	8.15
T_6	Captan	1250	37.66	52.34	58.15
		2500	34.66	55.34	61.48
T_7	Propineb	1250	76.33	13.67	15.18
		2500	70.00	20.00	22.22
T_8	Copper hydroxide	1250	88.66	1.34	1.48
		2500	88.00	2.00	2.22
T_9	Carbendazim + Mancozeb	1000	00.00	90.00	100.00
		2000	00.00	90.00	100.00
T_{10}	Control	-	90.00	00.00	00.00
			90.00	00.00	00.00
	SE±		**0.55**		
			0.98		
	C. D. (P=0.05 %)		**1.65**		
			2.93		

*Average of three replications, DAI= Days After Inoculation, Col. = Colony, Dia. = Diameter, Conc. = Concentration, Av. =Average, Figures in parenthesis are arc sine transformed value

At higher concentration, results showed that (Table 4.2), radial mycelial growth of the test pathogen ranged from 00.00 mm (Carbendazim + Mancozeb) to 88.00 mm (Copper hydroxide) as against 90.00 mm inn untreated control. However, in treatment of fungicides, Carbendazim + Mancozeb and Carbendazim there was no any fungus growth observed. This was followed by fungicides Thiram (11.00 mm), Captan (13.66 mm), Propineb (34.66 mm), Mancozeb (40.00 mm) and Copper oxychloride (70.00mm). Fungicides Thiophanate methyl (82.66 mm) and Copper hydroxide (88.00 mm) showed comparatively maximum mycelial growth (Plate VII B and Fig 3)

4.7.1.2 Mycelial growth inhibition

Result (Table 4.2) revealed that all the 9 fungicides tested significantly inhibited mycelial growth of *M. phaseolina*, over untreated control.

At lower concentration, per cent mycelial growth inhibition of the test pathogen ranged from 1.48 per cent (Copper hydroxide) to 100 per cent (Carbendazim + Mancozeb). However, significantly higher mycelial growth inhibition was recorded with Carbendazim + Mancozeb (100%). The second and third best fungicides found were Carbendazim (89.63%), Thiram (87.41%), respectively. This was followed by fungicides *viz*., Mancozeb (64.44%), Captan (58.15%), Thiophanate methyl (54.07%), Propineb (15.18%), Copper oxychloride (3.71%) and Copper hydroxide (1.48%).

At higher concentration (Table 4.2), mycelial growth inhibition was more as compared to lower concentration and it was ranged from 2.22 per cent (Copper hydroxide) to 100 per cent (Carbendazim + Mancozeb and Carbendazim alone). However, cent per cent inhibition was recorded with fungicides Carbendazim + Mancozeb and Carbendazim. It was followed by fungicides Thiram (87.77%), Mancozeb (84.82%), Captan (61.48%), Thiophanate methyl (55.55%), Propineb (22.22%), Copper oxychloride (8.15%) and Copper hydroxide (2.22%).

Thus, all the fungicides tested were found fungistatic against *M. phaseolina* and significantly inhibited its mycelial growth over untreated control. However, fungicides found most effective in the order of merit were Carbendazim 12 % + Mancozeb 63 %, Carbendazim, Thiram, Mancozeb, Thiophanate methyl, Propineb, Copper oxychloride and Copper hydroxide.

Similar fungistatic effects of the fungicides against *M. phaseolina* infecting maize and many other crops were reported earlier by several workers (Devi and Singh, 1997; Jha and Sharma, 2006; Verma and Ram, 2006; Zote *et al.,* 2006; Mondhe *et al*., 2008; Surywanshi *et al.,* 2008; Khalikar *et al.,* 2011; Gowdra *et al.,* 2012; Chaudhary *et al.,* 2017; Khan and Sahi, 2020).

4.7.2 *In vitro* evaluation of bioagents/antagonist

Results obtained on mycelial growth and inhibition of *M. phaseolina* with four fungal and two bacterial antagonists are presented in Table 4.3 and depicted in Fig.4.

The mycelial growth of the test pathogen recorded at seven days of incubation and per cent inhibition of mycelial growth of pathogen over control was calculated.

4.7.2.1 Radial Mycelial growth

At seven days after incubation, mycelial growth of the test pathogen was ranged from 33.00 to 64.00 mm. Significantly least growth was recorded with *T. harzianum* (33.00 mm). This was followed by *Trichoderma viride* (36.66 mm), *T. hamatum* (52.00 mm), *T. koningii* (54.00 mm), *Bacillus subtilis* (61.00 mm), *Pseudomonas fluorescens* (64.00 mm) as compared to 90.00 mm growth in untreated control (Plate VIII).

Table 4.3 Evaluation of bioagents against *M. phaseolina* under *in vitro* condition

Tr. No.	Bio agent	Mean Col. Dia. in mm in 7 (DAI*)	Percent inhibition over control
T_1	*Trichoderma viride*	36.66	59.26
T_2	*Trichoderma harzianum*	33.00	63.33
T_3	*Trichoderma hamatum*	52.00	42.22
T_4	*Trichoderma koningii*	54.00	40.00
T_5	*Bacillus subtilis*	61.00	32.22
T_6	*Pseudomonas fluorescens*	64.00	28.88
T_7	Control	90.00	00.00
	SE±	**0.59**	
	CD at 5%	**1.81**	

(*) = Average of three replication; Col.= Colony; Dia.=Diameter; DAI = Days After Inoculation

4.6.2.2 Mycelial growth inhibition

At seven days after incubation, the per cent inhibition of the test pathogen with the bioagents tested was ranged from 28.88 to 63.33 per cent. However, significantly highest inhibition was recorded with *T. harzianum* (63.33%). This was followed by *Trichoderma viride* (59.26%), *T. hamatum* (42.22%), *T. koningii* (40.00%), *Bacillus subtilis* (32.22%) and *Pseudomonas fluorescens* (28.88%).

