Studies on *Macrophomina* root rot of groundnut (*Arachis hypogaea* L.)

By

NEHA RANI

DEPARTMENT OF PLANT PATHOLOGY

BIHAR AGRICULTURAL UNIVERSITY, SABOUR
BHAGALPUR – 813 210

2014

Registration No. M/Pl. Path./91/BAC/2012-13

Dedicated

To

My Parents

&

Elder Brother

Whose perpetual

Affection Inspired Me for

Higher Ambition in Life

Neha Rani

BIHAR AGRICULTURAL UNIVERSITY, SABOUR, BHAGALPUR

Dr. Arun Prasad Bhagat
Assoc. Prof.-cum-Sr. Scientist

Deptt. of Plant Pathology
Bihar Agricultural College,
Sabour, Bhagalpur-813 210

Dated_____

Certificate-I

This is to certify that the thesis entitled **"STUDIES ON *MACROPHOMINA* ROOT ROT OF GROUNDNUT (*Arachis hypogaea* L.)"** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE IN THE SUBJECT OF PLANT PATHOLOGY** of the faculty of Agriculture, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, is genuine record of bonafide research work carried out by **Miss Neha Rani**, under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been fully acknowledged.

Bhagat 28/6/2014

(Arun Prasad Bhagat)
Chairman
Advisory Committee

Certificate-II

We, the undersigned members of the Advisory Committee of **Miss. Neha Rani**, a candidate for the degree of **MASTER OF SCIENCE IN AGRICULTURE IN THE SUBJECT OF PLANT PAHTOLOGY** have gone through the manuscript of the thesis and agree that the thesis entitled **"STUDIES ON *MACROPHOMINA* ROOT ROT OF GROUNDNUT (*Arachis hypogaea* L.)"** may be submitted in partial fulfilment of the requirement for the award of the degree.

28/6/2014

(Arun Prasad Bhagat)
Chairman
Advisory Committee

Endorsed :

29/6/2014

Head
Deptt. of Plant Pathology

Members

1. **Dr. Chanda Kushwaha**.....
   Asstt. Professor-cum-Jr. Scientist
   Deptt. of Plant Pathology

2. **Dr. S. N. Rai**.....
   Sr. Scientist-cum-Assoc. Prof.
   Deptt. of Entomology

3. **Mr. Manoj Kumar**.....
   Jr. Scientist-cum-Asstt. Prof.
   Deptt. of Plant Breeding and Genetics

4. **Dr. V. B. Patel**.....
   Univ. Prof.-cum-Chief Scientist
   Deptt. of Hort. (Fruit & Fruit Tech.)
   (Nominee of the Dean PGS)

Certificate – III

This is to certify that the thesis entitled **"Studies on *Macrophomina* root rot of Groundnut (*Arachis hypogaea* L.)"** submitted by **Miss Neha Rani** in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** of the faculty of Agriculture, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, was examined and approved on **14/09/2014.**

14.9.2014
Dr. Arun Prasad Bhagat
Chairman, Advisory Committee

14/09/14
Dr. N. Kudada
External Examiner

Endorsed :

14.9.2014
Head
Deptt. of Plant Pathology

Members

1. **Dr. Chanda Kushwaha**
   Asstt. Professor-cum-Jr. Scientist
   Deptt. of Plant Pathology

2. **Dr. S. N. Rai**
   Assoc.-Prof-cum-Sr. Scientist
   Deptt. of Entomology

3. **Mr. Manoj Kumar**
   Jr. Scientist-cum-Asstt. Professor
   Deptt. of Plant Breeding and Genetics

4. **Dr. V. B. Patel** 14/9/14
   Univ. Prof.-cum-Chief Scientist
   Deptt. of Hort. (Fruit & Fruit Tech.)
   (Nominee of the Dean PGS)

22.10.14
Assoc. Dean-Cum-Principal
Bihar Agricultural College, Sabour
Bhagalpur-813 210

Acknowledgement

It is proud privilege to express my most sincere and profound sense of gratitude to my Major Advisor Dr. Arun Prasad Bhagat, Sr. Scientist-cum-Assoc. Prof., Department of Plant Pathology, Bihar Agricultural College, Sabour, Bhagalpur for his inspiring, learned guidance, untiring help and constant encouragement in carrying out the research and preparation of this manuscript.

With the same spirit, I express my fathomless gratitude to benevolent and ever generous members of my Advisory Committee, Dr. Chanda Kushwaha, Asstt. Professor- cum-Jr.-Sci., Deptt. of Plant Pathology, Dr. S. N, Ray, Sr.-Scientist-cum-Assoc. Prof, Deptt. of Entomology and Mr. Manoj Kumar, Asstt. Prof.-cum-Jr. Scientist, Deptt. of PBG.

I am much obliged to my Nominee of Dean PGS Dr. V. B. Patel, University Professor-cum-Chief Scientist. Deptt. of Horticulture, (Fruit and Fruit Technology) for providing necessary facility to carry out the research work in an effective manner.

I am highly grateful to Dr. Gireesh Chand and Dr. J. N. Srivastava Assoc.-Cum- Sr. Scientist, Deptt. of Plant Pathology B.A.C., Sabour for providing adequate facilities and valuable suggestions during the course of investigation.

My special & heartiest thanks are also due to and Mr. C.S. Azad, Dr. Amrendra Kumar, Mr. R.N Gupta, Dr. Md. Arshad Anwer, Dr. Md Ansar, Dr. Santosh Kumar, Mr. Abhijeet Ghatak, and Mr. K. P. Singh., Assistant Professors, Deptt. of Plant Pathology for keen motivation and preparation of this manuscript.

My sincere thanks Mr. Subhashish Sarkhel, Asstt. Professor, Deptt. of Plant Pathology for their help and guidance during my study as well as during preparation of the manuscript.

I wish to record my thanks to Dr. R.N. Jha Sr. Scientist-cum-Associate Prof., Deptt. of Statistics, Mathematics and Computer Application for their constant supervision, valuable suggestions & fair guidance during the period of research work and writing of this thesis.

I am also grateful to Dean, (Agriculture), DRI-cum-Dean PGS, BAU, Sabour & Assoc. Dean-cum-Principle, BAC, Sabour for providing necessary facilities and encouragement to perform my research work for M. Sc. (Ag.) degree programme.

I am indeed very much obliged to the Hon'ble Vice-chancellor, BAU, Sabour (Bhagalpur) Bihar for providing all facilities during the course of my investigation.

I also pay my thanks to all staffs of the Department of Plant Pathology, Regional research sub-station Jalalghar, College, Library and University for their unending help during my research and study.

I am also extending my heartiest thanks to Dilip Kumar Sah the field overseer of the Department.

My acknowledgement would be incomplete if I do not pay thanks to batchmates, well wishers who helped me a lot in various ways during the entire period of my investigation.

I have no word to express my deepest and heartiest gratitude to my parents, Elder Brothers and Bhabhi for their love, affection, encouragement and blessings to achieve my goal.

I would like to record my special thanks to Ranjay Jee for his sincerity in helping in preparation of my thesis manuscript.

I wish to convey my heartiest thanks to all those who has helped me during my study programme.

At the last but not the least I am extremely grateful to the almighty who inspired and helped me to come up where I am today.

Place : Sabour

Dated: ...../06/2014 **(Neha Rani)**

ABSTRACT

TITLE OF THESIS	:	"**Studies on *Macrophomina* root rot of Groundnut (*Aarchis hypogaea* L.)"**
NAME OF THE STUDENT	:	Neha Rani
DEGREE PROGRAMME	:	M. Sc. (Ag.)
DEPARTMENT	:	Plant Pathology
REGN. NO	:	M/Pl.Path./91/2012-13
MAJOR SUBJECT	:	Plant Pathology
MINOR SUBJECT	:	Entomology
NAME OF THE MAJOR ADVISOR	:	Dr. Arun Prasad Bhagat Department of Plant Pathology
YEAR	:	2014
NAME OF THE UNIVERSITY	:	Bihar Agricultural University, Sabour, Bhagalpur

Key words;- Groundnut, *Macrophomina phaseolina*, cultural characteristics, disease incidence, poison food technique, seed treatment and dual culture.

Macrophomina root rot of groundnut (*Arachis hypogaea* L.) is an emerging problem in Bihar, where groundnut is extensively grow in large area as a major Kharif and spring oilseed crop. Its incidence varies from 0-32 percent in two seasons. On isolation from diseased plants, the associated pathogen was identified as *Macrophomina phaseolina* (Tassi.) Goid and its pathogencity was confirmed on Groundnut variety R-20 under artificially inoculated conditions. The typical symptoms of the disease may appear on roots, collar region, stem and branches of infected plants. The affected portions rotted, shriveled become darker or blakish in colour and plant collapsed and broke down from the rotted portion. Gradually affected plants showed general yellowing, drooping of leaves and ultimately death of plants before maturity. During cultural studies of the causal pathogen, *M. phaseolina,* Glucose asparagines agar medium supported maximum radial growth among nine different media tested fallowed by Czapek's (dox) agar, potato dextrose agar and carrot extract. Among five tested liquid media Richard's broth was found superior to other, in case biomass production which yielded 724.8

mg/flask after 21 days of inoculation, which was followed by Glucose asparagines, Czapek's (dox) medium, Potato dextrose and Asthana and Howker's agar medium.

During varietal screening, out of 20 entries of Groundnut, the cultivar, DH 86 (32.49 %) showed maximum root rot incidence percent during Kharif followed by CHICO (19.01 %). Minimum and no disease root rot percent was recorded in ICGV07214 (0.00 %). The cultivar, ICGV00338 (8.08%) showed maximum root rot incidence during Spring followed by ICGV02005 (5.71%). Minimum root rot disease incidence percent was recorded in the cultivar ICGV07210 (1.97%). Among the four fungicides namely Carbendazim, Mancozeb, Tricyclazole and Hexaconazole tested *in vitro* at four different concentration *viz.*, 1ppm, 5ppm, 10 ppm, 20 ppm, 30ppm,40ppm, 50 ppm, 100 ppm, 100 ppm and 200ppm, The systemic fungicide, Carbendazim was highly effective in inhibiting the growth of the fungus at all the concentrations followed by Hexaconazole, Mancozeb and Tricyclazole. Seed treatment with different fungicides and one biological agent, maximum germination percentage 66% were observed in seed treated with Carbendazim (12%) + Mancozeb (63%) followed by thiram (30%). Minimum germination percentage observed in seed treated with *Trichoderma* (20%). Maximum disease incidence percent was recorded in seed treated with *Trichoderma* (90%) followed by thiram and Copper oxychloride. Minimum disease incidence (14%) recorded in seed treated with Carbendazim (12%) + Mancozeb (63%). Under *in vitro* condition the ability of *Trichoderma* isolates to inhibit the mycelial growth of *M. phaseolina* in dual culture was determined on PDA medium. A clear zone of inhibition was noted between the *Macrophomina phaseolina and Trichoderma viride* which near the pathogen. The inhibition growth of pathogen were recorded after 24 and48 hrs. upto 47. 61% and 35.18%, respectively in dual culture experiments.

CONTENTS

CHAPTER	PARTICULARS	Page no.
1.	INTRODUCTION	1-4
II.	REVIEW OF LITERATURE	5-19
III.	MATERIAL AND METHODS	20-34
3.1	General method of sterilization & preparation of media	20-24
3.2	Isolation, Purification and maintenance of the pathogen	24-25
3.3	Morphological studies of the pathogen	25-26
3.4	Test of pathogenicity	26
3.5	Cultural studies	27-28
3.6	Screening of groundnut cultivars/entries against *M. phaseolina*	28-30
3.7	Evaluation of different fungicides against *M. phaseolina in vitro*.	31-33
3.8	Effect of different fungicidal treatment on the root rot disease of groundnut caused by *M. phaseolina*	33
3.9	Dual culture	34
IV	EXPERIMENTAL RESULT	35-43
4.1	Symptoms	35
4.2	Isolation and purification of the pathogen	35-36
4.3	Morphological studies	36
4.4	Pathogenicity	36
4.5	Cultural studies of the pathogen	37-38
4.5.1	Effect of different solid media on the radial growth of *M. phaseolina*	37
4.5.2	Effect of the different liquid media on growth of *M. phaseolina*	38
4.6	Screening of groundnut cultivars/entries against *M. phaseolina*	39-40
4.7	Chemical control	40-42
4.7.1	Evaluation of different fungicides against *M. phaseolina in vitro*	40-41
4.7.2	Effect of seed treatment on the root rot incidence of Groundnut caused by *M. phaseolina*	41-42
4.7.3	Dual culture	42-43
V	DISCUSSION	44-48
VI	SUMMARY	49-51
	REFERENCES	I-XIII

LIST OF TABLES

TABLE NO.	PARTICULARS	PAGE NO.
1.	List of fungicides evaluated	31
2.	Effect of different solid media on radial growth of *M. phaseolina*	37
3.	Effect of different liquid media on dry weight of mycelium of *M. phaseolina* after 21 days of incubation at 25± 1°C	38
4.	Screening of groundnut cultivars & entries against root rot under natural condition in field	39
5.	Evaluation of different fungicides against *M. phaseolina in vitro*	41
6.	Effect of different fungicidal seed treatment on the root rot disease of Groundnut	42
7	Dual culture of *M. phaseolina* with *Trichoderma viride*	43

LIST OF FIGURES

FIGURE NO.	PARTICULARS	AFTER PAGE
1.	Effect of different solid media on radial growth of *M. phaseolina*	37
2.	Effect of different liquid media on dry weight of mycelium of *M. phaseolina* after 21 days of incubation at 25± 1°C	38
3.	Screening of groundnut cultivars & entries against root rot under natural condition in field	39
4.	Evaluation of different fungicides against *M. phaseolina in vitro*	41

LIST OF PLATES

PLATES NO.	PARTICULARS	AFTER PAGE
1.	Symptoms of Dry root rot of groundnut caused by *Macrophomina phaseolina*	35
2.	Stem and root rot symptoms on artificially inoculated Groundnut plants	36
3.	A) Fungal growth of the pathogen *M. phaseolina* on different solid media B) Growth of mycelium mat of *M. phaseolina* in different liquid media	38
4.	Screening of groundnut cultivars/entries against *M. phaseolina*	39
5.	Evaluation of different fungicides against *M. phaseolina in vitro*	41
6.	Effect of different fungicidal seed treatment on the root rot disease of Groundnut	42
7.	Dual culture of *M. phaseolina* with *Trichoderma viride* after 5 days of incubation. (I) Inhibition zone	43

CHAPTER-I

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important legume crop cultivated world over for food & its oil use. Being a legume, it is also valued for its nitrogen fixing capacity through the root nodule bacteria (Rhizobium).

Groundnut is the 6th most important oilseed crop in the world. It contains 48-50% oil and 26-28% protein. It provides 12% recommended nutrients and has dietary fibre that reduce the risk of some kinds of cancer and helps control blood sugar. Among 13 essential vitamins, 7 are found in Groundnut and among 20 minerals necessary for growth, 7 are present in Groundnut.

The haulms are utilized as fodder and after extraction of oil, cake is used in the livestock feed industry. Groundnut shells are used as fuel, as filler industry, and in making cardboards. Being a leguminous crop, it enriches the soil with nitrogen and is therefore valuable in cropping system.

Groundnut is originated in the Northwest Argentina region in South America and is presently cultivated in 108 countries of the world. Groundnut is grown on nearly 21.70 million ha with the production of 41.18 million tons and an average yield of 1667 kg/ha in 2012 (FAOSTAT, 2012). In India during 2011-12 the production of Groundnut was 5.78 million tons from 4.90 million ha and average yield 1179 kg/ha. In Bihar, production of Groundnut was 1030 tons from 1020 ha and average yield 1010 kg/ha.

It is estimated that domestic demand of Groundnut by the end of 2020 will be 14 million tons in India Therefore, to meet the required demand of 8.2 million tons, we have to increase production by 2.2 % per year. This can be achieved through increase in yield and partly by regaining the lost area, although not the same area. The area under Groundnut cultivation has declined

by over 50% since 1990's and there is scope to expand Groundnut area in non-traditional areas where the Groundnut cultivation would be profitable.

In India Groundnut is cultivated largely in Kharif season & in some states it is cultivated in Rabi and Spring seasons. The yields in Rabi & Spring seasons are high owing to less incidence of disease & continuous availability of moisture through irrigation or other sources.

