Studies on Root & Stem Rot (*Macrophomina phaseolina*) (Tassi.) Goid. of Sesame and its Management through fungicides

THESIS

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By

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2020

CERTIFICATE – I

This is to certify that the thesis entitled "**Studies on root & stem rot (*Macrophomina phaseolina*) (Tassi.) Goid. of sesame and its management through fungicides**" submitted in partial fulfilment of the requirement for the degree of **MASTER OF SCIENCE (Ag.)** in **Plant Pathology** of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur is a record of the bonafide research work carried out by **Shivani Nagpure**, ID No. 180118016, under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instruction.

All the assistance and help received during the course of the investigation has been acknowledged by her.

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Place: Jabalpur **Shivani Nagpure**

Date :

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols	Legend
@	At the rate
Avg.	Average
Cm	Centimetres
et al.,	Co- workers
CD	Critical Difference
ºC	Degree centigrade
Fig.	Figure
Ha	Hectare
Kg	Kilograms
M	Meters
mµ	Micrometre
µ	Micron
Mg	Milligram
ml	Millilitre
Mt	Million tons
Viz.,	Namely
ppm	Parts per million
%	Per Cent
PDI	Per cent disease incidence
±	Plus or minus
Psi	Pond square inch
Spp.	Species
SEm±	Standard error of mean
i.e.	That is
SMW	Standard meteorological week
SC	Susceptible Check

Chapter-I

INTRODUCTION

INTRODUCTION

Sesame (*Sesamum indicum* L.) is commonly known as 'Til' also called as "Queen of oil seeds". It is belongs to family Pedaliaceae having Chromosome no. 2n=26 and originated from Africa. Sesame, probably the most ancient oil seed plant cultivated in many parts of the world. Sesame seeds are a rich source of protein (20%) and edible oil (50-52%) and contain about 47% oleic acid and 39% linoleic acid (Shyu and Hwang, 2002). Sesame seeds are used in culinary as well as in traditional medicines for the nutritive, preventive and curative purposes. Sesame is annual herb, height 2 to 3 feet. It is a short-day plant but also grows well in long-day areas. Normally the crop is grown in plains but also comes up successfully up to 1200 meter above mean sea level. It is grown as a rain fed crop throughout the tropics and subtropics. For maximum yields, sesame requires fairly high temperature and evenly distributed rainfall during its crop growth. It cannot withstand frost, prolonged drought, wet weather or water logged conditions more particularly at flowering and pod development stages. It can be grown on a wide variety of soils. It does best on sandy loams with adequate moisture. Acidic or alkaline soils are unsuitable for sesame (optimum pH range 5.5 -8.0).

Currently, China, India and Myanmar (Burma) are the world's largest producers of sesame (FAO, 2004). Sesame cultivated in about 1.56 million hectare area with a production of 0.78 million tons in India and productivity of 457 kg/ ha during 2018 (Anon, 2018). India is one of the largest exporters of sesame, exporting between 5 lakh to 6 lakh metric tonne of sesame annually. Almost all sesame cultivation and consumption occurs in developing countries with only 10 per cent entering the international trade (Kambikambi *et al*., 1997). Major sesame growing states are Madhya Pradesh (455 kg/ha), Rajasthan (562 kg/ha), Uttar Pradesh (226 kg/ha), Andhra Pradesh (309 kg/ha), West Bengal (933 kg/ha), Tamil Nadu (578 kg/ha), Maharashtra (262 kg/ha) and Orissa (237 kg/ha) during the year of 2018-19.

The main reason for the low productivity of sesame is attack of various diseases, such as Charcoal rot of sesame (*Macrophomina phaseolina*), Alternaria leaf spot (*Alternaria sesami*), Bacterial blight (*Xanthomonas*

compestris pv. *sesami*), Powdery mildew *(Erysiphae cichoracearum*), Cercospora leaf spot (*Cercospora sesami)* and sesame phyllody *(*Phytoplasma) Gupta *et al*., (2018).