The average per cent inhibition of the test pathogen over control with all the treatments was ranged from *T. harzianum* (63.33%) to *Pseudomonas fluorescens* (28.88%) as against 00.00% in untreated control. Significantly highest average per cent inhibition of the test pathogen was recorded with *T. harzianum* (63.33%). This was followed by *T. viride* (59.26%), *T. hamatum* (42.22%) and *T. koningii* (40.00%), *Bacillus subtilis* (32.22%) and *Pseudomonas fluorescens* (28.88%).

Results of the present study on antifungal activity of the *T. viride*, *T. harzianum*, *T. hamatum*, *T. koningii* and two bacterial antagonists *viz*., *P. fluorescens* and *B. subtillis* against *M. phaseolina* are in conformity with those reported earlier by several workers.

Gowdra *et al.* (2012) reported that *T. harzianum*, *Bacillus subilis* and *T. viride* were effective against *Macrophomina phaseolina*.

Similar results were also reported by (Sankar and Sharma, 2001; Pal *et al.,* 2001; Indra and Subbiah, 2003; Ramezani, 2008; Singh *et al.,* 2008; Shekhar and Kumar, 2010; Gowdra *et al.,* 2012; Arora and Dhurwe, 2013; Karthikeyan *et al,.* 2015 and Meena and Pandey, 2015).

4.7.3 Evaluation of fungicides and bioagents against *M. phaseolina* in pot culture

The results in respect of effect of fungicides and bioagents on charcoal rot of maize under glass house condition are presented in Table 4.4 and depicted Fig. 5. It is revealed that Carbendazim + Mancozeb and Carbendazim alone effectively controlled the charcoal rot of maize by 87.67 and 85.00 per cent respectively, followed by Thiram (79.34%) and Mancozeb (76.67%). Mancozeb seems to be less effective compared to other treatments. The charcoal rot incidence was 100% in untreated control.

Among the bioagents, *Trichoderma harzianum* controlled the maize charcoal rot by 46.67 per cent as against 100 per cent disease incidence in untreated control. *Trichoderma viride* was less effective as compared to *T. harzianum* which shows 42.00 per cent control of charcoal rot.

This clearly indicated that fungicides Carbendazim + Mancozeb and Carbendazim alone were effective in controlling charcoal rot of maize in glass house condition.

Table 4.4 Efficacy of fungicide and biocontrol agents against *Macrophomina phaseolina* in pot culture

Tr. No.	Treatment Name	Mean per cent charcoal rot*	Per cent charcoal rot control
T_1	Carbendazim + Mancozeb	12.33 (20.54)	87.67
T_2	Carbendazim	15.00 (22.77)	85.00
T_3	Thiram	20.66 (27.01)	79.34
T_4	Mancozeb	23.33 (28.86)	76.67
T_5	*Trichoderma harzianum*	53.33 (46.66)	46.67
T_6	*Trichoderma viride*	58.00 (49.58)	42.00
T_7	Control	100 (90.00)	00.00
	S.E. ±	**0.66**	
	C.D. (P=0.05 %)	**2.04**	

(*) = Average of three replications; CR= Charcoal Rot.
Figures in parenthesis are arcsine transformed values.

4.8 Screening of Genotypes

The screening of 53 genotypes was carried out *in vivo* by applying standard toothpick method and the rating were obtained from diseased plot (Plate IX). The data presented in Table 4.5 indicated that, out of fifty-three genotypes only two genotypes AH-8246R and VNR-4361 recorded least disease and hence categorized as resistant against charcoal rot. Four genotypes IT8582, PM10205L, Buland and G 25(C) recorded as highly susceptible. Twenty-four genotypes were found moderately resistant and twenty-three genotypes moderately susceptible reactions against *M. phaseolina*.

Table 4.5 Reaction of maize genotypes against charcoal rot

Sr. No.	Name of genotype	Rating scale*	Reaction
	Maturity Duration: Medium		
1.	CMH 9999	6.55	MS
2.	AH 8181	5.15	MS
3.	MMH17-22	5.2	MS
4.	DH 291	5.9	MS
5.	BLH 111	4.5	MR
6.	100K-18	4.6	MR
	Maturity Duration: Late		
7.	NMH-4320	5.50	MS
8.	NMH-4313	3.50	MR
9.	PM 18201L	3.11	MR
10.	PM 18202L	4.50	MR
11.	PM 18203L	4.22	MR
12.	PM 18204L	4.78	MR
13.	PM 18205L	6.11	MS
14.	PM 18206L	5.50	MS
15.	PM 18207L	6.22	MS
16.	PM 10208L	4.39	MR
17.	AH-8087	2.56	R
18.	AH-8246R	4.17	MR
19.	GK3228	6.94	MS
20.	GK3230	5.61	MS
21.	PSC4441	4.89	MR
22.	VNR-4361	2.39	R
23.	IT8582	7.22	S
24.	IMHSB-18K-19	6.44	MS
25.	IMHSB-18K-20	6.33	MS
26.	IMHSB-18K-21	3.61	MR
27.	PM 17208L	6.44	MS
28.	ADV 7043	4.83	MR
29.	Rasi 4181	4.78	MR
30.	DKC9197 (IS8638)	5.72	MS
31.	PM17201L	7.00	MS
32.	PM10205L	7.06	S

Table 4.5 contd….