A variety of stresses affect Groundnut production from planting to storage. Among these diseases are the major stresses. Different diseases hamper Groundnut production (Mayee, 1987; Mayee & Datar, 1988; Ganesan & Sekar, 2004). These include viral, bacterial, fungal and nematode diseases (Smith, 1994 and Subrahmanyam *et al*., 1980). The majority of diseases are caused by fungi and several of them caused reduction in yield varying in different them cause reduction in regions and seasons (Mayee, 1995). Among these, soil borne fungal pathogens causing serious losses have prime importance (Mathur & Cunfer, 1993). *Aspergillus flavus*, *A. niger, Cercospora arachidicola*, *Curvularia* sp., *Fusarium solani, F. oxysporum, Macrophomina phaseolina*, *Mucor, Rhizoctonia solani*, *Rhizophus* spp., *Penicillium* spp., *Puccinia arachidis*, *Pythium* spp., and *Sclerotium rolfsii* (Gibson, 1953; Clinton, 1960; Sadashivaiah *et al*., 1986; Parvathi *et al*., 1985 and Aliyu & Kutama, 2007) are serious pathogen of Groundnut. Generally these pathogens infect underground parts of the plant and reduce yield (Wisniewska & Chelkowski, 1999). Due to these above diseases not only the production of Groundnut is hampered but its quality is also deteriorated affecting our national income.

The Groundnut & other legumes in India often suffer from various type of root rot & wilt Among these the dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid has been noticed to cause 33.33 per cent seed rotting and 23.80 per cent post emergence mortality (Gupta and Kolte, 1982). *Macrophomina phaseolina* is a soil borne fungus causing the root rot disease on Groundnut and more than 500 plant species from more than

100 families (Mihail, 1992; Wyllie, 1988) distributed worldwide and is one of the cosmopolitan fungi.

The first symptom of disease is yellowing of the leaves which droop in next 2 or 3 days and withers off. The plant may wilt within a week after the appearance of first symptom. When stem is examined closely, dark lesion may be seen on the bark at the ground level. If the plants are pulled from soil the basal stem & main root. May show dry rot symptoms the tissues are weakened and break off easily in advanced cases Sclerotial bodies may be seen scattered on the affected tissues.

The causal organism *Macrophomina phaseolina* belongs to Division-Eumycota, Subdivision-Deuteromycotina, Class-Coleomycetes, Order-Sphaeropsidales, & Family-Sphaeropsidaceae.

The fungus invades the host both inter & intracellularly, it grows rather fast covering large areas of the host tissue & eventually killing them in short time. It produces numerous sclerotial bodies on host tissue, which measure about 110-130-µ in diameter. Often the conidial or pycnidial stage is produced on the host.

The fungus is a facultative parasite capable of living saprophytically on dead organic tissue, particularly on many of its natural hosts producing sclerotial bodies, which produces pycnidia. When atmospheric temperature is above 30˚C & the pycniospores remain viable for over a year since the fungus attack wide range of plant species .The fungus is mainly a soil dweller and spreads from plant to plant through irrigation water, food and implements and cultural operation. The sclerotia & pycniospore may also become air borne and cause further spread of the pathogen (Rangaswami and Mahadevan 2008).

Since there was little information on dry root rot of Groundnut, it was important to study biology of this pathogen and information relating to *in-*

vitro studies on effect of different fungicides on the growth of the pathogen will help to device suitable management practices for dry root rot of Groundnut. Therefore, following objectives of the study was outlined. Since use of resistant cultivars is one of the most effective means of disease management. Diverse germplasms were also collected and screened in field conditions under natural incidence of the disease to identify genotypes which are completely free from dry root rot or genotypes with very low levels of the disease.

Despite of its economic importance the disease has not been studied in details. In view of the seriousness of the disease and enormous losses in the yield of Groundnut, the present investigation was proposed with the following objectives :-.

1. Cultural Studies on *Macrophomina phaseolina*.
2. Screening of germplasm line of Groundnut against *Macrophomina phaseolina.*
3. Effect of different fungicides on growth of *Macrophomina phaseolina* under *in vitro* condition.

The result of the investigations are presented and discussed in the following chapters.

⁂⁂⁂⁂⁂

CHAPTER- II

REVIEW OF LITERATURE

Stem and root rot of 'Groundnut' (*Arachis hypogaea* L.) caused by *Macrophomina phaseolina* (Tassi) Goid is a disastrous disease whose occurrence has been reported form different states of India and abroad. The important findings of earlier studies related to the present investigations have been reviewed under the following heads.

1. History and nomenclature of pathogen,
2. Occurrence and distribution,
3. Host range
4. Symptomatological study,
5. Morphology, cultural and physiological studies
6. Varietal screening
7. Disease management

History and nomenclature of pathogen

The present name *Macrophomina phaseolina* of the fungus causing root root of Groundnut came in existence after several modifications. Due to presence of sclerotial stage, it was first named as *Sclerotium bataticola* by Taubenhaus (1913). Later on it was transferred under genus *Rhizoctonia bataticola* (Taub.) Butler in 1918. The Pycnidial stage was described by Maublanc as *Macrophomina phaseolina* (Maubl.) in 1905 Ashby (1927) showed that the fungus produces a pycnidial stage corresponding *Macrophomina philippinensis* Petrak; the type species of the genus *Macrophomina*. Goidanich suggested on earlier name; *Macrophomina phaseolina*. Tassi and Goid proposed the combination; *Macrophomina phaseolina* (Tassi) Goid for same fungus.

Occurrence and distribution:

The disease is very destructive in nature and has been reported from many parts of the world, *e.g*. India (Pearl, 1923), Burma and Ceylon (Small, 1927a and 1927b), Palestine (Reichert, 1930), Cyprus (Nattrass, 1934), Greece (Sarejanni and Cortzas, 1935), Uganda (Hansford, 1940), Turkey (Bremer, 1944), Pakistan (Prasad, 1944), Nigeria (Anon, 1955) Syria (Al-Ahmad and Saidawi, 1988), Iran (Mahdizadeh *et al*., 2011).

Sundararaman (1932) has reported that the yield from affected plants with 36.6 per cent of the normal. The disease may be present in all Sesamum growing areas of India, although it has been recorded mainly from Madhya Pradesh (Pearl, 1923), Bihar (Mc Rae, 1930), Madras (Sundararaman, 1931) and Uttar Pradesh (Mehta, 1951).

Patel and Patel (1990) observed that the optimum temperature for growth and Sclerotial formation by *Macrophomina phaseolina* was 35ºC, both declined at temperature below 15ºC and above 40ºC. Under field conditions disease intensity increased with a progressive rise in temperature and decrease in Relative humidity They observed maximum disease occurrence at 35ºC and 76 per cent Relative humidity

Singh *et al*. (1993) reported that the severity of stem rot caused by *Rhizoctonia bataticola (Macrophomina phaseolina)* was reduced by sowing Sesame between 10-20 July, resulting in increased yield as compared with crop sown on Ist July

The earlier planting recorded maximum charcoal rot (*M. phaseolina*) incidence in sorghum and it reduced with delayed sowing (Lukade, 1995).

Singh and Sandhu (1995) found that low and higher moisture levels of the soil induced pre-emergence charcoal rot of cowpea but seedling mortality was severe only under stress soil moisture (below 40%) and decreased with increasing moisture level.

Collar rot disease (*Aspergillus niger*) a reported from Andhra Pradesh during 1986-89 was more prominent than, *Rhizoctonia bataticola* [*Macrophomina phaseolina*] infection Rao *et al*. (1997)

Mittal (1997) observed in general, disease incidence was less in the mixed than in the sole crop and gradually declined from the first to last sowing date. Sowing on 19 October was most effective in reducing the disease and increasing yield of lentil.

Pathak and Barman (1998) reported that the highest root rot incidence was found in the May sown crop. Among the varieties, JRO-878 recorded the highest disease incidence. High temperature, high relative humidity and rainfall favoured disease development in the late sown crop.

Maheswari and Ramakrishnan (2000) reported that Saprophytic survival of *M. phaseolina* was maximum at low moisture levels of 40% recording a mean of 65.9% against 15.9 and 7.9% survival at 60 and 80%, respectively. At 65 days of incubation, the saprophytic ability was 3.4% at 80% moisture holding capacity (MHC) compared with 73.4% at 40% MHC while, it decreased progressively with increase in incubation period at 60 and 80% MHC in both the inoculum levels.

Umamaheswari *et al.* (2001) reported that Maximum disease incidence was recorded in sterilized soil at 15-45 days after sowing. A significant increase in disease incidence (48.7 and 98.5 per cent) was observed with increasing inoculum level from 500 to 1000 mg/kg of unsterilized and sterilized soil, respectively. In unsterilized soil, 50 per cent reduction in root incidence due to the antagonistic activity of bacteria and actinomycetes was observed compared to sterilized soil.

Thakare *et al.* (2002) reported that root rot incidence in groundnut was more in non-mulch with no seed treatment as compared to polythene mulch. Per cent root rot reduction in mulch was 41% caused due to *Sclerotium rolfsii* [*Corticium rolfsii*] and *Rhizoctonia bataticola* [*Macrophomina phaseolina*].

Khan *et al.* (2003) showed occurrence of the disease but Sahiwal showed high incidence and severity in 1999. Distribution of the disease in

Sindh, Punjab and NWFP was 85, 83 and 48% respectively. Among Provinces NWFP showed highest incidence 57% and Punjab exhibited highest severity 2.62 according to 0-5 severity rating scale. Continuous increasing trend of charcoal rot is alarming for farmers and authorities engaged in sunflower business.

Malathi and Doraisamy (2003) observed increased growth of the pathogen (*M. phaseolina*), and growth, sporulation and biomass production of the fungal antagonist (*Trichoderma* spp.) were observed between 25 and 35^0C.

Cardona (2006) observed the influence of temperature and humidity on the vertical distribution of *Macrophomina phaseolina* sclerotia in a naturally infested soil in Turen, Portuguesa state, Venezuela. Samples were collected from the soil at depths of 0-5, 5-10 and 10-20 cm in plots sown after maize-sesame. Linear regression analysis showed that the quantity of sclerotia in the soil was negatively correlated with humidity but positively correlated with temperature. The quantity of sclerotia decreased, the humidity increased and the difference between maximum and minimum temperatures decreased at higher soil depths.

Jaiman *et al.* (2009) reported storage condition has direct influence on seed mycoflora. 8% seed moisture for 6 months displayed a minimum incidence of *M. Phaseolina* and maximum seed germination. Storage temperature of 40˚C and RH of 90% for 6 months of storage restricted for incidence of *M. Phaseolina* in cluster bean.

Kale *et al.* (2009) reported that TG 51 also had a lower dry root rot (*Macrophomina phaseolina*) incidence (13.0%) than TAG 24 (43.0%) at Kadiri during the Rabi season of 2003-04.

Sagir *et al.* (2010) observed that sowing time and irrigation conditions influenced the incidence of Charcoal rot in sesame lines. The lowest diseases percentage of sesame lines were changed according to sowing time and irrigation conditions. The lowest disease percentage were recorded from B-

60 line (40.60%), and the highest from C-36 line (48.98%). When all factors were together evaluated, the lowest disease percentage were recorded from irrigated (27.56%) and late sowing time (34.73%), the highest from in dry condition (57.98%) and early sowing time (50.82%).

Moradia and Khandar (2011) reported that a field survey was done during 2002-2003 at Main Oilseeds Research Station, Junagadh Agricultural University, Junagadh to study the loss of yield of groundnut due to dry root rot. The maximum plant mortality (root rot) of 29.3 per cent due to *Macrophomina phaseolina* with highest yield loss of 435 kg/ha was found in Keshod tehsil of Junagadh district of Saurashtra region.

The survey in different locations of Cuddalore district revealed the endemic nature of the root rot disease incidence with the maximum incidence of the disease (31.68%) registered in Vengatakuppam (MP18) location. The disease incidence was more in improved cultivars like, VRI2, JL24; more in sandy loam soils and rainfed conditions (Raja Mohan and Balabaskar (2012).

Taliei *et al.* (2013) reported that in the seasons of 2009-2010 and 2010-2011, disease incidence ranged from 0 to 97% and 3 to 91% with the highest in Gorgan and Aliabad, (valley), respectively.

Host range:

Butler and Bishy (1931) cited many records on *Solanum tuberosum* L. *Gossypium spp., Carchorus capsularis* L., *C olitorius* L. *Cajanus cajan* L. *Millsp., Arachis hypogaea* L., *Alysicarpus spp., Carica papaya* L., *Citrullus vulgaris schard.* L. *Millsp., Crotalaria juncea* L. *Cucurbita maxima Duchesene, Dolichos biflorus* L., *D. lablab* L., *Hibiscus cannabinus* L., *Lycopersicon esculentum* L., *Medicago sativa* L., *Morus alba* L., *Nicotiana tabacum* L., *Phaseolus lunatus* L., *P. aureus* Roxb., *Solanum melongena* L. *and Vigna sinensis* L. as a root, stem and tuber parasite throughout India.

Subramanian (1952) and Ramakrishnan (1955) isolated the pathogen from black cotton soil from Udamalpet, and Vandalur state respectively from Madras.

Nema and Agrawal (1960) reported it on roots of *Cicer arietinum* L. and *Pisum sativum* L. from Jabalpur (M.P.).

Singh and Nene (1990) reported that *Rhizoctonia* incited diseases in wide range of hosts, especially under high temperature and drought stress conditions.

Winter rape, sesame, saffron, squash, lucerne, cotton, potato, sorghum, cucumber, okra and *capsicum* have been reported as new hosts for *M. phaseolina* in Romania (Ionita *et al.*, 1995).

Jayati-Bhowal *et al*. (2006) observed the phytopathogenic fungus *M. phaseolina* infects many plants, e.g. jute (*Corchorus capsularis*), soybean (*Glycine max*) and groundnut (*Arachis hypogaea*).

Mahdizadeh *et al.* (2011) observed on marigold (*Tagetes erecta*), cantaloupe (*Cucumis melo* var. *cantaloupensis*), cumin (*Cuminum cyminum*), hemp (*Cannabis sativa*), mung bean (*Vigna radiata*), okra (*Abelmoschus esculentus*), tomato (*Lycopersicon esculentum*), turnip (*Brassica rapa*), and watermelon (*Citrullus lanatus*), which was reported as new hosts for *M. phaseolina*

Symptomatology:

Thirumalachar (1953) observed grey discoloration in the beginning at the infection site followed by blackening of the stems, which bear numerous sclerotia of the fungus, as characteristic symptoms of the disease.

Jain and Kulkarni (1965) observed different types of symptoms at various stages of growth of sesame plant. In seedling stage, the roots may become brown and rot resulting in death of the whole plant. In other plants, the fungus attacks on the collar region of the stem showing brown colouration which later extends upwards from few mm to few inches. Slowly and slowly, the whole plants becomes brown coloured and small dot-like black pycnidial

structures containing fungal spores are seen on stem, branches, capsules and seeds.

Singh and Chouhan (1973) observed that M. phaseolina infected seedling showed seed rot, dark brown color patches on root shoot transitional zone and brown or black circular spot on cotyledonous leaves. *M. phaseolina* has been reported to induced root rot symptom also in cluster bean.

Okwulehie (2002) reported that investigations on the anatomical features of various parts of groundnut (*Arachis hypogaea* cv. *valencia*) plants infected with the fungus, *Macrophomina phaseolina* [*M. phaseolina*], in Nigeria were made with the aid of a binocular microscope. Anatomically significant changes were observed in the hypocotyl, root and stem. Hypocotyl tissues were completely destroyed by *M. phaseolina* infection. Internal necrosis extended into the stem tissue. Plugging of vascular tissues, especially xylem vessels, was observed which probably induced wilting. Cells were generally experienced hypertrophy while those of the root cortex were destroyed.

Rasheed *et al.* (2004) observed that in seedling symptom test, *M. phaseolina, Fusarium* spp., *Rhizoctonia solani, A. flavus* and *A. niger* caused pre-emergence and post-emergence rot resulting in root rot and damping-off in seedlings.

Morphology:

Brooks (1928) observed smooth, hard and black sclerotia measuring 50-1000µ in diameter which occurred mainly on the infected roots. The pycnidia formed on the stems were variable in size ranging from 16-30 x 5-10 µ.

Thirumalachar (1955) reported that the fungus at 30ºC produced grayish white fluffy mycelia which were pro-geotrophic in growth response.

Deshpande *et al.* (1969) observed white to brown fluffy mycelium in culture. Mycelium was branched and sparsely septate. Sclerotia connected by short strands or fibrillae with the rest of mycelium and were brown to black in colour and globose to irregular in shape. It measures 66.4 µ

to 315 µ in diameter (av 136.12 µ), the irregular ones were 166.4 µ to 498 µ in size.

Subramanayam (1971) reported that sclerotia were jet-black, minute smooth, externally composed of anastomosed black hyphae, interior light to dark brown, composed of free thick-walled cells. Sclerotia variable in shape, globose, oval, oblong, elliptical, curved or even forked, varying in size 25x22-152x32 µ, produced abundantly in the infected host tissues. Spores are single celled, hyaline somewhat elongated or cylindrical, 16-30 x 5-10 µ in size.