Macrophomina phaseolina, limiting the production of crop seedling mortality due to seed borne infection aggravates the disease problem by reducing the plant stand per unit area, resulting in low yield and 5-100% yield loss (Vyas and Patel, 1981). It has become a potential threat for the profitable cultivation especially in the changing warm climate and intensive farming situations (Saharan *et al*., 2005). Among the fungal diseases, root & stem rot, also called charcoal rot caused by *Macrophomina phaseolina* (Tassi.) Goid. is widely distributed and highly destructive from the establishment phase of crop (Dinakaran and Mohammed, 2001). It causes up to 50 per cent or more disease incidence in field resulting in heavy yield losses (Chattopadhyay *et al*., 2002). *Macrophomina phaseolina* is one of the destructive necrotrophic fungal pathogens that infect more than 500 plant species across 75 families (Su *et al*., 2001). *Macrophomina phaseolina* is a seed and soil borne pathogens causing root & stem rot on sesame. The disease is very important as infection occurs from seed germination and emergence to reproductive phase.

Yield losses have been estimated up to 57 per cent when there is about 40 per cent infection (Maiti *et al*., 1988). The crop is severely infected by *M. phaseolina* and is widely distributed in all sesame growing regions. The size of microsclerotia, 50-70 µm in diameter and 60-200 µm in diameter when produced in laboratory. They are black, smooth and round to oblong shape, uniformly reticulate, formed from hyphal aggregates. Pycnidia, when present, are immersed in the host tissue and erumpent when mature. On seeds, the fungus can be asymptomatic, remaining as microsclerotia on the tissues or under the coat tissue. Symptomatic seedlings show brownish to black discoloration at the soil limit and above. The seedlings eventually die. If older plants are infected, leaves lose vitality, turn yellow, wilt, & die and water absorption is blocked by the formation of numerous microsclerotia in xylem going up on the stems. Hyphal branches generally form at right angles. The

fungus produces pycnidia when the atmospheric temperature ranges of 25ºC to 35 ºC.

Not much research is carried out on sesame disease in Madhya Pradesh particularly on root & stem rot. Keeping in this view present investigations are undertaken with the following objectives.

1. Studies the cultural variability of root & stem rot isolates.
2. Evaluation of sesame genotypes against root & stem rot.
3. Management of root & stem rot of sesame through fungicides.

Chapter-II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The relevant literature available on management of root and stem rot (*M*. *phaseolina*) of sesame and other crops related to various aspects has been reviewed under following heads:-

2.1 The Disease

2.2 Pathogen

2.3 Taxonomy and Nomenclature

2.4 Cultural Variability

2.5 Morphological Variability

2.6 Varietal Screening

2.7 Germplasm Screening

2.8 Management of root & stem rot disease through fungicides

2.8.1 *In vivo*

2.8.2 *In vitro*

2.1 The disease

The disease has been found to occur frequently in India (Mc Rae, 1928; Mitra, 1932). *M. phaseolina* is one of the most destructive pathogens of sesame. It is known to cause complex disease syndrome like seed rot, seedling blight, root rot, stem rot and leaf spot.

In India charcoal rot is widely distributed in all sesame growing areas in Madhya Pradesh, Uttar Pradesh, Rajasthan, Gujarat, Punjab, Haryana Maharashtra, Bihar, Odisa, West Bengal, Tamil Nadu, Karnataka and Kerala (Saharan *et al*., 2005).

The root rot/charcoal rots of sesame caused by *M. phaseolina* (Tassi) Goid. was first reported from Uttar Pradesh by Mehta (1951). Later on, it was observed by Jain and Kulkarni (1965) from Gwalior and Jabalpur division of Madhya Pradesh.

The pathogen is both seed and soil borne. The sclerotia survive for many years in soil. *M. phaseolina* is primarily soil borne in nature, with heterogeneous host specificity, that is, the ability to infect monocots as well as dicots and non-uniform distribution in the soil (Mayek-Perez *et al*., 2003; Su *et al*., 2001). The genus causes stem canker, seedling blight, Charcoal rot, dry root rot, wilt, leaf blight, stem blight and pre-emergence and post-emergence damping-off (Singh *et al*., 1990); root and stem rot of softwood forest trees (McCain and Scharpf, 1989), fruit trees and weed species (Songa and Hillocks, 1996).

The infected seed produced infected plants, which die immediately (Gonzalez and Subero, 1984). The pathogen *M. phaseolina* affects the fibrovascular system of the roots and basal internodes of its host, impeding the transport of water and nutrients to the upper parts of the plant. As a result progressive wilting, premature dying, loss of vigour, and reduced yield are characteristic symptoms of *M. phaseolina* infection (Khan 2007). Chand and Khirbat (2009) observed that the disease is favoured by drought and high soil temperature. Murugesan *et al*., (1978) have found loss of 110 and 111 kg/ha in *kharif* and summer sesame crop, respectively.