Sr. No.	Name of genotype	Rating scale*	Reaction
33.	GK 3208	3.06	MR
34.	BLH 113	4.50	MR
35.	DKC 9188	3.83	MR
36.	Bio 305	4.94	MR
37.	ADV 7037	3.83	MR
38.	DKC 9181	5.17	MS
39.	HT 16047	4.89	MR
40.	PM 16202L	4.22	MR
41.	PM 162011L	5.06	MS
42.	VNR 32994	5.39	MS
43.	PM 16205L	6.22	MS
44.	Rasi 2015	3.83	MR
45.	BLH 116	5.33	MS
46.	MM 2033	5.72	MS
47.	DAS-MH-904	5.11	MS
48.	PM 16203L	4.56	MR
49.	Super 3366	4.44	MR
50.	HT 16052	5.06	MS
51.	NMH 713 (Filler)	4.11	MR
52.	Buland	7.2	S
53.	G 25(C)	7.5	S

* Mean of two replications

5. SUMMARY AND CONCLUSIONS

Maize (*Zea mays* L.) is third most important cereal crop cultivated in the country after rice and wheat is affected by several plant pathogens including fungi, bacteria and viruses. Charcoal rot incited by *Macrophomina phaseolina* (Tassi.) Goid., is one of the most destructive and widespread disease which cause average yield losses of 10 to 42.9 per cent. The fungus *M. phaseolina* cause seedling blight, root rot and basal stem rot disease. Maize plants affected with *M. phaseolina* became weak and dry, the lower stem exhibit typical charcoal, grey black discoloration which often gives wilted and defoliated appearance to field. When the stem is cut open numerous minute black speaks (microsclerotia) are visible on the shredded vascular bundles and on the inside of the stem giving a charred appearance.

The test pathogen, isolated from naturally charcoal rot diseased maize plants on PDA medium grew profusely, initially dirty white mycelium later turned brown to black with production of and sclerotia / microsclerotia. Pathogenicity *M. phaseolina* was proved successfully on susceptible maize cv. G-25 by sick soil method (pot culture) and applying Koch's postulates. Based on typical symptoms on root and nodal region, cultural characteristics of the pathogen, microscopic observations and pathogenicity test, the test pathogen was identified and confirmed as *Macrophomina phaseolina* (Tassi) Goid., the major cause of charcoal rot of maize.

All the eight-culture media tested exhibited better mycelial growth and poor to excellent sclerotial production of *M. phaseolina.* However, Potato dextrose agar was found most suitable and encouraged maximum radial mycelial growth (88.33 mm). The second and third best media found were Standard nutrient agar (70.00 mm) and Oat meal agar (68.66 mm). This was followed by the media *viz*., Malt extract agar (65.00 mm), Richards medium (63.00 mm), Czapek's dox medium (50.66 mm), Rose bengal medium (45.66 mm). Corn meal agar was found least suitable which recorded minimum mycelial growth (37.00 mm).Colour of the colonies produced was varied from dirty white to dark black, dark white to olive green, light brown or greyish black. Dark black to light olive grey, raised colonies, were produced on Potato dextrose agar. All the culture media tested exhibited a variable range of sporulation from poor (+) to excellent (++++).

However, only Potato dextrose agar recorded excellent (++++) sporulation. While good (+++) sporulation was recorded on Corn meal agar, Oat meal agar and Standard nutrient agar. Rose bengal medium exhibited fair (+ +) sporulation; whereas poor (+) sporulation was recorded on Richards medium and Czapek's dox medium.

All the nine fungicides tested were found fungistatic against *M. phaseolina* and significantly inhibited its mycelial growth over untreated control. At lower concentration, Carbendazim + Mancozeb (100%) inhibit cent per cent mycelial growth of the test pathogen. The next best fungicide was Carbendazim (89.63%). It was followed by Thiram (87.41%), Mancozeb (64.44%), Thiophanate methyl (54.07%), Propineb (15.18%), Copper oxychloride (3.71%) and Copper hydroxide (91.48%). While at higher concentration, Carbendazim + Mancozeb and Carbendazim exhibited cent per cent mycelial growth inhibition.

Among the six biocontrol agents evaluated, *T. harzianum* was found most effective antagonist with significantly least mycelial growth (33.00 mm) and highest mycelial growth inhibition (66.33%) of the test pathogen. The second and third best antagonists found were *T. viride* and *T. hamatum*, with second and third least mycelial growth of 36.66 mm and 52.00 mm and inhibition of 59.26 and 42.22 per cent respectively. *P. fluorescence* was found comparatively less effective with 64.00 mm mycelial growth and 28.88 per cent mycelial inhibition.

Under pot culture studies, as soil application and seed treatment, among the fungicides, Carbendazim + Mancozeb was found most effective with 87.67 per cent charcoal rot control. However, among bioagents *Trichoderma harzianum* remarkably manage the charcoal rot.

Under artificial epiphytotic conditions, all the 53 maize entries evaluated exhibited different reactions against *M. phaseolina*. However, two entries were found resistant; 24 entries were moderately resistant; 23 entries were moderately susceptible and four entries were susceptible against *M. phaseolina*.

Conclusion

From the results obtained on various aspects during present investigations on maize charcoal rot (*M. phaseolina*) disease, following conclusions are being drawn:

1. The fungal pathogen *Macrophomina phaseolina* (Tassi) Goid., was found associated with charcoal rot on maize, one of the major constraints in the production of maize, causing heavy quantitative losses.
2. The pathogen, *M. phaseolina* grew better and excellent sclerotial production on a wide range of synthetic and non-synthetic culture media tested. However, pathogen grew well and produced excellent sclerotia on Potato dextrose agar medium.
3. All the fungicides evaluated *in vitro* were found fungistatic / antifungal to *M. phaseolina.* However, Carbendazim 12% + Mancozeb 63% and Carbendazim alone were found most effective.
4. All the six bioagents evaluated *in vitro* were proved potential antagonists against *M. phaseolina*. However, *Trichoderma harzianum* was found most effective.
5. Under pot culture studies, as soil application and seed treatment, among the fungicides Carbendazim + Mancozeb was found most effective. However, among bioagents *Trichoderma harzianum* was remarkably manage the charcoal rot.
6. Out of 53 maize genotypes evaluated under artificial epiphytotic conditions (Tooth pick method) in field, 2 entries i.e., AH-8246R and VNR-4361 were found resistant against *M. phaseolina.*

6. LITERATURE CITED

Anis, M., Zaki, M.J. and Dawar, S. 2011. Screening of sunflower (*Helianthus annuus* L.) varieties against charcoal rot fungus, *M. phaseolina* (Tassi) Goid. *Fuuast J. Biol.,* **1**(1):43-46.