Smits and Noguera (1990) reported that the formation of sclerotia in *M. phaseolina* began with branching and inter-winning of adjacent hyphal filaments. The pycnidial stage began with the merging of hyphal filaments towards a common point, followed by the development of ringed primodia and finally pycnidia. Matured pycnidia were sugblobose with a reticulate appearance, a short neck and a circular ostiole.

Cultural and physiological studies:

Various aspects of morphology, cultural, and physiological parameteters of *Macrophomina phaseolina* was studied by many workers on different host species. These studies assisted in characterized of the pathogen as well as its variation.

Paracer and Bedi (1962) observed the linear growth rate of the fungus and found that it increased as the nutritive status of the culture medium increased. Sclerotial formation started as early as 48 hours after inoculation on Brown's agar reinformed with 2 per cent potato (starch), while it took 168 hours or one week to start on the poorest medium, *viz.*, water agar on the other hand, rich media like Richard's agar and nutrient glucose agar, the formation of sclerotia were delayed considerably. Shanmugam and Govindaswamy (1973) observed that *Macrophomina phaseolina* grew best on Richard's medium at pH 5, with dextrose and asparagines as carbon and nitrogen sources. Cultural studied indicated that *Rhizoctonia bataticola* prefered.

Rhizoctonia bataticola grew most rapidly on potato dextrose agar and Richards'medium and showed profuse growth (Gupta and Kolte,1981). This study was further spported by Jha (1996) where potato dextrose agar and Richard's agar media supported excellent Sclerotial formation.

Okwulehie (2004) reported that the best synthetic medium and inoculation technique for the growth and development of *M. phaseoli* [*M. phaseolina*] was investigated. The physiological changes in groundnuts under infection with the pathogen were analysed. The media tested were potato dextrose agar (PDA), peanut leaflet oatmeal agar (POMA), Czapeks-Dox agar (CDA) and corn meal agar (CMA). The best growth was observed on POMA and PDA media.

Okwulehie (2005) observed some synthetic media were tested to investigate the best media that would best support the growth of *Macrophomina phaseoli* [*M. phaseolina*]. The fungus grew in both solid and liquid synthetic media, but the best growth was recorded in Peanut (groundnut) leaflet oat meal agar (POMA), and potato dextrose agar (PDA).

Varietal screening:

Li-ZhengChao and Qiu-QingShu (2000) reported that Huayu 16 is resistant to root rot, caused by *Macrophomina phaseolina*, drought and waterlogging, and tolerant of peanut stripe virus. This new cultivar is recommended for cultivation in medium or high fertility sand loam soils.

Gopal *et al.* (2006) reported that twenty groundnut genotypes were evaluated in Jagtial, Andhra Pradesh, India, for resistance to pod rot disease (caused by soilborne organisms, such as *Rhizoctonia solani, Fusarium solani, Sclerotium rolfsii* [*Corticium rolfsii*], *Fusarium oxysporum and Macrophomina phaseolina*). Based on percent disease index (PDI), the cultivars were classified as immune (disease-free), resistant (0.10-10.0 PDI), moderately resistant (11.1-30.0 PDI), susceptible (30.1-50.0 PDI), and highly susceptible (>50.0 PDI). PDI, percent incidence (PI), and percentage of pods

infected (PPI) ranged from 38.8 to 65.9, 39.9 to 94.4, and 9.3 to 90.7%, respectively. The lowest PDI values were recorded for INS 9013 and R 8808 (38.8% for each), followed by R 8972 (38.9%) and ICGV 86885 (39.2%). PI was lowest in R 8972 (39.9), followed by ICGS 11 (53.6%), ICGV 86885 (55.9%) and R 8806 (61.2%). R 8972, R 8808, ICGV 86885, R 8806 and ICGS 11 registered the lowest PPI (9.3, 10.4, 11.8, 13.1 and 15.1, respectively).

Vijay Mohan *et al*. (2006) said that twelve chickpea cultivars were evaluated for resistance against dry root rot disease (*Macrophomina phaseolina*) under natural and artificial conditions. Screening under natural conditions were conducted during the Rabi seasons of 2001-02 and 2002-03 in Jharkhand, India. For artificial conditions, potted soils were inoculated with mycelial bits of the pathogen and seeds were sown at 4 seeds per pot three days after soil inoculation. Under natural conditions, the mean disease incidence was lowest (8.0%) in G-543, followed by BG-360 and GNG-1365 which recorded mean disease incidence of 9.6 and 9.8%, respectively. susceptible.

Kale *et al.* (2007) observed that TG 38 showed resistance to stem rot (*Sclerotium rolfsii* [*Corticium rolfsii*]) and dry root rot (*Macrophomina phaseolina*).

Prasanthi (2007) observed sixty-one indian bean (*Phaseolus vulgaris*) lines collected from different sources were screened for resistance to dry root rot disease (*Rhizoctonia bataticola* [*Macrophomina phaseolina*]) under natural field conditions of Tirupati, Andhra Pradesh, India, during kharif, 2005.

Moradia (2012) conducted a pot experiment was to assess the varietal resistant of seventy one groundnut varieties against dry root rot disease (*Macrophomina phaseolina*). Out of seventy one varieties, 28 varieties were found resistant which are spreading type, 6 varieties were moderately resistant and rest 37 varieties were found susceptible to *M. phaseolina* which were bunch type of varieties.

Chemical control:

Spraying of Derosal, J K Stein and Topsin-M gave the best result against stem blight of cowpea induced by *Macrophomina phaseolina* and increased the yield (Sharma *et al.*, 1995).

Shalaby (1997) studied the effect of benlate , vitavax, Rhizolex T and Tecto TBZ (thia bendazole) on disease severity and as seed treatments on soil fungi causing sesame root rot and concluded that benlate and vitavax were the most effective seed dressings against the *Macrophomina phaseolina* under both laboratory and green house conditions.

Rao *et al.* (1998) reported that Seed treatments consisting of carbendazim WP, carbendazim SD + captan, carbendazim + thiram, captan, thiram, mancozeb, tolclofos-methyl, pyroquilon, TCMTB, carbedazim-Jkstein, quintozene, captafol, carboxin and triadimenol were tested for the management of these fungi under artificial infection of the seed. The seed treatments were found to control the damage due to these fungi. Among the treatments, carbendazim SD (0.05% + captan 0.125%) was the best in reducing seed rot and pre- and post-emergence seedling blight and in increasing pod yields significantly in all the three seasons of field experimentation, followed by treatment with captafol (0.25%).

Malathi and Doraisamy (2003) reported that the interaction of *T. harzianum* (TH-5) with different fungicides (captan, thiram and carbendazim) in controlling *M. phaseolina* infecting groundnut. The antagonist and the fungicides were tested individually and in combination on the pathogen. The toxicity of the fungicides at 5, 10, 50 and 100 micro g/ml was also tested on *T. harzianum*. Growth of TH-5 and the pathogen were completely inhibited by carbendazim at 5 and 100 micro g/ml, respectively.

Choudhary *et al.* (2004) observed the effectiveness of four fungicides, *i.e.* Bavistin [carbendazim], Antracol [propineb], Indofil M-45 [mancozeb + thiophanate-methyl] and Ridomil MZ [mancozeb + metalaxyl], applied at 300,

400, 500 and 1000 ppm, in inhibiting the mycelial growth of *Macrophomina phaseolina*, the causal agent of stem and root rot of sesame, was studied *in vitro* using the poisoned food technique. All fungicides dose-dependently inhibited mycelial growth compared with the untreated control, with Bavistin being the most effective.

Rai *et al.* (2005) observed maximum germination was recorded when seeds were treated with Dividend or *T. viride* combined with Vitavax. Seed treatments with mancozeb and thiram were the least effective, but were superior to the control.

Vijay Mohan *et al*. (2006) reported that in fungicidal trials on management of dry root rot of chickpea caused by *Macrophomina phaseolina*, Carbendazim (0.2%) and Etaconazole (0.1%) used as seed treatment, soil drenching and seed treatment plus soil drenching recorded lowest disease incidences of 15.6 and 18.2 per cent and highest grain yield of 192 and 18.9 q/ha respectively, during Rabi, 2001-2002 and 2002-2003 crop seasons.

Bainade *et al.* (2007) observed that the effects of chemical and biological control of *Macrophomina phaseolina*. Treatments comprised: mancozeb, quintal, carbendazim, Benlate [benomyl], tricyclazole and benomyl, and *Trichoderma viride, T. harzianum* and *T. lignorum* [*T. viride*]. Mancozeb (2.5%), quintal (2%) and tricyclazole (1%) completely inhibited the growth of *Macrophomina phaseolina* in *in vitro* conditions.

Khan *et al.* (2008) reported that carbendazim and captan were highly inhibitory in culture of *M. Phaseolina. P. fluorescence* strain PFBC-25 was comparatively less sensitive to carboxin, chlorothalonil and carbendazim. Captan and carboxin were relatively more toxic to strain PFBC-26 .The mycelia growth of *M. phaseolina* was significantly reduced by both the strains of *P. fluorescence.*

Ammajamma and Hegde (2009) reported that Carboxin (0.05 and 0.1) and Hexaconazole, Metalaxyl and Triadimefon at 0.1 per cent completely

(100%) inhibited the growth of *R. bataticola* and among the non-systemic fungicides tested, Thiram at 0.1, 0.2 and 0.3 per cent concentrations was found effective against *Rhizoctonia bataticola*.

Hegde and Chavhan (2009) reported that effective fungicide for the management of root rot. Drenching with fungicides like carbendazim @ 0.1%, hexaconazole @ 0.1%, mancozeb @ 0.2% and carboxin+thiram @ 0.1%, have managed the root rot effectively.

Rajani and Parakhia (2009) observed that soil application of neem cake with *T. harzianum* was found most effective in reducing the disease and in increasing seed yield (2126 kg/ha). It gave the highest net return (1:7.91). Mustard cake with *T. harzianum* was the next best treatment (ICBR1:5.05). Soil application of neem or mustard cake @ 500 kg/ha along with talc based preparation of *T. harzianum* @ 5kg/ha may be applied just before sowing in furrow for an effective management of root rot disease and for improving yield of castor.

Jaiman and Jain (2010) reported that efficacy of fungicides *viz*., bavistin, raxil, topsin M, captan, indofil M-45 and thiram were tested against *M. phaseolina* causing root rot in cluster bean both *in vitro* and *in vivo*. Maximum inhibition of fungal growth was found with bavistin followed by topsin M. the fungicides checked the disease as compared to control.

Tandel *et al*. (2010) observed that for the control of the disease, seven fungicides were tested. Among them Carbendazim + mancozeb (Sixer) was found significantly superior over the rest as it resulted minimum (8.13%) disease intensity. This suggested that leaf blight of mung bean (*Macrophomina phaseolina*) can be controlled very effectively by spraying of carbendazim + mancozeb (Sixer) and the huge crop loss can be saved if sprayed at the time of disease initiation.

Moradia (2011) reported that poisoned food technique was employed to study the efficacy of different nine systemic fungicides at 250, 500

and 1000 ppm against *Macrophomina phaseolina* (groundnut isolate) under *in vitro* conditions. All the fungicides were capable of inhibiting the growth of the fungus at all the concentrations tried. Difenconazole (Score 25% EC), carboxin (Vitavax 75% WP) and saaf (SAAF 75%) were found to be the best, which caused cent per cent inhibition of growth at all the concentrations tried.

Sreedevi *et al*. (2011) said that five *Trichoderma* spp. were isolated from the rhizosphere soil of healthy groundnut plants, identified using morphological and microscopic characteristics and were evaluated for *in vitro* antifungal activity against *M. phaseolina* by dual culture plate technique and bioassay methods (*in vitro* antibiosis). Scanning electron microscopy was used to study the conidial surface of *T. harzianum*. Among the five isolates *T. harzianum* (T_3), *T. viride* (T_1) had maximum antifungal activity against *M. phaseolina* compared to the other *Trichoderma* spp. In dual culture technique *T. viride* and *T. harzianum* reduced mycelial growth by 61.1% and 64.4%, respectively. Based on the dual culture technique, *T. harzianum* (T_3), *T. viride* (T_1) were selected for further research.

Nageswararao *et al.* (2012) reported that pot culture studies with groundnut cv TCGS 888 under sterilized soil conditions indicated efficacy of mancozeb seed treatment @ 3 g/Kg seed (66.7%) and neem cake soil application @ 0.51/ha (66.7%) applied either alone or in integration with *T. virens* in decreasing groundnut root rot incidence.

⁂⁂⁂⁂⁂

CHAPTER- III

MATERIALS AND METHODS

Present investigations were carried out in the Department of Plant Pathology, B A U, Sabour, Bhagalpur. The field trials were conducted at the Jalalgar, Regional Research Sub-station (RRS), Purnia. The Jalalgar RRS is situated at 25^0 13' N to 25^0C latitude and 87^0 12' to 88^0C 5'E longitude with an attitude of 32.66 meter above mean sea level. The plot had a fairly uniform topography and the soil was deep well drained sandy loam. Recommended package of practices was followed to raise a good crop of groundnut. Detailed methodologies followed to study the various aspects of the disease and the casual organism are described hereunder.

3.1 General method of sterilization & preparation of media:

Cleaning and Sterilization of glass wares:

Different types of glassware like petridishes, flasks, pipettes, test tubes, Thistle funnels to be used during experiments were washed and cleaned. After that these were treated with strong cleaning solution prepared by dissolving 80 grams of potassium dichromate ($K_2C_2O_7$) in 400 ml of concentrated H_2SO_4 and diluted in 300 ml of distilled water, again washed thoroughly, first in running tap water and finally with sterilized water as described by Riker and Riker (1936). After normal air drying the glasswares were sterilized at 180^0C for 1¼ hours in the hot air oven. The inoculating needle was dipped in spirit and heated over the flame of sprit lamp until red for 2-3 times. Inoculating needle was used for inoculating and transferring inoculum from one culture tube and Petriplates to another, whereas 6 mm cork-borer was exclusively used for transferring measured quantity of inoculum either in solid or liquid media wherever required.

Sterilization of sand and pot filling:

Sand was sterilized for two consecutive days in an autoclave at 15 lb/sq. inch pressure for 30 minutes. The pots were washed thoroughly with water, rinse with one per cent (1%) formalin and dried in sun before use. Pots were filled up with the sterilized sand covered with polythene sheets in order to prevent aerial contamination.

Preparation of media:

The following culture media were used in various cultural and physiological studies. The media were prepared according to the standard formula given by Riker and Ricker (1936) and Anisworth and Bisby (1967). The constituents of different media used during investigation were as follows:

(A) Non-synthetic media

(1) Oat meal agar

Oat meal	:	50 g
Agar-agar	:	20 g
Distilled water	:	1 L

(2) Maize meal agar

Maize meal	:	50 g
Agar-agar	:	20 g
Distilled water	:	1 L

(3) Carrot medium.

Carrot (grated)	:	50 g
Distilled water	:	1 L

(B) Semi senthetic media

(4) **Potato-dextrose –agar**

Potato (peeled)	:	200 g
Dextrose	:	20 g
Agar-agar	:	20 g
Distilled water	:	1 L

(C)Synthetic media

(5) Czapek's (dox) medium

Sucrose	:	30 g
Sodium nitrate	:	2 g
Potassium dehydrogen phosphate	:	1 g
Potassium chloride	:	0.5 g
Magenesium sulphate	:	0.5 g
Ferrous sulphate	:	0.01 g
Distilled water	:	1 L

(6) Asthana and Howker's medium.

Glucose	:	5.00 g
Potassium nitrate	:	3.50 g
Potassium dehydrogen phosphate	:	1.75 g
Magenisium sulphate		75 g
Distilled water	:	1 L

(7) Glucose asparagine medium.

Glucose	:	30.00 g
Asparagine	:	1.00 g
Magenesium sulphate	:	0.5 g
Potassium dehydrogen phosphate		1.50 g
Distilled water	:	1 L

(8) Glucose peptone medium

Glucose	:	10.00 g
Peptone	:	2.00 g
Potassium dehydrogen phosphate		50 g
Distilled water	:	1 L

(9) Richard's medium

Sucrose	:	50.00 g
Potassium	:	10.00 g
Potassium dehydrogen phosphate		5.0 g
Magnesium sulphate	:	2.50 g
Ferric chloride	:	0.02 g
Distilled water	:	1 L

Preparation of solid media:

For preparation of synthetic media, various ingredients were dissolved in 500 ml of distilled water in one litre conical flask and agar-agar at the rate of 2 per cent was boiled in another 500 ml distilled water. The contents of both the flasks were mixed together in one flask and volume was made upto one litre. The media contained in flask was autoclaved at 15 lbs/sq. inch pressure for 15 minutes and after sterilization required quantities of media were poured aseptically in different sterilized petriplates and test tubes.

For preparation of PDA, 200 gm of peeled potatoes were cut into slice and boiled in about 500 ml of distilled water for half an hour. It was then filtered through muslin cloth and the filterate was taken in a flask. In Another flask 500 ml of distilled water with 20 g agar-agar powder was boiled. Filtrates of potaoes and boiled agar were mixed thoroughly in one litre beaker. Then, 20 g of dextrose was mixed in it and volume was made up to one litre by adding distilled water. The flask was plugged with non-absorbent cotton and autoclaved at 15 lbs/sq. pressure for 15 minutes. For slant preparation 10 ml. of medium were poured into test tubes separately. The test tubes were plugged

carefully with non-absorbent cotton and autoclaved. Then, the test tubes were Spread over the slanter for solidification.

In case of non-synthetic media, like carrot, corn meal or oat, all the procedures followed in preparation of PDA were same except dextrose which was not added in these media.

Autoclaved media in which agar had been mixed was cooled down to around 45^{o}C and 20 ml of it was poured in sterilized petriplates of 90 mm diameter in aseptic condition to ensure equal depth and diameter. Sufficient number of petriplates were poured with medium to conduct the investigation in three replications for each medium/treatments.

The test tubes containing 10 ml solid medium were carefully slanted at 10-15^{o} angle by putting it on a wooden slanter after autoclaving mixing it thoroughly by rotating the tube between palms. After solidification they were used for inoculationing the pathogen and preparing the tubed cultures.

Preparation of liquid media:

All the ingredients of a solid media were used for preparation of liquid media omitting agar-agar. The required quantities of ingredients were weighed and dissolved in 1000 ml. Erlenmeyer flask. The flasks were then plugged with non-absorbent cotton and finally autoclaved at 10 lbs/inch pressure for 15 minutes.

3.2 Isolation, Purification and maintenance of the pathogen:

Isolation were made on PDA Medium from different parts of the diseased plants showing characteristic symptoms of stem and root rots. The specimens were first washed by passing it through running tap water to remove dust or soil particles if any. Diseased parts just touching the healthy portion were chosen and separated with the help of sterilized blade and were cut into smaller pieces (2-5 mm in size). These pieces were washed thoroughly in sterilized water in order to remove surface-contaminates and then surface

sterilized with 0.1 per cent mercuric chloride solution for 30 seconds only with the help of camel hair brush. These pieces were washed thoroughly in three consecutive changes of sterilized distilled water to remove the residue of $HgCl_2$ completely. Excess moisture was removed by putting the pieces pressed in between two folds of sterilized blotting paper. Then it was transferred to PDA slants aseptically in laminar flow. The inoculated PDA slants were incubated in BOD incubator at temperature. As soon as the mycelial growth was visible around the inoculated pieces, growing fungal tips were transferred to the sterilized medium previously poured into sterilized petridishes. After 3-4 days, they were again transferred to a fresh PDA slant to obtain pure culture by hyphal tip isolation method. Regular transfer of hyphal tip pure culture of the pathogen was done during the period of investigation for maintenance of the pathogen.

Maintenance and preservation of culture:

The stock culture was maintained on PDA slant and preserved in refrigerator at 5-6^{o}C. The pathogen was subcultured at a regular intervals of 30 days to keep the fungus in active stage.

3.3 Morphological studies of the pathogen:

Morphological studies of the pathogen was done from the pure culture of the fungus made earlier. Mycelial suspension in sterilized water was made from pure culture grown on one PDA. One drop of the mycelia suspension was kept on slide and morphological studies of pathogen were carried out by examining the slide through a research microscope. Character of mycelium viz., branching patterns, width and colour, texture, shape, and size were recorded with the help of ocular and stage micrometers calibrated on a research microscope.

3.4 Test of pathogenicity:

To test the pathogenicity, pot experiment were conducted in Department of Plant Pathology, B.A.U., Sabour. The disease free Groundnut seeds of variety, R-20 were sown in 3" trays, previously washed with one percent formalin solution and filled with sterilized sand. After germination, 5-6

seedlings per tray was maintained. Tray were watered frequently to maintain sufficient moisture in the trays.

Preparation of inoculum suspension:

A 6 mm disc from 10 days old culture of the plated fungus (*M. phaseolina*) grown on PDA medium was transferred to each of 250 ml Erlenmeys flask containing 100 ml potato dextrose broth and were incubated at 25±1^{o}C for 15 days. Mycelium and spore were collected by filtering through Whatman filter paper No.1 which were macerated intermittently for 2-3 minutes in mortar and pestle and diluted in sterilized water to obtain 200 ml of mycelia matle suspension of fungus.

Inoculation of groundnut plants:

The one week seedlings were taken and the roots were washed in sterilized distilled water. The roots were dipped in mycelia suspension for 10 min and then incubated in wet towel paper at 25^{0}C for 48 hrs. Observations were recorded till 5 days of inoculation in disease incidence. The microorganism was reisolated from plants showing rotting symptom and brought into pure form by comparing their cultural and morphological characters with those of original one.

3.5 Cultural studies:

Method of inoculation:

Eight days (8) old culture of the fungus growing uniformly on potato dextrose agar plate was used as inoculum with precautions that the quality of the mycelium could be more or less same. The size of the inoculum was standardized by cork-borer having an internal diameter of 9 mm. the inoculums grown uniformly on PDA was cut-out with sterilized cork borer from one zonation. The piece of inoculum was placed with the help of sterilized inoculating needle at the centre of the petridishes containing medium, in an inverted position to bring the inoculum into direct contact with the

surface of the medium. Whereas in case of culture flask, the inoculums *i,e* equal volume of spore suspension made from pure culture in sterilized pipettes and shaked well in order to mix the inoculum thoroughly. Inoculation was made in aseptic conditions in laminar flow chamber. After inoculation, the petridishes and the flasks were incubated at room temperature 25 ± 1^0C.

Measurement of radial growth:

Radial growth of mycelium in petridishes on different solid media were measured by drawing two lines passing through centre of the cultured plates at right angle to each petridishes. The diameter of the fungal growth on these two lines were measured and average of two expressed as diameter of the developing colony in mm. Thus, linear growth of the colony was measured into two directions. In the case of wavy, irregular growth, colony averaging growth was measured by averaging the largest and shortest diameters of the fungal growth (Brown, 1923).

Estimation of dry weight of mycelia growth:

The spore suspension from 8 days old culture grown on PDA was prepared in sterile distilled water for inoculation of liquid media. One drop of spore suspension were added to 25 ml media contained in 100 ml. Erlenmeyer flask aseptically with the help of sterilized pipettes. The flasks thus inoculated were incubated for 21 days at 25 ± 1^0C.

At the end of the required incubation period, the mycelium mats were filtered through Whatman filter paper No. 42. The filter paper containing mycelia mats were dried in the hot air oven at 36^0C for 96 hours and subsequently cooled in desiccators containing concentrate H_2SO_4 before finally recording their constant weight over a balance. For five each and every treatment, three replications were kept and mycelia weights were obtained accordingly.

The dry weight of the mycelium mats of the fungus was calculated in mg as follows:

$$DW = W_2 - W_1$$

Where,

DW = Dry weight of fungal mass.

W_2 = Weight of fungal mass along with filter paper

W_1 = Weight of filter paper alone.

3.6 Screening of groundnut cultivars/entries against *M. phaseolina:*

The following 20 cultivars & entries of groundnut collect from Regional Research Sub-station, Jalalgar, Purnia were used for screening under natural condition against *Macrophomina* root rot pathogen : ICGV00338, ICGV02OO5, ICGV02038, ICGV02125, ICGV4017, ICGV06237, ICGV06279, ICGV06285, ICGV06319, ICGV07210, ICGV07211, ICGV07213, ICGVO7214, ICGVO7214, Chico, R20, DH86, ICGV91114 ,ICGV02266, JL24.

Experiments were carried out for screening the groundnut cultivars & entries against *Macrophomina* root rot incidence:

Under field condition at Regional research sub-station Jalalgar, Purnia

For screening the entries against Macrophomina root rot disease, these entries were sown in row at Regional Research Sub-station, Jalalgar, Purnia as per following specification. All the agronomic requirements were met with utmost care as standard recommendations for groundnut crop. Final disease observation in terms of incidence per cent was recorded at flowering stage.

Conduction of performance trial : Kharif and Spring season cultivation

Date of sowing : 24th July'2013

	:	10th March'2014
Number of entries	:	20
Design	:	Randomized Block Design (RBD)
Replication	:	3
Spacing	:	30cm x 10cm

The observation regarding infection of plants were recorded. A 0-5 point rating scale (as given below) was used for scoring the reaction of entries/lines against the pathogen (Anonymous, 1998).

Scoring scale

0 - No symptoms on the plant

1 - 1 or less plant/surface affected

2 - 1-10 plants/surface affected

3 - 11-25 plants/surface affected

4 - 26-50 plants/surface affected

5 - 51 or more plants/surface affected

Interpretation of scores:

Score	**Per cent infection**	**Reaction**
0	No symptoms	Immune
1	1% or less infection	Most resistant
2	1-10% infection	Resistant
3	11-25 infection	Moderately susceptible

4	26-50 infection	Susceptible
5	51% or more infection	Highly susceptible

Field trial on Groundnut germplasm was carried out at Regional Research Sub-station Jalalgar, Purnia during 2013, Kharif season and 2014, spring. 20 Groundnut germplasm were provided by Bihar Agricultural University, Sabour used for screening against root rot. The disease incidence of root rot will be recorded for each germplasm lines.

$$\text{Disease incidence} = \frac{\text{No. of infected Plants}}{\text{Total Plants}} \times 100$$

3.7 Evaluation of different fungicides against *M. phaseolina in vitro:*

The efficiency of various fungicides belonging to different groups were tested against the pathogen *in vitro* as well as *in vivo*.

The details of the fungicides evaluated in the present investigation are given below :

Table- 1: List of fungicides evaluated :

Trade/ common Name	Chemical Name	Active Ingredients	Place of Manufacturing
Bavistin	Carbendazim	50% WP	Saraswati Agro Chemical (Indian) Pvt. Ltd. Lone-2, Phase- 1,SIDW Industrial complex, Bari Brahmana, Dist- Jammu & (J & K)
Hexaconazole Malinda		5% EC	Crystal Phosphates Ltd.
Dithane M-45	Mancozeb		
Hillblast	Tricyclazole	75%WP	Hindustan Insecticides Ltd. (A Government of India Enterprise) An ISO 9001:2008 Certified Company

Thiram		75 % WS	GSP Crop Science Pvt. Ltd 531, phase 11. G.I.D.C, Kathwada, Odhav Road. Ahmadabad -382430. Gujarat
Trichoderma – viride (Panther-TV)			Liebigs Agrochem Pvt. Ltd. 3A, Dhar Madas Row, Kolkata – 700 026
Combiplus	Carbendazim + Mancozeb	12% WP + 63% WP	Nagarjuna Agrochem Limited plot No. 61, Nagarjuna Hills, Punja-gutta, Hyderabad-83 Andhra prudish Factory Ethalkota- 533238, E.G. Dist, A.P

Preparation of stock solution of fungicides evaluated:

Effect of different fungicides on radial growth of *M. Phaseolina* was studied *in vitro* using poisoned food technique (Nene and Thapliyal, 1979). 100 ml of stock solution of 40,000 ppm 10,000ppm, 20,000 ppm and 5000were prepared for all the four fungicides viz., Carbendazim, Mancozeb, Tricyclazole and Hexaconazole used in experiments. For this 400 mg,100mg, 200mg and 1ml of the fungicide was incorporated in 10 ml, of sterilized distilled water in 100 ml Erlenmeyer flask. To obtain desired concentration of fungicide in the medium, an amount of stock solution to be added in PDA was calculated by using the following formula:

$$C_1V_1 = C_2V_2$$

Where,

C_1 = Concentration of stock solution

C_2 = Concentration of fungicide desired.

V_1 = Volume (ml) of stock solution to be added

V_2 = Measured Volume (ml) of PDA in which fungicides is to be added.

Four concentrations of Carbendazim, Mancozeb, Tricyclazole and Hexaconazole fungicides *i.e.* 1ppm, 5ppm, 10ppm, 20ppm, 30ppm, 40ppm, 50ppm, 100ppm and 200ppm were used against the pathogen, to examine the

inhibitory effect on the mycelium growth. The requisite quantities of fungicides calculated from formula given above were taken from stock solution already prepared with the help of micro pipette and incorporated into sterilized unsolidified potato dextrose agar medium. The mixture was shaken well to make it homogeneous. PDA poisoned with different fungicides was poured aseptically into 90 mm sterilized petriplate @ 20 ml per plate in Laminar flow chamber.

These plates were allowed to solidify and then inoculated with 9 mm disc of *M. phaseolina, c*ut from 7 days old cultures, by placing the inoculums in the centre of the petriplates as mentioned earlier. PDA plates without fungicide were also inoculated with test fungus inoculums to serve as check. Three replications for each treatment were maintained. All petriplates were inocubated at 25 ± 1^0C for 10 days. The relative efficacies were known by measuring the radial growth of the mycelium and the data were converted to per cent inhibition of growth over check by using following formula :

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

Where,

C = Average colony diameter in check (mm)

T = Average colony diameter in the treatment (mm) in fungicide amended medium.

3.8 Effect of different fungicidal treatment on the root rot disease of Groundnut caused by *M. phaseolina:*

Three fungicides and bio control agent *viz.*, thiram, copper oxychloride, combiplus (Carbendazim (12% WP) + Mancozeb (63%WP)) and biocontrol trichoderma were tested as seed treatment. Before sowing, the seeds treated with required quantity of fungicide. The disease free Groundnut seeds of variety R-20 were sown in 3" trays, previously washed with one percent

formalin solution and filled with sterilized sand. After germination, 20 seedlings per treatment were artificially inoculated as described earlier and incubated at 25^0C by paper towel method for 5 days. Observations were recorded on germination percentage and disease incidence.

3.9 Dual culture:

Dual culture plate technique (Sreedevi *et al*., 2011) was used to study the antagonistic effects of the *Trichoderma* isolates on *M. phaseolina.* All antagonistic pathogen combinations were examined on 20 ml of PDA in 9-cm petriplates, with four replicate plates per treatment. For dual culture technique, a mycelial plug (0.5cm in diameter), taken from actively growing 3 days old culture of *M. phaseolina* and *Trichoderma* isolates placed 8cm apart from each other on the PDA. For control treatments, a plug of *M. phaseolina* was placed on the PDA medium. The plates were incubated at 28^0C. Observations on the antagonistic activities of *Trichoderma* isolates on *M. phaseolina* were recorded after every 24 hrs for 5 days and inhibition percentage was calculated using the following formula (Edington, 1971).

$$\text{Inhibition percentage (\%)} = \frac{A1-A2}{A1} \times 100$$

Where, A_1 is the colony area of uninhibited *M. phaseolina* in the control and A_2 is the colony area of *M. phaseolina* in dual culture.

⁂⁂⁂⁂⁂

CHAPTER- IV

EXPERIMENTAL FINDINGS

The present investigations were carried out on *Macrophomina* root rot of groundnut. The results obtained during investigations have been described characterisation and management of pathogen under following subheads:

4.1. Symptoms:

The characteristic symptom of the disease, as observed on stem and roots of infected groundnut plants is described as under:

On stems:

Initially, dark cortical lesion were observed near the collar region of stem at about just before flowering i.e. 20-25 days after sowing. Rapidly, these lesions enlarge in size from few mm to few centimeter. In advanced stage of infection the lesion extended upward and downward, gridling the affected portion from all sides, gradually the whole plant become brown coloured, rotted at affected portion, which eventually shrivels and collapses. The affected plants showed general yellowing, drooping of leaves and ultimately death of plants at premature stage. (Plate 1, B and C)

On roots:

When infected plants were carefully uprooted, black coloured roots were observed. It appeared as black charcoal. The root becomes brittle and broke easily when bent. Roots infection was found extend upto collar region in some of the infected plants.

Plate 1: Symptoms of Dry root rot of groundnut caused by *Macrophomina phaseolina* (A) Healthy plant (B) Plant infected by *M.phaseolina*.(C) Symptom on stem (D) Culture of *M. phaseolina*

4.2 Isolation and purification of the pathogen:

Isolation of the causal pathogen was made on PDA medium from different diseased specimens collected from Regional Research Sub-station, Jalalgar, Purnia by technique was described earlier. The isolated fungus was purified by sub culturing and the purified culture maintained in PDA slants for further studies.