Anonymous. 2000. CGIAR Research, Areas of Research: Maize (*Zea mays* L.). <http://www.cgiar.org/areas/maize.htm>.

Anonymous. 2012. Inoculation Methods and Disease Rating Scales for Maize Diseases. Directorate of Maize Research, Pusa Campus, New Delhi

Anonymous. 2017. NCoMM Special report: September 2017, National Collateral Management Services Limited.

Anonymous. 2020. www. indiaagristat.com.

Anwar-ul-Haq and Nashir, M. A. 1994. Screening of different maize cultivars for resistance against stalk rot. *Pak. J. Phytopathol.,* **6**(1):31-34.

Arora Manjeet and Dhurwe Umesh. 2013. Biochemical control of charcoal rot of *Sorghum bicolor* (L.) Moench. *Int. J. Curr. Microbiol. App. Sci*., **2**(11):19-23.

Arora, D.K. and Upadhyay, R.K. 1978. Effect of fungal staling substances on colony interactions. *Plant Soil*. **49**:685-690.

Ashraf, W., S.T. Sahi, I.Ul Haq and S. Ahmed, 2015. Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with maize (*Zea mays*) in Punjab-Pakistan. *Int. J. Agric. Biol*., **17**:1037-1042.

Bashir, MR. 2018. Evaluation of New Chemistry Fungicides against Charcoal rot of Sesame caused by *Macrophomina phaseolina* in Pakistan. *J. Hortic*., **5**:109.

Chambers, K.R. 1987. Stalk rot of maize: Host-pathogen Interaction. *Phytopathol.,* **118**:103-108.

Chaudhary, D.H., Pathak, D.M. and Chaudhary, M. 2017. *In vitro* Efficacy of Fungicides against Dry Root Rot (*Macrophomina phaseolina*) of Soybean. *Int. J. Curr. Microbiol. App. Sci.,* **6**(8):1298-1301.

Chopra, V.L. (eds.) 2001. Maize. In Breeding Field Crops. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi, pp. 147-185.

Coser SM, Chowda Reddy RV, Zhang J, Mueller DS, Mengistu A, Wise KA, Allen TW, Singh A and Singh AK. 2017. Genetic Architecture of Charcoal Rot (*Macrophomina phaseolina*) Resistance in Soybean Revealed Using a Diverse Panel. *Front. Plant Sci.,* **8**:1626.

Cramer, H. H. 1967. Plant Protection and World Crop Production. Bayer, Leverkusen, Germany. pp. 524.

Crous, P.W., Slippers, B., Wingfield, M.J., Rheeder, J., Marasas, W.F.O., Philips, A.J.L., Alves, A., Burgess, T., Barber, P. and Groenewald, J.L. 2006. Phylogenetic lineages in the Botryosphaeriaceae. *Stud. Mycol*., **55**:235–253.

Csondes, I. 2012. Effect of culture media on the growth and morphology of Hungarian *Macrophomina phaseolina* isolates. *Acta Agron. Hung.,* **60**(2):109-129.

Das, N. D. 1988. Effect of different sources of carbon, nitrogen and temperature on the growth and sclerotial production of *Macrophomina phaseolina* causing root rot / charcoal rot disease of castor. *Indian J. Plant Pathol*., **6**:97-98.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species groups of *Trichoderma*: production of volatile and non-volatile antibiotics. *Trans. Br. Mycol. Soc*., **57**:41-48.

Desai, S., Hegde, R.K. and Desai, S. 1991. A preliminary survey of incidence of stalk rot complex of maize in two districts of Karnataka. *Indian Phytopathol.,* **43**:575–576.

Deshmukh, M. A, Gade, R.M, Belkar, Y.K. and Koche, M.D. 2016. Efficacy of bioagents, biofertilizers and soil amendments to manage root rot in greengram. *Legume Res*., **39** (1):140-144.

Devi, T.P and Singh, R.H. 1997. Screening of fungicides against seedling mortality of black gram caused by *Macrophomina phaseolina. Legume Res.,* **20**(2):71-76.

Devlash, R., Guleria, S. K. and Thakur, D. R. 2011. Evaluation of seed dressing fungicides for the management of banded leaf and sheath blight of maize. *Plant Dis. Res.,* **26**(2):169.

Dhillon, B. S and Prasanna, B. M. 2001. Maize; In "Breeding Food Crops." Ed. Chopra V.L. Oxford & IBH, New Delhi. pp 147-185.

Dhingra, O.D. and Sinclair, J.B. 1978. Biology and Pathology of *Macrophomina phaseolina.* O.D. Dhingra and J. B. Sinclair, eds. Universidade Federal De Vicosa, Minas Gerais.

Doebley, J., Goodman, M. M. and Stuber, C. W. 1987. Patterns of isozyme variation between maize and Mexican annual teosinte. *Econ. Bot*., **41**(2):234–246.

Dowswell, C. R., Paliwal, R. L. and Cantrell, R. P. 1996. Maize in the Third World. West view Press, Boulder, USA.

Elad, Y., Zvieli, Y. and Chet, I. 1986. Biological control of *Macrophomina phaseolina* (Tassi) Goid., by *Trichoderma harzianum. Crop Prot*., **5**(4):288-292.

Emayavarman, P., Renukadevi, P., Ravikesavan, R. and Nakkeeran, S. 2019. Antifungal Efficacy and Growth Promotion by *Trichoderma virens* TRI 37 and *Bacillus amyloloiquefaciens* (VB7) against *Macrophomina phaseolina*-the Maize Charcoal Rot Pathogen. *Int. J. Curr. Microbiol. App. Sci*., **8**(11):2682-2693.

Fouad Almomani, Mohammed Alhawatema & Khalid Hameed. 2013. Detection, identification and morphological characteristic of *Macrophomina phaseolina*: the charcoal rot disease pathogens isolated from infected plants in Northern Jordan. *Archives of Phytopathol. and Pl. Protect.,* **46**:9, 1005-1014.