4. 3 Morphological studies:

Morphological studies, to characterize of *Macrophomina phaseolina* was 10 days old culture, grown on PDA were taken (The slides

were prepared in Lacto phenol and examined under research microscope). Mycelium was seen as white flaffy in the beginning, which changed to dirty white with age. Mycelium was septate, branched near the distal septum of the fungal cell often at right or acute angled in older hyphae and the branches were slightly. Constricted at or near the point of origin with a septum placed nearby. The diameter of hyphae varied from 1.52 to 2.21 µm. Microsclerotia were not seen under *in vitro* conditions.

On the basis of the above characters and close symptom the pathogen was identified as *Macrophomina phaseolina* (Tassi.) Goid. (Plate 1, D).

4.4 Pathogenicity:

After artificial inoculation and incubation period of seedling at 25^0C for 10 days, the inoculated plants were kept in B.O.D for disease development. Regular observations were made for recording gradual development of symptoms. 10 days after artificial inoculation, symptoms were clearly observed. The collar region showed browning and rotting which extended to root also and distinguished the infected portion from the uninfected one (plate 2). The organism was re-isolate from the infected portion of the stem of the inoculated plants by the methods already stated earlier. Then their cultural and morphological characters were compared with those of original one, which was found similar. Thus satisfying the Koch's postulates, and the pathogenicity of the fungus *Macrophomina phaseolina* was established.

Plate- 2: Stem and root rot symptoms on artificially inoculated plants

4.5 Cultural studies of the pathogen:

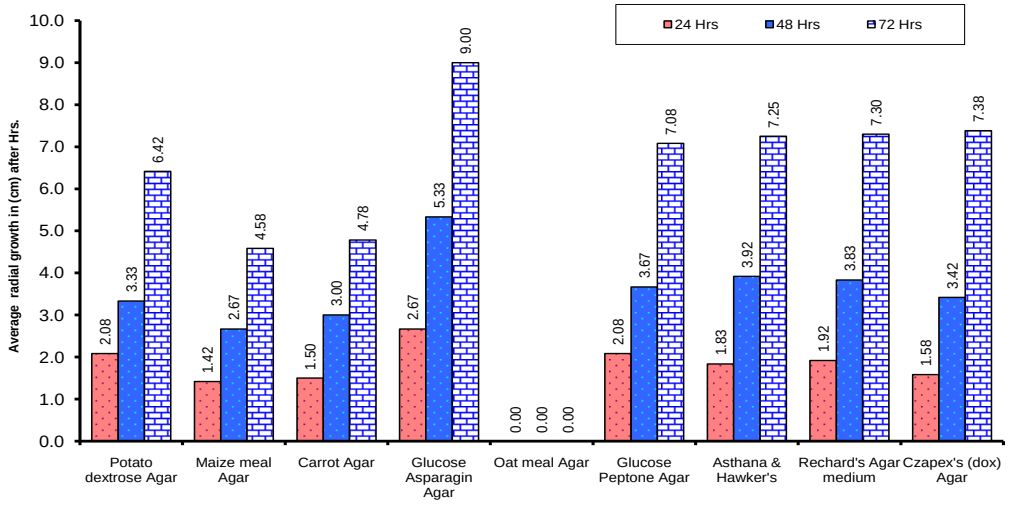
4.5.1. Effect of different solid media on the radial growth of *M. phaseolina:*

Different constituents are required by various fungi in varying quantities for their growth and hence a particular medium may be suitable for a particular fungus and other for another one. Keeping this very fact in view, an experiment was planned and conducted to find out a suitable culture medium for growth and conidial production of *M. phaseolina*, the causal pathogen of root rot disease of groundnut. Therefore, altogether nine solid media of various origin and composition were used for this purpose, which were potato dextrose agar, maize meal agar, carrot agar, glucose asparagines agar, oat meal agar, glucose peptone agar, Asthana and Howker's agar, Richard's agar, and Czapek's agar medium. Petri- plates containing 20 ml of medium were inoculated with 8 days old culture, bit of 6mm size and studied according to the method described earlier. Radial growth was measured after 24, 48, and 72 hours of inoculation. The results so obtained are presented in Table-1 and illustrated graphical in Figure-1 & Plate -3. (a).

Table-2: Effect of different solid media on radial growth of *M. phaseolina* at 25± 1ºC.

Sl. No.	Media	*Average radial growth (in mm)		
		24hrs	48hrs	72hrs
1.	Potato dextrose agar	20.8	33.3	64.2
2.	Corn meal agar	14.2	26.7	45.8
3.	Carrot agar	15.0	30.0	47.8
4.	Glucose Asparagin agar	26.7	53.3	90.0
5.	Oat agar	00.0	00.0	00.0
6.	Glucose peptone agar	20.8	36.7	70.8
7.	Asthana and Hawker's	18.3	39.2	72.5
8.	Richard's agar	19.2	38.3	73.0
9.	Czapek's dox agar	15.8	34.2	738
	SEm±	0.26		
	C.D. at 5%	0.53		
	C.V. (%)	9.92		

*Average of three replications



Result tabulated above in the Table-2 revealed that all the solid media under test supported mycelia growth of *M. phaseolina*. Glucose asparagines agar medium proved to be the best medium in respect of radial growth of mycelium covering full plate with compact colony growth in 90 mm petri- plate after 72 hrs of inoculation followed by Czapek's dox agar (73.8 mm), Richard's agar (73.0 mm), Asthana and Howker's agar (72.5 mm), and glucose peptone agar (70.8mm). No and scanty fungal growth was obtained on oat meal agar medium measuring only 0 mm in diameter. Other media in order of superiority were potato dextrose agar (64.2 mm), carrot agar (47.8 mm) and maize meal agar (45.8 mm).

4.5.2. Effect of the different liquid media on growth of *M. phaseolina:*

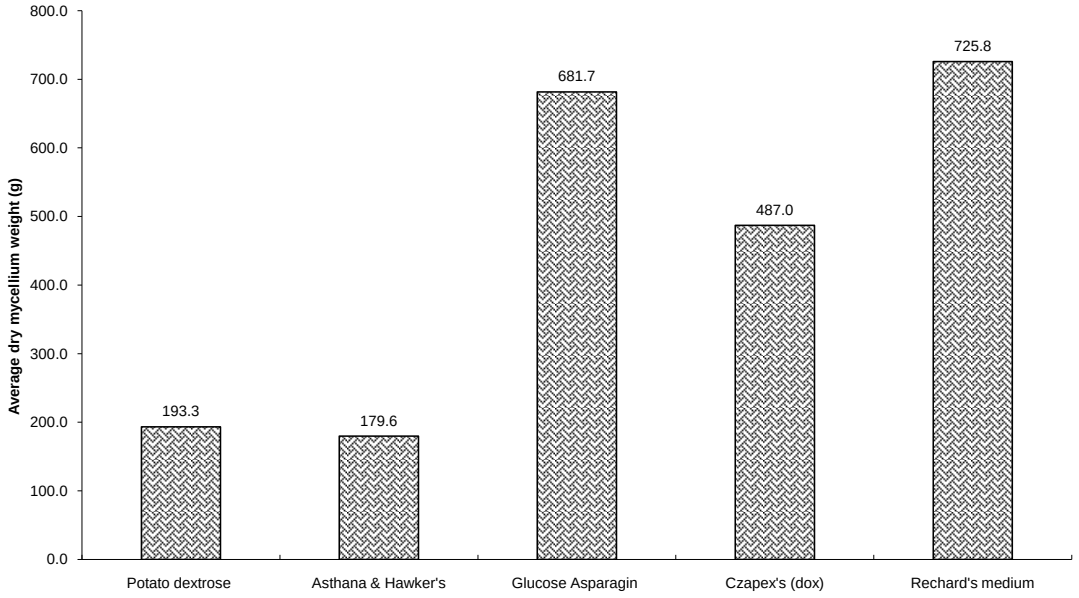
100 ml of each liquid medium was pipette in 100 ml sterilized Erlenmeyer flask in triplicate. Flasks containing media were inoculated and incubated at 25 ± 1°C for 21 days and dry weight of mycelial mat were recorded as per methods described earlier and the results so obtained were presented in Table-3 and illustrated graphical in Figure-2 & Plate-3 (b).

Perusal of data presented in Table-3 indicated that Richard's broth was found superior to other media under test, in terms of mycelial mat production yielding 725.8 mg/flask after 21 days of inoculation, which was followed by Glucose asparagines (681.8 mg). Asthana and Howker's medium supported the least (179.6 mg) fungal growth of the pathogen where as potato dextrose and Czapek's dox yielding 193.3mg and 487.1 mg, respectively.

Table-3: Effect of different liquid media on dry weight of mycelium of *M. phaseolina* after 21 days of incubation at 25± 1°C.

Sl. No.	Media	*Average dry weight of mycelium (in mg)
1.	Potato dextrose	193.3
2.	Asthana and Hawker's	179.6
3.	Glucose Asparagin agar	681.8
4.	Czapek's dox	487.1
5.	Richard's	725.8
SEm±		0.00025597
C.D. at 5%		0.00077
C.V. (%)		0.11

*Average of four replications



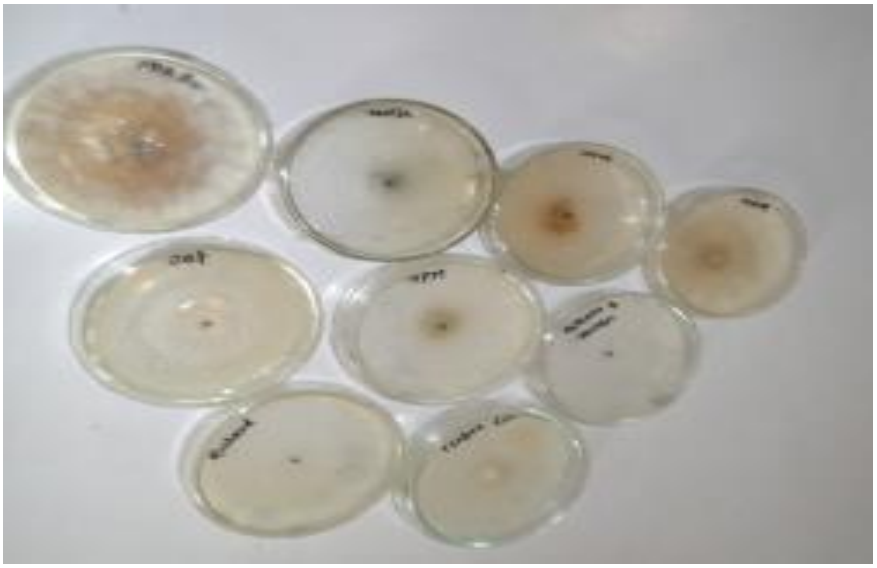


Plate 3(a) Fungal growth of the pathogen *M. phaseolina* on different solid media

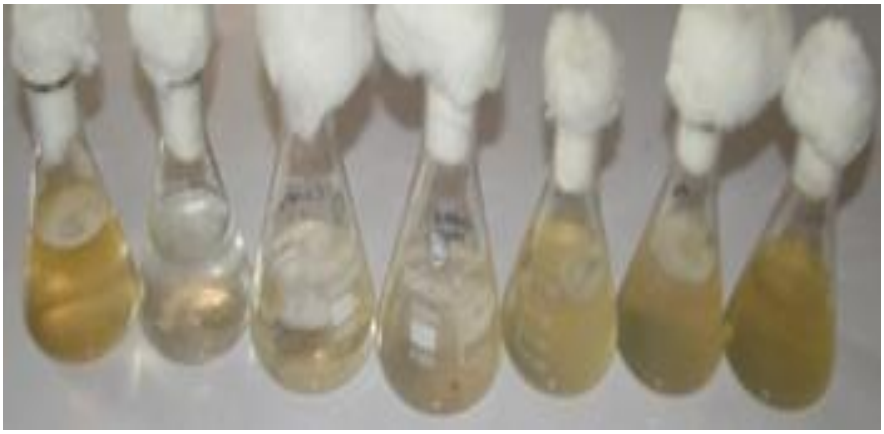


Plate3 (b) Growth of mycelium mat of *M. phaseolina* in different liquid media

Plate - 3

4.6 Screening of groundnut cultivars/entries against *M. phaseolina:*

The field experiments on screening of groundnut cultivars against root rot incidence.

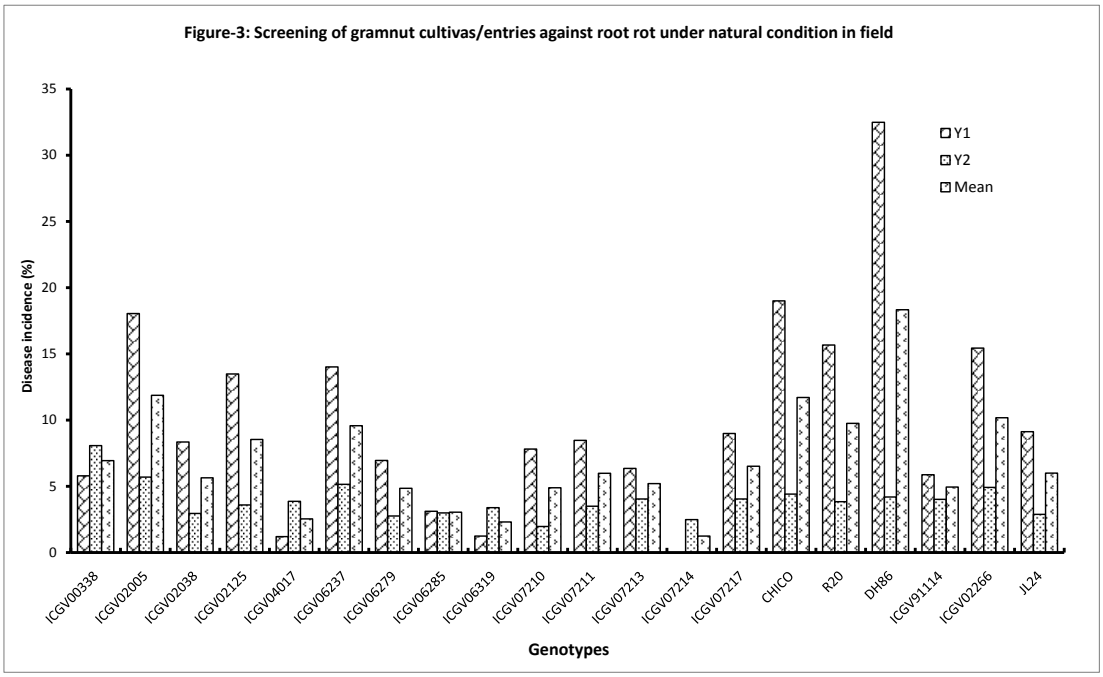
The final observations with respect to incidence of the disease was recorded at pre- maturity stage as per described in material and method. The data so obtained have been tabulated in Table-4 & illustrated graphical in Figure-3 & Plate-4.

Table-4: Screening of groundnut cultivars & entries against root rot under natural condition in field.

Genotype	Kharif *(mean of disease incidence (%)	Spring (mean of disease incidence (%)	Mean
ICGV00338	5.80 (3.55)**	8.08 (4.06)**	6.94
ICGV02005	18.05 (5.04)	5.71 (3.76)	11.88
ICGV02038	8.35 (4.00)	2.95 (3.22)	5.65
ICGV02125	13.49 (4.69)	3.60 (3.38)	8.54
ICGV04017	1.20 (2.60)	3.88 (3.33)	2.54
ICGV06237	14.02 (4.63)	5.15 (3.64)	9.59
ICGV06279	6.95 (3.15)	2.76 (3.17)	4.86
ICGV06285	3.12 (3.25)	3.00 (3.22)	3.06
ICGV06319	1.24 (2.62)	3.40 (3.33)	2.32
ICGV07210	7.81 (3.94)	1.97 (2.90)	4.89
ICGV07211	8.47 (3.82)	3.50 (3.34)	5.99
ICGV07213	6.36 (3.88)	4.05 (3.45)	5.20
ICGV07214	0.00 (0.71)	2.49 (3.07)	1.24

ICGV07217	8.99 (4.18)	4.05 (3.46)	6.52
CHICO	19.01 (4.80)	4.41 (3.55)	11.71
R20	15.68 (4.87)	3.84 (3.43)	9.76
DH86	32.49 (5.86)	4.20 (3.44)	18.34
ICGV91114	5.88 (3.79)	4.02 (3.38)	4.95
ICGV02266	15.45 (4.84)	4.92 (3.57)	10.18
JL24	9.13 (4.24)	2.88 (3.20)	6.01
SEm±	0.46		
C.D. at 5%	1.32		
C.V. %	20.51		

*Mean of three replications **Figures in parenthesis are transformed angular values



The result indicated that the Groundnut entries differed in their susceptibility to the pathogen during Kharif-2013 and spring-2014 crop seasons. The mean disease incidence ranged from 00.00 to 32.49 percent during Kharif season. The cultivar, DH86 (32.49 %) showed maximum disease incidence during Kharif followed by CHICO (19.01). Minimum and no disease incidence was recorded in the cultivar ICGV07214 (0.00 %). The cultivar, ICGV00338 (8.08%) showed maximum disease incidence percent during spring followed by ICGV02005 (5.71%). Minimum disease incidence found in cultivar ICGV07210 (1.97%).

4.7 Chemical control:

4.7.1. Evaluation of different fungicides against *M. phaseolina in vitro:*

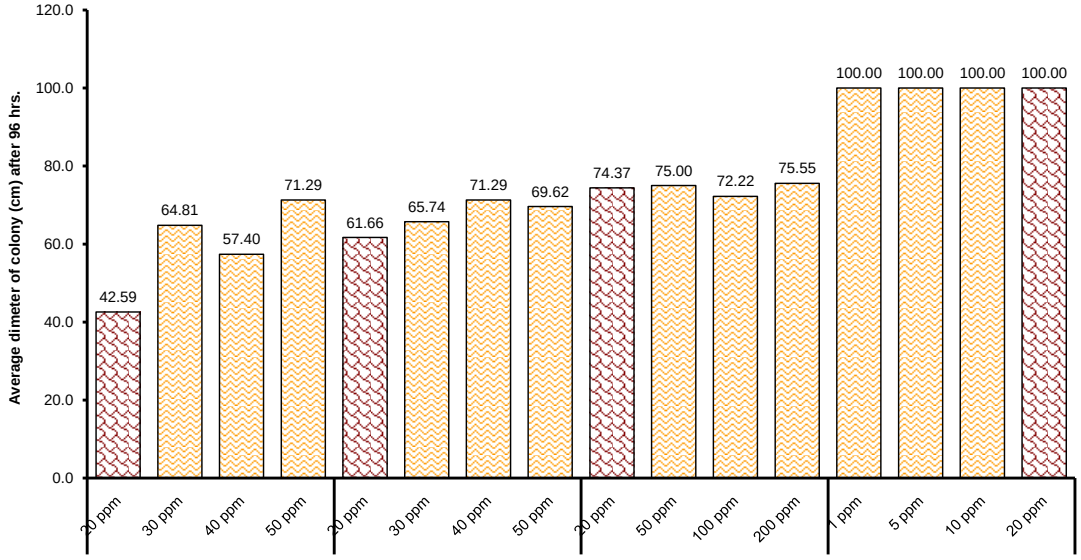
An experiment was conducted to determine the effect of four fungicides namely, Tricyclazole, Mancozeb, Hexaconazole and Carbendazim at different concentration *viz.*, by poisioned food technique the radial growth of the pathogen were recorded at 72 and 96 hrs after incubation. Percent inhibition of the radial growth of the pathogen at different concentration of fungicides are presented in the Table-5 & illustrated graphical in Figure-4 & Plate-5.

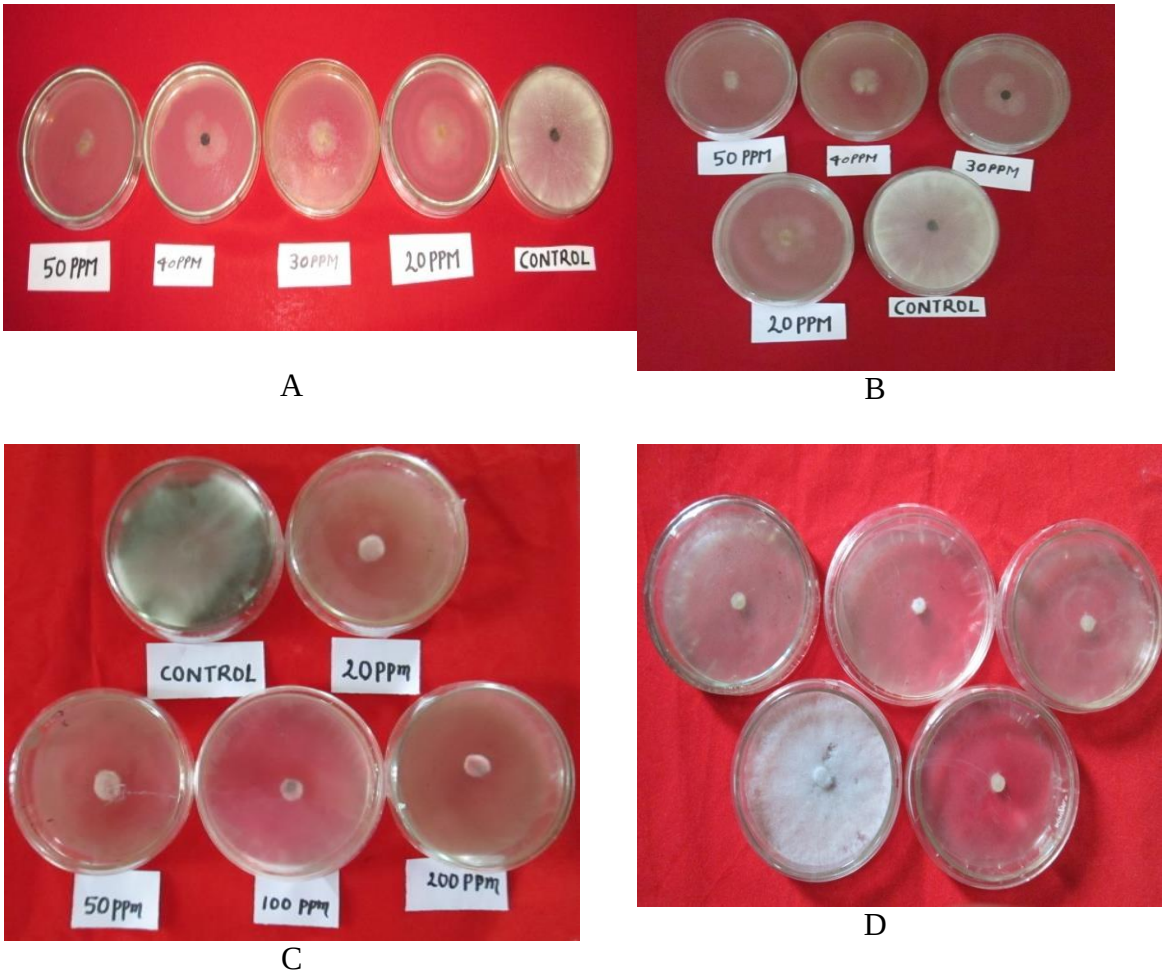
The result showed that all the fungicides significantly inhibited radial growth of the pathogen the superior over the control reducing the radial growth of the pathogen. It is evident from table 5 that the systemic fungicide Carbendazim was highly effective in inhibiting the radial growth at all the concentration under in vitro test, followed by Hexaconazole, Mancozeb sand Tricyclazole. All the fungicides mycelial growth of the pathogen. However at all the concentrations Hexaconazole proved to be superior than remaining three fungicides in suppressing the growth of the pathogen followed by Mancozeb and Tricyclazole.

Table-5: Evaluation of different fungicides against *M. phaseolina in vitro*.

Fungicides	Concentration	Percentage inhibition over control after hours	
		72	**96**
Tricyclazole	20 ppm	47	43
	30 ppm	70	65
	40 ppm	64	57
	50 ppm	74	71
Mancozeb	20 ppm	70	62
	30 ppm	77	66
	40 ppm	82	71
	50 ppm	80	70
Hexaconazole	20 ppm	76	74
	50 ppm	82	75
	100 ppm	84	72
	200 ppm	83	76
Carbendazim	1 ppm	100	100
	5 ppm	100	100
	10ppm	100	100
	20 ppm	100	100
SEm±		2.84	3.10
C.D. at 5%		8.18	8.94
C.V.(%)		6.10	7.16

Figure-04: Evaluation of different fungicide against Macrophomina phaseolina in vitro at 25±1^{O}C after 96 hrs of incubation





A B

C D

Plate 5: Evaluation of different fungicides against *M. phaseolina in vitro*
(A) Mancozeb (B)Tricyclazole (C) Hexaconazole (D) Carbendazim

4.7.2. Effect of seed treatment on the root rot incidence of Groundnut caused by *M. phaseolina:*

To study the effect of different fungicide in minimizing the root rot disease of groundnut under lab conditions an experiment was conducted in Department of plant pathology, BAU, Sabour, Bhagalpur. Three fungicides and biocontrol agent *viz.*, Thiram, Copper oxychloride, Carbendazim (12%) + Mancozeb (63%) and Bio control *Trichoderma* were tested as seed treatment as mentioned in earlier. The result so obtained have been presented in Table-6 & illustrated in Plate-6.

Table-6: Effect of different fungicidal seed treatment on the root rot disease of Groundnut.

Sl. No.	Fungicides	Germination (%)	Disease incidence
1.	Thiram	30	75
2.	Copper oxychloride	24	66
3.	Combiplus (Carbendazim (12%) + Mancozeb (63%)	66	14
4.	*Trichoderma*	20	90

The result clearly indicated that all the fungicides used for seed treatment protect seedling from *M. phaseolina* to some extent. After artificial inoculation maximum germination percentage (66 %) were observed in seed treated with Carbendazim (12%) + Mancozeb (63%) followed by thiram (30%). Minimum germination percentage observed in seed treated with *Trichoderma* (20%). Maximum disease incidence was recorded in seed treated with *Trichoderma* (90%) followed by thiram and Copper oxychloride, Minimum disease incidence (14%) recorded in seed treated with Carbendazim (12%) + Mancozeb (63%). However, the seed treatment with Carbendazim (12%) + Mancozeb (63%) was superior than other @ 2g/kg of seed.

Plate 6 : Effect of different fungicidal seed treatment on the root rot disease of Groundnut.

4.7.3. Dual culture:

To study the ability of *Trichoderma* isolates to inhibit the mycelial growth of *M. phaseolina* dual culture experiment was conducted on PDA medium. A clear zone of inhibition was noted between the *Macrophomina phaseolina and Trichoderma viride* which near the pathogen. The inhibition growth of pathogen were recorded after 24 and 48 hrs. upto 47. 61% and 35.18%, respectively in dual culture experiments. The result so obtained have been presented in Table-7 & illustrated in Plate-7.

Table-7: Dual culture of *M. phaseolina* with *Trichoderma viride.*

Treatments	Radial growth of *Macrophomina phaseolina* (mm)		% Inhibition	
	24 hrs	48 hrs	24 hrs	48 hrs
T.viride + *M.phaseolina*	45.00	58.33	47. 61	35.18
Control (*M. phaseolina*)	70.00	90.00		

Each value is an average of 3 replicate.

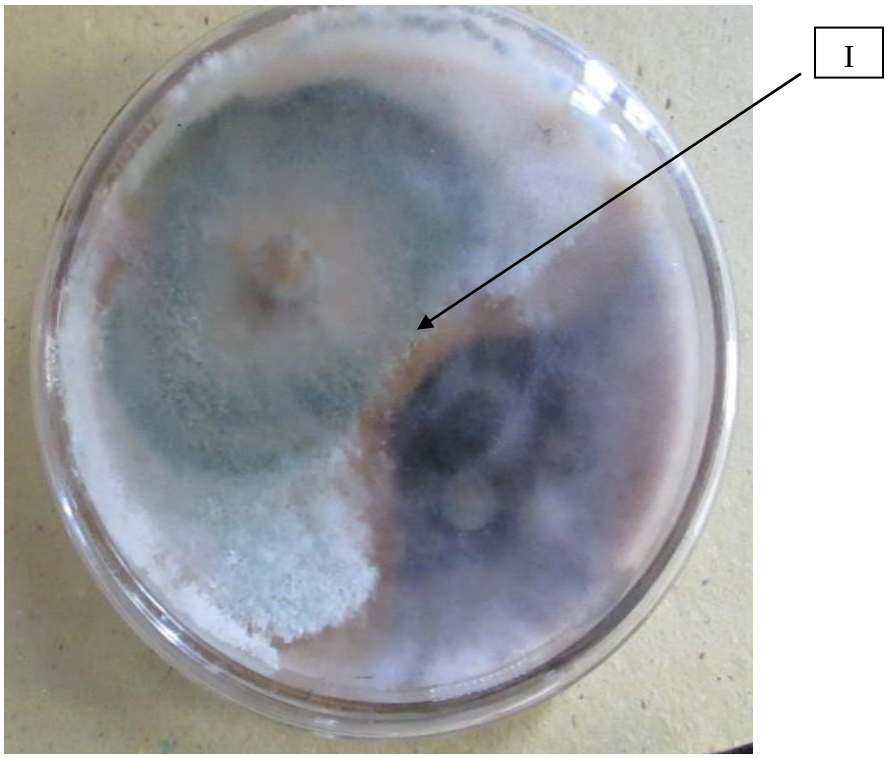


Plate.-7: Dual culture of *M. phaseolina* with *Trichoderma viride* after 5 days of incubation. (I) Inhibition zone

⁂⁂⁂⁂⁂

CHAPTER-V

DISCUSSION

Groundnut (*Arachis hypogaea* L.) is an important legume crop cultivated worldwide for food & its oil use. Groundnut originated in the Northwest Argentina region in South America and is presently cultivated in 108 countries of the world including India. In India Groundnut is cultivated in Gujrat been reported to be subjected to a number of diseases, of which '*Macrophomina phaseolina* root rot' has gained importance in recent years, particularly in the areas where it is extensively grown in Kharif season.

Macrophomina root rot is very destructive in nature and has been reported from many parts of the world. It has been reported Groundnut from India by Pearl in 1923. The disease has recorded its presence in all most all the growing areas in this country. In Bihar, this disease has been reported for the first time by Mc Rae in 1930.

Rotting of stems as well as roots are the characteristics symptom of the disease. It was felt imperative to investigate whether these symptoms are produced by a single pathogen due to its close occuring with soil. With this view, the investigations were undertaken, to find out the main organism responsible for the expression of the characteristics rotting symptoms on stems, roots as well as other aerial parts of the plants; its morphology, cultural characters, of the pathogen and evolve measures effectively manage this disease through application of suitable fungicides.

The specimens collected from Regional research sub-station Jalalgar, Purnia. showed characteristics symptoms of the disease. The isolated pathogen's behavior was confirmed on the basis of Koch's postulates. Its morphological characters particularly mycelium, having close similarity with earlier reports given by many workers *viz.*, Thirumalachar (1955), Deshpandey

et al. (1969), Subramanayam (1971) mentioned the fungal pathogen to be identified as *Macrophomina phaseolina* (Tassi.) Goid. Koch's postulates proved positive and revealed that *Macrophomina phaseolina* was responsible for stem and root rot of groundnut in this part of Bihar under Groundnut cultivation. The typical symptoms of root rot appeared on stems and roots of the infected groundnut plants. In the beginning dark cortical lesions were formed near the collar region on stem. Rapidly, these lesions increased in size from few mm. to few inches. In advanced stage the lesion extended upward and downward, girdling the affected portion from all sides and gradually the whole plant becomes is brown coloured, rotted at affected portion, shrivelled and collapsed, breaking down the upper parts from initially infected portion. The above ground and below ground symptom expressed in Groundnut upon infection by *Macrophomina phaseolina* where characteristically similar to the previous reported by the presence of dark charcoal black collar in disease survey field experiments. The affected plants showed general yellowing, drooping of leaves and ultimately death of the plants at pre- mature stage which is with the reports.

When infected plant were carefully uprooted, black coloured roots. It appeared as charcoal had been sprinkled on roots. The roots become brittle and broke easily. These symptoms are somewhat in close conformity with the symptoms observed earlier by Thirumalachar (1953).

Morphological characters of the fungus, studied on infected plants as well as on culture medium showed close similarities. Some variation from previous reports in respects of mycelium sclerotia where obtained. Fungus produced dirty white mycelium which was septet, long and branched the diameter of hyphae varied from 1.52 to 2.21 µm. somewhat similar results were also obtained by Thirumalachar (1955), Deshpande *et al*. (1969) and Smits and Noguera (1990). The isolate in Groundnut did not form microsclerotia but it occurs in some of the isolates.

The fungus was cultured on nine solid media of various origin to study its growth. The Glucose asparagines agar medium proved to be the best among all under test, in respect radial growth of mycelium covering full plate size with compact colony growth in 90 mm petri-plate after 72 hrs of inoculation, followed by Czapek's (dox) agar (73.8 mm), Richard's agar (73.0 mm), Asthana and Howker's agar (72.5 mm) and glucose peptone agar (70.8 mm). No and scanty fungal growth was obtained on oat meal agar medium. PDA media might to be used for rapid multiplication of inoculum for disease screening.