Freije, A. and Wisen, K. 2016. Diseases of corn. *Formerly Purdue Extension publication* BP-59.

Gaikwad, S. N. and Rajurkar, S. K. 2018. Cultural studies of *Macrophomina phaseolina* causing Root Rot disease of Groundnut (*Arachis hypogaea* L.). *Int. J. Acad. Res. Dev.,* **3**(5):105-108.

Gavali, M. T., Mane, S. R., Kumbhar, V. R., Birajdar, G. M. and Bhale, U. N. 2017. Cultural and morphological variability of *Macrophomina phaseolina*

(Tassi) Goid. Causing charcoal rot of jowar in Solapur district of Maharashtra, India. *International J. Current Adv. Res.,* **6**; ISSN: O: 2319-6475, ISSN: P: 2319-6505.

Goidanich, G. 1947. A revision of the genus *Macrophomina phaseolina* Petrak type species: *Macrophomina phaseolina* (Tassi.) Goid. *Macrophomina phaseolina* (Maubl) Ashby. *Ann. Sper. Agr.,* (NS), **1**: 449-461.

Gowdra, N., Muhammad S. and Narendrappa, T. 2012. *In vitro* evaluation of fungicides, bio agents and botanicals against *Macrophomina phaseolina* (Tassi) Goid the causal agent of dry root rot in chickpea. *Mysore J. Agric. Sci.,* **46**(4):778-783.

Groves, C. and Smith, D. 2013. Disease Profile: Charcoal Rot of Soybean. University of Wisconsin, Extension.

Harlapur, S. I., Wali, M. C., Anahosur, K. H. and Murali, K. S. 2000. A report on survey and surveillance of maize diseases in north Karnataka. *Karnataka J. Agril. Sci.,* **13**(3):750-751.

Hooda, K. S., Bagaria, P. K., Khokhar, Mukesh, Kaur, Harleen and Rakshit, Sujay. 2018. Mass Screening Techniques for Resistance to Maize Diseases. ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana- 141004, 93pp.

Iltis, H. H. and Doebley, J. F. 1980. Taxonomy of *Zea* (Gramineae). II. Subspecific categories in the *Zea mays* complex and a generic synopsis. *Amer. J. Bot.,* **67**(6):994-1004.

Indra, N. and Subbiah, G. 2003. Management of black gram root rot caused by *Macrophomina phaseolina* by antagonistic microorganisms. *Madras Agric. J.*, **90**(7-9):490-494.

Iqbal, U. and Mukhtar, T. 2014. Morphological and Pathogenic Variability among *Macrophomina phaseolina* Isolates Associated with Mungbean (*Vigna radiata* L.) Wilczek from Pakistan. *The Scientific World J.*, 2014.

Iqbal, U., Mukhtar, T., Iqbal, S.M., Ul-Haque, I. and Malik, S.R. 2010. Host plant resistance in black gram against charcoal rot (*Macrophomina phaseolina* (Tassi) Goid). *Pak. J. Pathopathol.,* **22**:126-129.

Jana, T., Sharma, T. and Singh, N. 2005. SSR-based detection of genetic variability in the charcoal root rot pathogen *Macrophomina phaseolina*. *Mycol. Res.,* **109**(1):81-86.

Jha, K. M. and Sharma, N. D. 2006. Studies on factors affecting *Rhizoctonia bataticola*. IV. Fungicide. *J. Mycopathol. Res*., **44** :67-71.

Kaiser, S.A.K.M and Mukherjee, N. 1979. Stalk rot complex of maize in West Bengal and their management. *Indian J. Mycol. Res.,* **17**(2):77-83.

Kaiser, S.A.K.M. and Das, S.N. 1988. Physical Factors that Influence the Growth and Spread of Charcoal Rot Pathogen (*Macrophomina phaseolina*) Infecting Maize. *J. Phytopatol.,* **123**(1):47-51.

Karthikeyan, V., Brindha, S., Annadurai, B. and Gangwar, S.K. 2015. Biological control of *Macrophomina phaseolina* (Tassi) Goid. Root rot in *Vigna mungo* (Black gram) with *Trichoderma spp. Int. J. Adv. Res. Biol. Sci.,* **5**(2):118-127.

Kaur, H., Malhi, N. S. and Chawla, J. S. 2010. Evaluation of maize genotypes for resistance to maydis leaf blight and charcoal rot under artificial epiphytotic conditions. *Indian Phytopathol*., **63**(1):21-22.

Khadim Hussain Wagan, Muhammad Ibrahim Khaskheli, Jamal-U-DdinHajano and Abdul Ghani Lanjar. 2018. Isolation and characterization of *Macrophomina phaseolina* isolates prevailing in Sindh, Pakistan. *Pure Appl. Biol*., **7**(4):1309-1315.

Khaire, P. B., Hingole, D. G., Gayakwad, A. M. and Sontake, P. L. 2018. Field screening of mungbean genotypes against leaf blight intensity of pathogen *Macrophomina phaseolina*. *Int. J. Curr. Microbiol. App. Sci*., Special. **6** : 2228-2232.

Khaire, P.B. 2016. Effect of weather parameters on incidence of leaf blight of mung bean (*Vigna mungo* L.) caused by *Macrophomina phaseolina* Tassi (Goid). Agricultural Research Station, Badnapur Dist Jalna 431202 (MS), India.

Khalikar, P.V., Gholve, V.M. and Adsul, A.K. 2011. *In vitro* management of *Macrophomina phaseolina* by chemicals. *Int. J. Plant. Prot.,* **4**(1):201-203.

Khan, M.A. and Sahi, S.T. 2020. Chemical control of charcoal rot of soybean caused by *Macrophomina phaseolina* (Tassi) Goid. *Pak. J. Agri. Sci.,* **28**(2):18-20.

Khokhar, M.K., Hooda, K.S., Sharma S.S. and Singh, V. 2014. Post Flowering Stalk Rot Complex of Maize - Present Status and Future Prospects. Maydica electronic publication. **59**:226-242.