The fungus was cultured on five liquid media were inoculated with mycelia suspension and incubated for desired period for maximum harvest of biomass and conidial production. Richard's broth was found superior to other media under test, in terms of mycelia mat production yielding 725.8 mg/flask after 21 days of inoculation, which was followed by Glucose asparagines 681.8 mg. Asthana and Howker's medium supported the least (179.6mg) fungal growth of the pathogen where as potato dextrose and Czapek's (dox) yielding 193.3 mg and 487.1 mg, respectively. The earlier finding was reported by Shanmugam and Govindaswamy (1973) and Jha (1996). Such liquid media that promotes mycelia weight might to help in appropriate experiments.

Groundnut entries differed in their susceptibility to the pathogen during Kharif – 2013 and Spring-2014 crop seasons. The mean root rot incidence ranged from 00.00 to 32.49 percent. The cultivar, DH 86 (32.49 %) showed maximum root rot incidence percent during Kharif followed by CHICO (19.01 %). Minimum and no disease root rot percent was recorded in the cultivar ICGV07214 (0.00 %). The cultivar, ICGV00338 (8.08%) showed maximum root rot incidence percent during Spring followed by ICGV02005 (5.71%). Minimum root rot incidence percent was recorded in the cultivar ICGV07210 (1.97%). These variations in reaction of cultivar/entries may be due to fact, that they have different genetic make- up. Many workers *viz.*, Li-

ZhengChao and Qiu-QingShu (2000), Gopal, Ahamed and Babu (2006) and Moradia (2012) and others in past had screened a large number of groundnut cultivars/entries and had obtained some sources of groundnut against *M. phaseolina*, the causal pathogen of root rot disease in different parts of the country.

All the four fungicides *viz.*, Carbendazim, Mancozeb, Tricyclazole and Hexaconazole were evaluated *in vitro* at four concentration *viz.*, 1ppm, 5ppm, 10 ppm, 20 ppm, 30ppm,40ppm, 50 ppm, 100 ppm, 100 ppm and 200ppm,were found to be significantly superior over the check, in inhibiting the radial growth of the fungus *M. phaseolina.* The fungicidal efficacy of the chemicals showed that increased doses of fungicides were directly proportionate to the inhibition of mycelia growth of the pathogen. Carbendazim completely inhibited at 1ppm and higher concentrations. The best action of Carbendazim against the mycelia growth, may be attributed due to its systemic nature and more toxic fungicidal values. Choudhary *et al.* (2004) had also reported the maximum inhibition of radial growth of *M. phaseolina* by carbendazim *in vitro*. Mancozeb, Tricyclazole and Hexaconazole were also found in higher concentrations. These findings were in close similarities with the earlier observations recorded by Choudhary *et al*. (2004) who also had obtained the effective inhibition in growth of *Macrophomina phaseolina* at higher concentration of Bavistin [carbendazim], Antracol [propineb], Indofil M-45 [mancozeb + thiophanate-methyl] and Ridomil MZ [mancozeb + metalaxyl], applied at 300, 400, 500 and 1000 ppm, in inhibiting the mycelial growth of *Macrophomina phaseolina.* The variation in toxicity levels of these fungicides at particular concentration may be due to difference in efficacy of the fungicides in reducing the mycelia growth of the fungus.

Study under lab conditions, on the fungicidal and biological control of the root rot of groundnut indicated that, in all fungicidal treatments, the seed treatment with Thiram, Copper oxychloride, Carbendazim (12%) + Mancozeb (63%) and *Trichoderma* @ 2 g/kg seed. After artificial inoculation

maximum germination percentage (66 %) were observed in seed treated with Carbendazim (12%) + Mancozeb (63%) followed by thiram (30%). Minimum germination percentage observed in seed treated with *Trichoderma* (20%). Maximum disease incidence was recorded in seed treated with *Trichoderma* (90%) followed by thiram and Copper oxychloride, Minimum disease incidence (14%) recorded in seed treated with Carbendazim (12%) + Mancozeb (63%). The best action of Carbendazim (12%) + Mancozeb (63%) against of *M. phaseolina* may be attributed due to its systemic nature and more toxic fungicidal values. These findings were in close similarities with the earlier observations recoded by Rao *et al.* (1998) who also had obtained the carbendazim SD (0.05%+captan 0.125%) was the best in reducing seed rot and pre- and post-emergence seedling blight . disease was observed in seed treated with Thiram followed by *Trichoderma* and Cuopper oxychloride,. However, the seed treatment with combiplus was best seed treatment. Which also, no disease was found after seedling deep in mycelium suspension.

Dual culture technique the studied the ability of *Trichoderma* isolates to inhibit the mycelial growth of *M. phaseolina*. In dual culture technique *T. viride* reduced mycelial growth in 24 hrs and 48 hrs by 47.61 % and 35.18 %, respectively. These findings were in close similarities with the earlier observations recoded by Sreedevi *et al.* (2011) who also had obtained in dual culture technique *T. viride* and *T. harzianum* reduced mycelial growth by 61.1% and 64.4%, respectively. Based on the dual culture technique, *T. viride* were selected for further research.

⁂⁂⁂⁂⁂

CHAPTER- VI

SUMMARY

Macrophomina root rot of groundnut (*Arachis hypogaea* L.) is a emerging disease in Bihar, where groundnut is extensively grown in large area as a major Kharif and emerging in spring oilseed crop. Its incidence varies from 0-32 per cent in two season. Present investigations were undertaken to identify the pathogen, to study the sequence of symptoms produced by the pathogen, its morphological and cultural status and to evolve effective management of the disease through seed treatment with different fungicidal treatments. Screening of available cultivars/entries to identify resistant source for use in future breeding programme was also done under natural conditions.

The typical symptoms of the disease may appear on roots, collar region, stem and branches of infected plants. In the beginning, dark cortical leasions were formed near the collar region on stem. Rapidly these leasion increases in size from few mm to few inches. In advanced stage, the lesion extended upward and downward, gridling the stem from all sides and gradually the whole plant become brown coloured and ultimately dried prematurely. The affected portions rotted, shriveled become more darker or blakish in colour and plant collapsed and broke down from the rotted portion. Gradually affected plants showed general yellowing, drooping of leaves and ultimately death of plants before maturity.

The pathogen was isolated on potato dextrose agar medium and pathogenicity was proved following Koch's postulates. On the basis of morphological character of mycelium produced in nature as well as in the culture and artificially infected plants, the pathogen was identified as *Macrophomina phaseolina* (Tassi.) Goid.

The fungus was grown on nine solid and five sets of liquid media separately under *in vitro* conditions. Synthetic and semi- synthethic media were found to be superior over natural media in terms of radial growth and biomass production. Highest radial growth (9mm) was found in glucose asparagines agar medium followed by Czapek's (dox) agar, Richard's agar, Asthana and Howker's agar and glucose peptone agar. no and scanty fungal growth was obtained on oat meal agar medium. All the liquid media behaved more or less similarly as solid media in terms of biomass production Richard's broth was found superior to other media pertaining to biomass production which yielded 725.8g/flask after 21 days of inoculation, which was followed by Glucose asparagines, Czapek's (dox) medium, Potato dextrose and Asthana and Howker's agar medium.

The screening of cultivars/entries of groundnut against *Macrophomina phaseolina* root rot under natural condition revealed that, out of 20 cultivars/entries. The mean root rot incidence ranged from 00.00 to 32.49 percent. The cultivar, DH 86 (32.49 %) showed maximum root rot incidence percent during Kharif followed by CHICO (19.01 %). Minimum and no disease root rot percent ICGV07214 (0.00 %). The cultivar, ICGV00338 (8.08%) showed maximum root rot incidence Spring followed by ICGV02005 (5.71%). Minimum root rot incidence was found in the cultivar ICGV07210 (1.97%).

All the four fungicides *viz.,* Carbendazim, Mancozeb, Tricyclazole and Hexaconazole tested *in vitro* at four different concentration *viz* 1ppm, 5ppm, 10 ppm, 20 ppm, 30ppm,40ppm, 50 ppm, 100 ppm, 100 ppm and 200ppm, were found to be significantly superior over control, in checking the radial growth of the pathogen. The systemic fungicide carbendazim was highly effective in inhibiting the growth of the fungus at all the concentrations followed by Hexaconazole, Mancozeb and Tricyclazole.

Seed treatment with different fungicides and one biological agent maximum germination percentage (66 %) were observed in seed treated with Carbendazim (12%) + Mancozeb (63%) followed by thiram (30%). Minimum germination percentage observed in seed treated with *Trichoderma* (20%).

Maximum disease incidence was recorded in seed treated with *Trichoderma* (90%) followed by thiram and Copper oxychloride, Minimum disease incidence (14%) recorded in seed treated with Carbendazim (12%) + Mancozeb (63%).

Under *in vitro* condition the ability of *Trichoderma* isolates to inhibit the mycelial growth of *M. phaseolina* in dual culture was determined on PDA medium. A clear zone of inhibition noted between the *Macrophomina phaseolina and Trichoderma viride* was near the pathogen. The inhibition growth of pathogen were recorded after 24 and48 hrs. upto 47. 61% and 35.18%, respectively in dual culture experiments.

⁂⁂⁂⁂⁂

REFERENCES

Ainsworth, G.C. and Bisby, G. R. (1967). *Dictionary of fungi*. Common Wealth Mycological Institute, Kew, Surrey, England.

Al-Ahmad, M and Saidawi, A. (1988). *Macrophomina* (charcoal) root rot of sesame in Syria. *Arab J. Pl. Protec*. **6(2) :** 88-93.

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

Aliyu, B. S. and Kutama, A. S. (2007). Isolation and identification of fungal flora associated with groundnut in different storage facilities. *Science World J.* **2(2) :** 34-36.

Ammajamma, R. and Hegde, Y. R. (2009). Efficacy of fungicides against *Rhizoctonia bataticola* causing wilt of *Coleus forskohlii* (Wild) Briq. *Int. J. Pl. Protec*. **2(1) :** 31-32.

Anon (1955). Annual Report on the Department of Agriculture of the Northern Region of Nigeria, 1952-53, 76 pp.

Anon (1998). All India Co-ordinated Research Project on Sesame & Niger Tech Prog. & Guidelines for Implementation. Project Co-ordinating unit (Sesame & Niger). J.N. K. V.V., Jabalpur, M.P. ,65-68.

Ashby, S. F. (1927). *Macrophomina phaseoli* (Maubl.) Comb. Nov. pycnidial stage of *Rhizoctonia bataticola* (Taub.) Butler. *Trans Brit. Myc. Soc.* **12 :** 141-147.

Bainade, P. S., Tripathi, B. P. and Khare, N. (2007). Effect of chemical and biological control of *Macrophomina phaseolina in vitro* and *in vivo*. *Environment and Ecology*. **25(1) :** 29-31.

Bremer, H. (1944). Uber Welkrantheiten in Sudwest-Anatolien (On wilt diseases in South-West Anatolia). *Istanbul yaz*, 18, 40 pp (*Rev. Appl. Mycol*. **25 :** 255, 1946).

Brooks, F. T. (1928). *Plant Disease*. Oxford Univ. Press, New York, pp.304.

Brown, W. (1923). Experiments on the growth of fungi in culture media. *Ann. Bot*. **37 :** 105-109.

Butler, E. J. (1918). *Fungi and Diseases in plants*. Thacker, Spink and Co., Calcutta.

Butler, E.J. and Bisby, G. R. (1931). *The fungi of India*, ICAR Sci. Monograph, No. II, New Delhi, pp 158.

Cardona, R. (2006). Vertical distribution of sclerotia of *Macrophomina phaseolina* in a natural infested soil in Portuguesa state. *Revista de la Facultad de Agronomía, Universidad del Zulia*. **23 (3) :** 285-293.

Choudhary, C. (2000) studies on stem and root rot of Til (*Sesamum indicum L*.) caused by *Macrophomina phaseolina* (Tassi) Goid**. M.Sc (Ag)** Thesis submitted to Deptt. of Mycol. & PI, Patho RAU, Pusa.

Choudhary, C. S., Prasad, S. M. and Singh, S. N. (2004). Effect of sowing date and fungicidal spray on *Macrophomina* stem and root rot and yield of sesame. *J. Appl. Biol*. **14 (2) :** 51-53.

Choudhary, C. S., Singh, S. N. and Prasad, S. M. (2004). *In vitro* effectiveness of chemicals to control *Macrophomina phaseolina* (Tassi.) Goid, causing stem and root rot of sesame. *J. Appl. Biology*. **14 (1) :** 46-47.

Choudhary, Sumuti, Pareek Savita and Saxena Jyoti (2010). Efficacy of biocontrol agent singly and in combination against Dry root rot of *Macrophomina phaseolina* of mungbean. *J. myco. pl. pathol*. **40(1) :** 141-144.

Christopher, B. J., Usharani, S. and Udhayakumar, R. (2008) Management of dry root rot (Macrophominaphaseolina (Tassi.) (GOID)) ofurd bean

(Vignamungo (L.) Hepper) by the integration of (Trichodermavirens) and organic amendments. *Myesor J. Agril Sci.* **42 (2) :** 241-246.

Christopher, D. J., Usharani, S. and Udhayakumar, R. (2008). Management of dry root rot (*Macrophomina phaseolina* (Tassi.) Goid) of peanut (*Arachis hypogeae L.*) by the integration of antagonistic (*Trichoderma virens*) and organic amendments. *Advances Pl. Sci.* **21(2) :** 389-392.

Clinton, R. S. S. (1960). Seed bed pathogen of groundnut in Sudan, and an attempt to control with an artificial testa. *Empire J. Exptl. Agr***. 28 :** 211-222.

Deshpande, A. L., Agrawal, J. P. and Mathur, B.N. (1969). *Rhizoctonia bataticola* causing a root rot of opium in Rajasthan. *Indian Phytopath*. **22(4) :** 510.

Edington, L.V., Khew, K.L. and Barren, G.I. (1971). Fungitoxic spectrum of benzimidazole compounds. *Phytopath.* **61 :** 42-44.

FAO, 2013. production year Book 2012. Food and agricultural organization, Roam, Italy.

Ganesan, S. and Sekar. **R.** (2004). Biocontrol mechanism of Groundnut (*Arachis hypogaea L.*) Diseases-*Trichoderma* system. In: *Biotechnological Applications in Environment and Agriculture*, (Eds.): G.R. Pathade and P.K. Goel, ABD Pub. Jaipur, India, pp. 312-327.

Gibson, I. A. S. (1953). Crown rot, a seedling disease of groundnut caused by *Aspergillus niger.* II. An anomalous effect of organo-mercurial seed dressings. *Trans. Brit. Mycol. Soc*.**36 :** 19-122.

Gopal, K., Ahamed, S. K. and Babu, G. P. (2006). Relative resistance in groundnut genotypes to pod rot disease. *Legume-Research*. **29(3) :** 205-208

Gopal, K., Vijayakumar, S., Ahmed, N. N. and Nargund, V. B. (1994). Screening of groundnut against pod rot. *Groundnut-News*. **6(1) :** 3.

Gupta, S. C. and Kolte, S. J. (1981). Cultural characteristics of leaf and root isolates of *M. phaseolina* (Tassi) Goid from groundnut. *Indian J. Microbiol.* **21 (4) :** 345-346.

Ionita, A., Iliescu, H., Jinga, V. And Iordache, E. (1995). *Macrophomina phaseolina* a dangerous parasite of cropped plants-possibilities for control. *Problem-de-protectia-plantelor*, **23 (2) :** 179-196.

Hansford, C.G. (1940). Report of the senior plant pathologist. *Rept. Dept. Agic.* 1938-39 **(2) :** 28-29.

Hegde, Y. R. and Chavhan, T. L. (2009). Management of root rot of *Jatropha curcas* in Karnataka. *Int. J. Pl. Protec.* **2 (2) :** 243-244.

Jain, A. C. and Kulkarni, S. N. (1965). Root and stem rot of sesamum. *Indian Oilseed J.* **9(3) :** 201-203.

Jaiman, R. K. and Jain, S. C. (2010). Effect of fungicides on root rot of cluster bean caused by *Macrophomina phaseolina*. *Environment and Ecology*, **28 (2A) :** 1138-1140.

Jaiman, R. K., Jain S. C and Sharma Pankaj (2009). Effect of storage condition on Macrophomina phaseolina root rot in cluster bean. *J. myco. Pl. pathol*. **39(1) :** 82-85.

Jayati-Bhowal, Ghosh, Sumita, Guha, A. K. and Chatterjee, B. P. (2006). Infection of jute seedlings by the phytopathogenic fungus *Macrophomina phaseolina* mediated by endogenous lectin. *Res. J. Microbio.* **1(1) :** 51-60.

Jha, A. K. (1996). Studies on collar rot of okra caused by *Macrophomina phaseolina* (Taffi) Goid. M. Sc. (Ag.) Thesis submitted in Deptt. of Mycology & Plant Pathology BAU, Ranchi, Bihar.

Kale, D. M., Murty, G. S. S. and Badigannavar, A. M. (2007). New Trombay groundnut variety TG 38 suitable for the residual moisture situation in India. *J. SAT-Agril Res.* **3(1) :** 1-2.

Kale, D. M., Murty, G. S. S., Badigannavar, A. M. and Dhal, J. K. (2009). New trombay groundnut variety, TG 51, for commercial cultivation in India. *J. SAT-Agril Res.* **7 :** 1-2.

Khan, Mudasser Ahmed and Gangopadhaya, (2008). Efficiency of Pseudomonas fluorescens in controlling Root rot of chickpea caused by *Macrophomina phaseolina. J. myco. Pl. pathol*. **38(3) :** 580-587.

Khan, S. N., Ayub, N. and Ahmed, I. (2003). A re-evaluation of geographical distribution of charcoal rot on sunflower crop in various agroecological zones of Pakistan. *Mycopath*. **1 (1) :** 63-66.

Lukade, G. M. (1995). Effect of sowing time on incidence of charcoal rot and yield of released sorghum cultivars. *Indian J. Mycol. Pl. Pathol.* **25(1&2) :** 53.

Li-Zheng Chao and Qiu-QingShu Huayu (2000). A new high-yielding, improved quality groundnut cultivar with wide adaptability for northern China. *Int. Arachis-Newsletter*, **20(16) :** 31-32.

Mahdizadeh V., Safaie N. and Aghajani, M.A. (2011). New hosts of *Macrophomina phaseolina* in Iran. *J. Pl. Pathology*. **93** (4, Supplement), S4.63-S4.89

Maheswari, C. U. and Ramakrishnan, G. (2000). Factors influencing the competitive saprophytic ability of *Macrophomina phaseolina* in groundnut. *Madras Agril. J.* **86(10/12) :** 552-553.

Malathi, P. and Doraisamy, Sabitha (2003). Compatibility of *Trichoderma harzianum* with fungicides against *Macrophomina phaseolina*. *Plant-Disease-Research-Ludhiana*. **18(2) :** 139-143.

Malathi, P. and Doraisamy, Sabitha (2003). Effect of temperature on growth and antagonistic activity of *Trichoderma spp*. against *Macrophomina phaseolina. J. Biol. Control*. **17(2) :** 153-159.

Maryam Aghakhani and Dubey, S. C. (2009). Morphological and pathogenic variation among isolates of *Rhizoctonia bataticola* causing dry root rot of chickpea. *Indian Phytopath*. **62 (2) :** 183-189.

Maublanc, A. (1905). *Macrophoma phaseoli*. *Bul. Soc. Myc. France*, **21 :** 90.

Mathur, S. B. and Cunfer, B. M. (1993). Seed borne diseases and seed health testing of wheat. Danish government institute and pathology for developing countries. Copenhagen, pp.168.

Mayee, C. D. (1995). Current status and future approaches for management of groundnut diseases in India. *Indian Phytopath*. **48 :** 389-401.

Mayee, C.D. (1987). Diseases of groundnut and their management. In: Plant protection in field crops, (Eds.): M.V.N. Rao and S. Sitanantham, PPSI, Hyderabad. pp. 235-243.

Mayee, C. D. and Data, V. V. 1988. Diseases of groundnut in the tropics. *Review Trop. Pl. Path.* **5 :** 169-198.

Mc. Rae, W. (1930). Report of the Imperial Mycologist, *Scient Repts. Agric. Res. Int.* Pusa, 1928-29.

Mehta, P. R. (1951). Observations on new and known diseases of crop plants of the Uttar Pradesh. *Plant Prot. Bull*., New Delhi. **3 :** 7-12.

Mihail, J. D. (1992).”*Macrophomina*”.Pp. 134-136. In: L.L.Singleton, J.D. and C.M Rush (Eds.) Methods for **Research on soil borne phytopathogenic fungi**. APS press, USA.

Mittal, R. K. (1997). Effect of sowing dates and disease development in lentil as sole and mixed crop with wheat. *J. Myco. Pl. Pathol.* **27 (2) :** 203-209.

Moradia, A. M. (2011). Management of *Macrophomina phaseolina* in groundnut through systemic fungicides. *Internat. J. Agric. Sci*. **4(1) :** 212-213.

Moradia, A. M. and Khandar, R. R. (2011). Loss of yield of groundnut (*Arachis hypogaea* L.) due to dry root rot (*Macrophomina phaseolina*) and their management under *in vivo* condition. *Internat. J. agric. Sci.* **7(2) :** 282-285.

Moradia, A. M. (2012). Effect of source of varietal resistance against of *Macrophomina phaseolina* on groundnut. *Int. J. Pl. Protec*. **5(2) :** 438-439.

Nageswararao, G., Patibanda, A. K. and Ranganathswam, Y. M. (2012). Studies on the efficacy of fungicides, organic amendments and biocontrol agent on dry root rot (*Rhizoctonia bataticola*) of groundnut *in vivo*. *J. Mycopath. Res*. **50(2) :** 285-289.

Nattrass, R. M. (1934). Annual Report of the Mycologist for the year 1933.
Ann. Rept. Deptt. Agric. Cyprus for the year 1933 : 48-57.

Nema, K. G. and Agarwal, G. P. (1960). *M. phaseolina* on roots of *Cicer arietinum* L. and *Pisum sativum* L. *Proc. Nat. Acad. Sci. India,* **30 :** 57.

Nene, Y. L. and Thapliyal, P. N. (1979). *Fungicides in plant disease control*. Oxford & IBH publishing Company, New Delhi (India): 507 pp.

Oilseeds: *The Hindu Survey of Indian Agriculture*. 1999 pp 61-65.

Okwulehie, I. C. (2002). Anatomical changes in groundnut due to infection with *Macrophomina phaseolina* (Maub.) Ashby. *J. Sustain. Agril. and Environment,* **4(2) :** 249-257.

Okwulehie, I. C. (2004). Studies on *Macrophomina phaseoli* (Maub.) Ashby growth and some physiological aspect of groundnut *(Arachis hypogaea L*) plant infected with the fungus. *Global J. Pure-and Applied-Sci.* **10(1) :** 23-29.

Okwulehie, I. C. (2005). Physiological studies in groundnuts (Arachis hypogaea L.) infected with *Macrophomina phaseoli* (Maub.) Ashby. *Int. J. Tropical-Plant-Diseases,* **19(1/2) :** 25-37.

Paracer, C. S. and Bedi, P. S. (1962). Studies on wilt disease of linseed in Punjab-II. *Indian Oilseed J*. **6(4) :** 304-306.

Parvathi, K., Kateswarlu, K. Ven and Rao, A. S. (1985). Influence of root rot infecting fungi on development of *Glomus mosseae* in Groundnut. *Current Sciences, India,* 54 **(19) :** 1006- 1007.

Patel, K. K. and Patel, A. J. (1990). Control of charcoal rot of sesamum. *Indian J. Mycol. Pl. Pathol*. **20 (1) :** 62-63.

Patel, K. K. and Patel, A. J. (1990). Meterological correlation of charcoal rot of sesamum. *Indian J. Mycol. Pl. Pathol*. **20 (1) :** 64-65.

Pathak, D. and Barman, B. (1998). Effect of dates of sowing on the incidence of root rot disease of jute caused by *Macrophomina phaseolina* (Tassi.) Goid. *Annals of Biology* (*Ludhiana*). **14 (2) :** 185-187.

Pearl, R. T. (1923). Report of the mycologist to the Government of the Central Provinces and Berar. *Rept. Deptt. Agri.,* Central Provinces and Berar for the year ending 30th June, 1922:19-20.

Prasad, N. (1944). Studies on root rot of cotton in Sind. II Relation of Root-rot on cotton with root-rot of other crops. *Indian J. Agric. Sci.***, 14 :** 388-399.

Prasanthi, L. (2007). Evaluation of Indian bean (field bean) lines for resistance to dry root rot caused by *Rhizoctonia bataticola*. *J. Arid Legumes*. **4 (2) :** 154-155.

Rai, M., Ved Ratan and Srivastava, S. S. L. (2005). Management of root rot of urd bean [*Vigna mungo* (L.) Hepper] caused by *Macrophomina phaseolina*. *Farm Sci. J.,* **14 (1) :** 89.

Raja Mohan, K. and Balabaskar, P. (2012). Survey on the incidence of groundnut root rot disease in cuddalore district of Tamilnadu and

assessing the cultural charecters and pathogenicity of *Macrophomina phaseolina* (Tassi.) Goid.. *Asian J. Sci and Tech.*, **3 (4) :** 90-94.

Rajani, V. V. and Parakhia (2009). Management of root rot disease (*Macrophomina phaseolina*) of castor (Ri) with soil amendments and biocontrol agent. *J. Myco. Pl. Pathol*. **39(2) :** 290-293.

Ramkrishnan, K. (1955). Report of *M. Phaseolina* from soil of Vandalur, Madras State. *Proc. Indian Acad. Sci. B***. 41:**112.

Rao, V. V. R., Reddy, M. S., Shantaram, M. V. and Rao, K. C. (1997). Survey on the occurrence of seedling diseases of groundnut (*Arachis hypogaea L.*). *Indian J. Pl. Protec.* **25(1) :** 75-77.

Rao, V. V. R., Rao, K. C., Shantaram, M. V. and Reddy, M. S. (1998). Management of seed and seedling diseases of groundnut (*Arachis hypogaea L.*) through seed treatment. *Indian J. Pl. Protec*. **26(1) :** 1-8.

Rangaswami, G. and Mahadevan, A. (2008). *Diseases of crop plants in India (4th ed).* New Delhi, PHI Learning Private Limited, page no. 275-278.

Reichert, I. (1930). Palestine, Root disease caused by *Rhizoctonia bataticola*. *Int. Bull. Plant. Prot*.**4 :** 17.

Riker, A. J. and Riker, R. S. (1936). *Introduction to Research on Plant Disease*. John S. Swift Co. No.4, New York, U.S.A.

Sadashivaiah, A. S., Ranganathaiah, K.G. and Gowda, D. N. (1986). Seed health testing of *Helianthus annus* with special refrence to *Macrophomina phaseolina*. *Indian Phytopathol*, **39 :** 445-447.

Sagır, P., Sagır, A.and Sogut, T. (2010). The effect of sowing time and irrigation on Charcoal rot disease *(Macrophomina phaseolina*), yield and yield components of sesame. *Bitki Koruma Bülteni*. **50 (4) :** 157-170.

Sarejanni, J. A. and Cortzas, C. B. (1935). Note sur le parasitisme du *Macrophomina phaseoli* (Maubl.) Ashby [A note on the parasitism of *Macrophomina phaseoli* (Maubl) Ashby] *Ann. Inst. Phytopath*. **1 (3) :** 38-44.

Shalby, S. I. M. (1997). Effect of fungicidal treatment of sesame seeds on root rot infection, plant growth and chemical components. *Bulletin of Faculty of Agril. Univ. of Cairo*, **48(2) :** 397-411.

Shanmugam, N. and Govindaswamy, C.V.(1973). Physiological studies on *M. phaseolina* causing groundnut root rot. *Indian J. Mycol. Pl. Pathol.* **3(1) :** 1-7.

Shazia Rasheed, Dawar, Shahnaz and Ghaffar, Abdul (2004). Location of fungi in groundnut seed. *Pakistan J. Bot.* **36 (3) :** 663-668.

Singh, Paramjit, Gupta, T. R. and Singh, P. (1993). Effect of sowing dates on the development of disease and seed yield in sesamum (*Sesamum indicum* L.) *Plant Disease Research*, **8(1) :** 61-63.

Singh, R. D. and Sandhu, A.C. (1995). Factors affecting development of charcoal rot of cowpea. *Indian J. Mycol Pl. Pathol*. **25 (1 & 2) :** 74.

Singh, S. K. and Nene, Y. L. (1990). Cross inoculation studies on *R. bataticola* isolate from different crops. *Indian Phythopath*, **43 :** 446-448.

Small, W. (1927a). Further notes on *Rhizoctonia* (Taub.) Butler. Trop. Agriculturist, **69 (1) :** 9-12.

Small, W. (1927b). Further occurrence of *Rhizoctonia* (Taub.) Butler. Trop. Agriculturist, **69 (4) :** 202-203.

Smith B.W. (1994). Foliar Diseases. In: Compendium of Peanut Disease. American Phytopathological Society, pp. 77.

Smits, B. G. and Noguera, R. (1990). The ontogeny and morphogenesis of Sclerotia & pycnidia of *M. phaseolina*. *Agronomica Tropica* (Maracay).

Smith, G. S. and Carvil, O. N. (1997). Field screening of commercial and experimental soybean cultivars for their reaction to *Macrophomina phaseolina. Plant Disease*. **81 (4) :** 363-368.

Sreedevi, B., Charitha Devi, M. and Saigopal, D.V.R. (2011). Isolation and screening of effective *Trichoderma* spp. against the root rot pathogen *Macrophomina phaseolina. J. Agric. Techno*. **7(3) :** 623-635.

Subramanayam, C. V. (1971). Hyphomycetes. ICAR, New Delhi pp 881-82.

Subramanian, C. V. (1952). *M. phaseolina* from black cotton soil, udamalpet, Madras State. *J. Madras Univ. B*., **22 :** 220.

Subrahmanyam, P., Mehan, V. K., Nevill,S D. J. and Donald, D. Mac (1980). Research on Fungal Diseases of Groundnut. ICRISAT. In: A Proceeding of an International Workshop on Groundnut. ICRISAT, Patancheru, A.P., India, pp. 189-197.

Sundararaman, S. (1931). Administration Report of the Mycologist for the year 1929-30:30 pp (*Deptt. Agric*., Madras).

Sundararaman, S. (1932). Administration Report of the Mycologist for the year 1930-31:20 pp (*Deptt. Agric*., Madras).

Taliei, F., Safaie, N., Aghajani, M. A. (2013). Spatial distribution of M*acrophomina phaseolina* and soybean charcoal rot incidence using Geographic Information System. *J. Agric. Sci. and Techno*, **15 :** 1523-1536.

Tandel, D. H., Sabalpara, A. N., Pandya, H. V.and Naik, R. M. (2010). Effect of leaf blight [*Macrophomina phaseolina* (Tassi.) Goid.] on growth parameters and yield of greengram and its chemical control. *Int. J. Pl Protect*. **3 (2) :** 329-331.

Taubenhaus, J. J. (1913). *Sclerotium bataticola. Phytopathology*, 3:164f.

Thakare, A. R., Chavan, P. N., Raut, B. T., Tini-Pillai, and Paulkar, P.K. (2002) Effect of polythene mulch and seed treatment on diseases and yield of groundnut. *Research-on-Crops*, **3(1) :** 159-163.

Thirumalachar, M. J. (1953). Pycnidial stage of charcoal rot inciting fungus with a discussion on its nomenclature. *Phytopath*. **43 :** 608-610.

Thirumalachar, M. J. (1955). Incidence of charcoal rot of potato in Bihar (India) in relation to cultural conditions. *Phytopath*. **45(2) :** 91-93.

Umamaheswari, C., Ramakrishnan, G. and Nallathambi, P. (2001). Role of inoculum level on diseases incidence of dry root rot caused by *Macrophomina phaseolina* in groundnut. *Madras Agril. J.* **87(1/3) :** 71-73.

Vijay, Mohan, Prasad, S. M., Barnwal, M. K. and Kudada, N. (2006). Fungicidal management of dry root rot disease and yield of chickpea. *J. of Appl. Bio*. **16(½) :** 42-44.

Vijay, Mohan, Prasad, S. M. and Kudada, N. (2006). Varietal screening of chickpea against dry root rot disease. *J. Res, Birsa Agril University*. **18 (1) :** 145-147.

Wisniewska, H. and Chelkowski, J. (1999). Influence of exogenic salicylic acid on *Fusarium* seedling blight reduction in barley. *Acta Physiologiae Plantarum*, **21 :** 63-66.

Wyllie, T. D. (1988). Charcoal rot of soyabean-current status. Pages 106-113 *In*: **I. D. wyllie and K. H Scott**, (Eds.). Soyabean Diseases of the North Central Region. *The American Phytopathology Society, St. Paul . MN.*

⁂⁂⁂⁂⁂