Knox-Davies, P.S. 1966. Further studies on pycnidia production by *Macrophomina phaseoli*. *S. Afr. J. Agric. Sci.,* **9**:595-600.

Koehler, B. 1960. Corn stalk rot in Illions. Ill *Agric. Exp. Stn. Bull*. 658. pp 90

Krishna, K.M., Chikkappa, G.K. and Manjulatha, G. 2013. Components of genetic variation for *Macrophomina phaseolina* resistance in maize. *J. Res. ANGRAU,.* **41**(3):12-15.

Kulkarni, S. and Anahosur, K.H. 2011. Integrated management of dry stalk rot disease of maize. *J. Pl. Dis. Sci.,* **6**(2):99-106.

Kumar P. and Gaur V. K. 2010. Variation among isolates of *Macrophomina phaseolina* causing root rot of Groundnut. *Pl. Dis.* Res., **25** (2):155-161.

Kumar, A., Jat, S.L., Kumar, Ramesh and Yadav, O.P. 2013. Directorate of Maize Research, Pusa Campus, New Delhi- 110 012, 123p.

Kumar, B., Hooda, K.S., Singh, V., Sekhar, J.C., Kumar, K., Parihar, C.M., Jat, S.L., Aditya, K., Singh, A.K., Kaul, J., Kaur, H., Kaur, H. and Yadav, O.P. 2017. Multi-environment field testing to identify stable sources of resistance to charcoal rot (*Macrophomina phaseolina*) disease in tropical maize germplasm. *Maydica.* **62.**

Kumar, B., Jat, S.L., Mukri Ganapati and Yatish, K.R. 2013. Maize production systems for improving resource-use efficiency and livelihood security. Directorate of Maize Research, Pusa Campus, New Delhi. 88p.

Lal, S. and Singh I. S. 1984. Breeding for resistance to downy mildews and stalk rots in maize. *Theor. Appl. Genet.,* **69**:111-119.

Ledencan, T., Simic, D., Brkic, I., Jambrovic, A. and Zdunic. Z. 2003. Resistance of maize inbreds and their hybrids to *Fusarium* stalk rot. *Czec. J. Genet. Plant Breed.,* **39**:15-20.

Livingston, J. E. 1945. Charcoal Rot of Corn and Sorghum. *Historical Research Bulletins of the Nebraska Agricultural Experiment Station* (1913-1993). **149**:135-140.

Mahadevakumar, S. and Janardhana, G. R. 2016. First report of leaf blight caused by *Macrophomina phaseolina* on *Jasminium multiflorum* in India. *J. Plant Pathol*, **98**(1):171-185.

Malathi, P. and Doraisamy, V. 2003. Compatibility of *Trichoderma harzianum* with fungicides against *Macrophomina phaseolina*. *Pl. Dis. Res*., **18**(2):139-143.

Malcom, C.S. 1980. Compendium of corn diseases. APS press, Minnesota. pp:105.

Matsuoka, Y., Vigouroux, Y., Goodman, M.M., Sanchez G., Buckler, E. and Doebley J. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. *Proc. Natl. Acad. Sci*., USA. **99**:6080–6084.

Meena, B. and Pandey. R. N. 2015. *In vitro* evaluation biological control agents against *Macrophomina phaseolina* and *Rhizoctonia solani*. *Trends Biosci*., **8** (3):669-671.

Mondhe, M.K., Parate, R.L., Thakur, K.D. and Bhoye, B. B. 2008. Evaluation of leaf extracts and fungicides against *Macrophomina phaseolina* (Tassi) Goid causing green gram leaf blight. *J. Plant Dis. Sci.,* **3**(2):239-240.

Mukerji, K.G. and Manoharachary, C. 2010. Taxonomy and Ecology of Indian Fungi. I.K. International publishing House Pvt. Ltd. ISBN: 978-93-80026-92-3.

Mukerji, K.G. and Manoharachary, C. 2010.Taxonomy and Ecology of Indian Fungi. Page No. 60.

Mukesh-Kumar, Lal, H.C. and Jha, M.M. 1996. Assessment of yield loss due to post-flowering stalk rot in maize. *J. Appl. Biol*., **8**(1):90-92.

Murali Krishna, K., Chikkappa, G.K. and Manjulatha, G. 2013. Components of Genetic Variation for *Macrophomina phaseolina* Resistance in Maize. *J. Res. ANGRAU.,* **41**(3):12-15.

Murali Krishna, K., Kulakarni, N. and Sai Kumar, R. 2013. Genetic analysis of post flowering stalk rot resistance in maize (*Zea mays* L.). *Indian J. Genet*., **73**(3):78-81.

Murthy Krishna, Niranjana, S.R. and Selty, H.S. 2003. Effects of chemical fungicides and biological agent on seed quality improvement in pulses. *Seed Res*., **31**(1):121-124.

Nagamma, Gowdra, Saifulla, Muhammad, Pavithra, S. and Suresh, S.R. 2015. Morphological variability of *Macrophomina phaseolina* (Tassi) Goid, causal agent of dry root rot disease of chickpea (*Cicer arietinum* L.). *Internat. J. Plant Protec.*, **8**(1):69-72.

Nene, Y.L. and Thapliyal, R.N. 1993. Evaluation of fungicides. In: Fungicides for Plant Disease Control (3rd ed.). Oxford, IBH Pub. Co. New Dehli. pp. 331.

Pal, K.K., Tilak, K.V.B.R., Saxena, A.K., Dey, R. and Singh, C.S. 2001. Suppression of maize root diseases caused by *Macrophomina phaseolina, Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiol. Res*., **156**:209-223.

Parmar, H., Kapadiya, H.J., Bhaliya, C.M. and Patel, R.C. 2018. Effect of Media and Temperature on the Growth and Sclerotial Formation of *Macrophomina phaseolina* (Tassi) Goid causing Root Rot of Castor. *Int. J. Curr. Microbiol. App. Sci.,* **7**(2):671-675.

Parmar, H.V., Kapadiya, H.J. and Bhaliya, C.M. 2017. Efficacy of different fungicides against *Macrophomina phaseolina* (Tassi) Goid causing castor root rot. *Int. J. Chem. Stud.,* **5**(5):1807-1809.

Patil, S.J., Wali, M.C., Harlapur, S.I. and Prashanth, M., 2000. Maize research in North Kamataka (Bulletin). University of Agricultural Sciences, Dharwad, 2000. pp. 22-23.

Payak, M.M. and Sharma, R.C. 1985. Maize diseases and approaches to their management in India. *Trop. Pest Manag*., **31**:302-310.

Pearson, C.A.S., Leslie, J.F. and Schawenk, F. W. 1987. Host preference correlated with chlorate resistance in *Macrophomina phaseolina*. *Plant Dis.,* **71**:828-831.

Priya Santosh. 2006. Studies on leaf blight of patchouli, M.Sc. Thesis submitted to Dr. B.S.K.K.V., Dapoli, Dist. Ratnagiri (M.S.), India.

Ramezani Hesamedin. 2008. Biological control of root-rot of eggplant caused by *Macrophomina phaseolina*. *American-Eurasian J. Agric. & Environ. Sci.,* **4**(2):218-220.

Reichert, I., and Hellinger, E. 1947. On the occurrence, morphology and parasitism of *Sclerotium bataticola*. *Palestine J. Bot.,* **6**:107-147.

Salik Nawaz Khan. 2007. *Macrophomina phaseolina* as causal agent for charcoal rot of sunflower. *Mycopath.,* **5**(2):111-118.

Sankar, P. and Sharma, R.C. 2001. Management of charcoal rot of maize with *Trichoderma viride. Indian Phytopath.,* **54**(3):390-391.

Satpathi, Ashish Kumar and Gohel, N.M. 2018.Cultural and morphological variability among the isolates of *Macrophomina phaseolina* (Tassi) Goid. Causing stem and root rot of sesame (*Sesamum indicum* L.). *Int. J. Chem. Stud.,* **6**(6):2890-2893.

Sayyad, S.I., Mogle, T.R. and Sonkamble, M.M. 2015. Cultural characteristics of *Macrophomina phaseolina*. *Ann. Pl. Prot. Sci*., **23**(2):309-417.

Shaw, R.H. 1988. Climate requirement. In Sprague G.F., Dudly J.W (eds.) Corn and Corn 638 Improvement, 3rd ed Madism, WI: ASA 609.

Shekhar M. 2004. Studies on variability in *Macrophomina phaseolina* (Tassi) Goid incitant of charcoal rot of maize (*Zea mays* L.). Ph.D. thesis. Meerut: Department of Botany, Meerut College, C.C.S. University.

Shekhar, M. and Kumar, S. 2012. Inoculation methods and disease rating scales for maize diseases. ICAR- Directorate of Maize Research, Pusa Campus, New Delhi – 110 012 (India), 31 pp.

Shekhar, M. and Sangit Kumar. 2010. Potential biocontrol agents for the management of *Macrophomina phaseolina*, incident of charcoal rot in maize. *Archives of Phytopathology and Plant Protection.,* **43**(4):379-383.

Shekhar, M., Kumar, S., Ramesh, C., Sharma, C., and Rathore, Singh. 2010. Sources of resistance against post flowering stock rots of maize. *Archives of Phytopathol. and Plant Protect*., **47**(3):259-263.

Shekhar, M., Sharma R.C., Singh Lokendra and Dutta Ram. 2006. Morphological and pathogenic variability of *Macrophomina phaseolina* (Tassi.) Goid. Incitant of Charcoal rot of maize in India. *Indian Phytopath.,* **59**(3):294-298.

Shoaib, A., Ahmed, J., Akhtar, S. and Awan, Z.A. 2019. Comparative resistance of maize cultivars to charcoal rot disease. *Pak. J. Sci.,* **71**(4):202-202.

Simay, E. I. 1990. A contribution to the host range of *Macrophomina phaseolina* in Hungary. *Novenytermeles,* **39**(1):23-27.

Singh, N., Pandey, P., Dubey, R.C. and Maheshwari, D. K. 2008. Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* (Sarg.) by rhizosphere competent *Bacillus subtilis* BN1. *World J. Microbiol. Biotech.*, **24**(9):1669.

Singh, N., Rajendran A., Meena, S. and Mittal, G. 2012. Biochemical response and host-pathogen relation of stalk rot fungi in early stages of maize (*Zea mays* L.) *African J. Biotech.* **11**(82):14837-14843.

Singh, R.D.N. and Kaiser, S.A.K.M. 2000. Biological control of charcoal rot pathogen (*Macrophomina phaseolina*) which infects maize by nonpathogenic *Fusariumsolani* f. sp. *Psidii.* Der Tropenlandwirt, Beltrage zur tropischen Landwirtlschaft und Veterinarmedizin. 101. Jhargang, **S**. 153-161.

Singh, R.N., and Kaiser, S.A.K.M., 1994. Histopathological study of roots and stalk of maize plant invaded by the charcoal rot pathogen *Macrophomina phaseolina*. *Advan. Pl. Sci.,* **7**:125-130.

Srinivas, T., Indira, S. and Bhadraiah. 2016. Isolation of *Macrophomina phaseolina* pathogen from infected parts of sorghum stems. *J.R.B.A.T.*, **4**(1):176-178.

Su, G., Suh, S.O., Schneider, R.W. and Russin, J.S. 2001. Host Specialization in the Charcoal Rot Fungus, *Macrophomina phaseolina*. *Phytopathol.,* **91**:120-126.

Subedi, Subash, Subedi, Himalaya and Neupane, Sarswati. 2016. Status of maize stalk rot complex in western belts of Nepal and its integrated management. *J. Maize Res. and Develop.,* **2**(1) : 30-42.

Suriachandraselvan, M. and Seetharaman, K. 2003. Effect of culture media on growth and sclerotial production of different isolates of *Macrophomina phaseolina* infecting sunflower. *J. Mycol. Pl. Pathol*., **33**(2):226-229.

Suryawanshi, A.P., Gore, D.B., Pawar, A.K. and Wadie, A.G. 2008. Efficacy of fungicides against macrophomina blight of mung bean. *J. Pl. Dis. Sci.,* **3**(I):40-42.

Suryawanshi, A.P., Mule, V.P., Utpal, D. and Wagh, S.S. 2014. Cultural and morphological studies of *Macrophomina phaseolina* on different media. *J. Pl. Dis. Sci.,* **9**(2):273-275.

Tandel, D.H., Sabalpara, A.N. and Pandya, J.R. 2010. Efficacy of fungicides and phytoextracts against green gram leaf blight caused by *Macrophomina phaseolina*. *J. Pl. Dis. Sci.,* **5**(1):48-50.

Tetali, S. and Karpagavalli, S. 2016. Effect of culture media on the growth of *Macrophomina phaseolina* isolate. The International Conference on Integrating Climate, Crop, Ecology–The Emerging Areas of Agriculture, Horticulture, Livestock, Fishery, Forestry, Biodiversity and Policy Issues. ISBN: 978-81-930585-9-6.

Thombre, B.B. and Kohire, O.D. 2018. Efficacy of systemic and non-systemic fungicides against *Macrophomina phaseolina* (Tassi) Goid causing mungbean blight. *Int. J. Chem. Stud.,* **6**(2):3054-3057.

Thori, H.R., Bunker, R.N., Mathur, K. and Sharma, S.S. 2012. Integrated management of post flowering stalk rot of maize caused by *Fusarium moniliforme. Indian Phytopath.,* **65**(2):151-154.

Umer Iqbal and Tariq Mukhtar. 2014. Morphological and pathogenic variability among *Macrophomina phaseolina* isolates associated with mungbean (*Vigna radiata* L.) Wilczek from Pakistan. *The Scientific World Journal* Volume 2014, Article ID 950175, 9 pages.

Verma, S.C. and Ram, R.C. 2006. Inhibitory effect of some fungicides and neem product on mycelial growth and sclerotial development of *R. solani* causing sheath blight of rice. *Indian Phytopathol*., **24**(1&2):62-55.

Vincent, J.M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors, Nature: 159-180.

Watson, L. and Dallwitz, M.J. 1992. Grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, photochemistry, cytology, classification, pathogens, world and local distribution, and reference.

White, D.J. 1999. Fungal stalk rots. Compendium of corn diseases. 3rd Edition. D.G. White ed. APS Press, St. Paul, M.N.

Zote, K.K., Gore, D.D., Deshpande, G.D., Zagade, S.N., Mulla, S.M. and Chavan, A.A. 2006. Efficacy of fungicides against macrophomina blight of mungbean. Paper present in national symposium on recent trends in diagnosis and management of chronic and emerging plant disease of IPS zonal meet LPS, Nagpur. pp.74.

7. APPENDIX

Potato Dextrose Agar

Peeled Potato	:	200 g
Agar	:	20 g
Dextrose	:	20 g
Distilled water	:	1000 ml

Corn Meal Agar

Corn meal extract (From 50g of whole maize)	:	2.0 g
Agar	:	15.0 g
Distilled water	:	1000 ml

Oat Meal Agar

Rolled oats	:	30.0g
Agar	:	15.0g
Distilled water	:	1000 ml

Czapek's Dox Agar

Sucrose	:	30.0g
Sodium nitrate	:	2.0g
Dipotassium phosphate	:	1.0g
Magnesium sulphate	:	0.5g
Potassium chloride	:	0.5g
Ferrous sulphate	:	0.01g
Agar	:	15.0g
Final pH (at 25°C)	:	7.3±0.2
Distilled water	:	1000 ml

Richard's Agar Medium

Potassium nitrate	:	10.0g
Monopotassium dihydrogen phosphate	:	5.0g
Magnesium sulphate	:	2.5g
Ferric Chloride	:	0.02g
Sucrose	:	50.0g
Agar	:	15.0g
Distilled water	:	1000 ml

Malt Extract Agar

Malt extract	:	30.000
Mycological peptone	:	5.000
Agar	:	15.000
Distilled water	:	1000 ml
Final pH (at 25°C)	:	5.4±0.2

Rose Bengal Medium

Papaic digest of soybean meal	:	5.0g
Dextrose	:	10.0g
Monopotassium phosphate	:	1.0g
Magnesium sulphate	:	0.5g
Rose bengal	:	0.05g
Agar	:	15.0g
Distilled water	:	1000 ml
Final pH (at 25°C)	:	7.2±0.2

Standard Nutrient Agar

peptone	:	10.0g
Beef extract	:	10.0g
Sodium chloride	:	5.0g
Agar	:	20.0g
Distilled water	:	1000 ml

7. VITAE

Mr. CHARDE UTTAM MOHAN

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

2021

Title of thesis		**:**	"Studies on charcoal rot of maize caused by *Macrophomina phaseolina* (Tassi) Goid."
Major field		**:**	Plant Pathology
Biographical information		**:**	
Personal	**Date of Birth**	**:**	24 July, 1994
	Place of Birth	**:**	Panora, Tah. Gondpipari, Dist. Chandrapur.
	Father's Name	**:**	Shri. Mohan Tukaram Charde
	Mother's Name	**:**	Sau. Shema Mohan Charde
Educational	**Bachelor's degree Obtained**	**:**	Received Bachelor of Science (Agriculture) degree from Manoharbhai Patel College of Agriculture, Hiratola, Dist. Gondia during the year 2014 – 2018
	Class	**:**	First class (88.30%)
	Name of the University	**:**	Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra State, India
Address		**:**	At. Panora Post. Dhamangaon, Tah. Gondpipari Dist. Chandrapur. Pin code – 442 702.
	Email- id	**:**	uttamcharde45@gmail.com
	Contact No.	**:**	9823082195