2.2 The Pathogen

The pathogen (*M. phaseolina*) causes number of diseases in several crops. The wide spread occurrence and destructive nature of the pathogen have caught attention of several scientist of the world.

The basic characteristics of the species are the formation of sclerotia in the host tissue as well as in the culture. On the infected plants sclerotia was seen in the pith region and on outer surface of roots just below the bark region (Subramanian,1971). *Rhizoctonia bataticola* is believed to be a synonym of *Macrophomina phaseolina.* (Holliday and Punithalingam,1988).

Colony colour of *Macrophomina* varies in culture from black to brown or grey and becomes dark in colour with age. Hyphae are septate, initially hyaline turning to a honey or black colour. Numerous dark brown to black coloured sclerotia can be seen on the reverse side of the culture plate. The vegetative mycelium is characterized by the formation of monilid or barrel-shaped cells and the formation of septum near the branching of the mycelium.

Branching occurs at right angle to parent hyphae, but branching at acute angles is also common (Dhingra and Sinclair, 1973). Microsclerotia are formed from the aggregation of hyphae with 50 to 200 individual cells coupled by a melanin pigment. *M. phaseolina* generally produce globose or flattened pycnidia that range from 100 to 200 µm in diameter. Pycnidia are initially embedded in the host tissue and are dark to greyish in colour but become black and erumpent with maturity. The pycnidia produced in the culture are typically dark brown to black, subglobose to lageniform with the diameter of around 300 µm and composed of several layers of cells. The inner layer is hyaline while the outermost layer of cells is dark brown to black in colour. Conidiophores are septate or branched and simple. Conidia are characteristically hyaline, ovoid, with truncate base that ultimately becomes rounded and measures around 5–10 × 14–30 µm (Punithalingam, 1982). The apex is usually rounded and covered with a thin membrane that avert and stay at the apex and measures up to 16–24 × 5–9 µm. Eversion and gelatinization of the outer layer of conidial wall results in the formation of apical cap or funnel-shaped conidial appendage.

2.3 Taxonomy and Nomenclature

The systematic position of *Macrophomina phaseolina* is as follows, given by Kirk *et al*., (2008)

Kingdom	:	Fungi
Division	:	Ascomycota
Class	:	Dothideomyctes
Order	:	Botryosphaeriales
Family	:	Botryosphaeriaceae
Genus	:	Macrophomina
Species	:	*M*. *phaseolina*

Halsted (1890) described the sclerotial state as *Rhizoctonia bataticola* (Taub.) Butler on sweet potato (*Ipomoea batatas*). The pycnidial state of the fungus was originally named *Macrophoma phaseolina* by Tassi (1901) and *Macrophoma phaseoli* by Maublanc (1905).

The genus *Macrophomina* was first established by Petrak (1923) with the description of *M. philippinensis* from the dried specimens of *Sesamum orientale* collected by G.M. Reyes in Philippines in 1921.

Ashby (1927) critically examined and compared the type specimens of the fungus from beans with other related genera and established the binomial species *Macrophomina phaseoli* (Maubl.).

Later, Goidanich (1947) changed the binomial *Macrophomina phaseoli* to *Macrophomina phaseolina* (Tassi.) Goid., since the original specimen of *Macrophomina* was collected by Tassi in 1901. Hence, the two names, that is, *Macrophomina phaseoli* (Maubl.) Ashby and *Macrophomina phaseolina* (Tassi.) Goid. became widely accepted in the literature.

2.4 Cultural Variability

Monga and Sheo Raj (1994) studied Thirteen isolates of *Rhizoctonia bataticola* were categorised into four distinct groups on the basis of cultural characteristics. Seven isolates of *R. bataticola* causing root rot of cotton differed in cultural characters such as colour of the mycelium.

Shekhar *et al.,* (2006) studied cultural and pathogenic behaviour of seven isolates of *Macrophomina phaseolina* incitant of charcoal rot of maize, obtained from different agro-ecological zones of India. Cultural variability was shown by pathogen on the basis of colour of colony and colony appearances.

Endraki and Banihashemi (2010) studied sixty isolates of *Macrophomina phaseolina*, the cause of charcoal rot, from different parts of Iran on various plants including cantaloupe, long melon, soybean, cucumber, apricot, rosemary and sesame. Isolates were grouped in four phenotypes: fluffy with abundant sclerotia, fluffy with few sclerotia, partially fluffy, and appressed growth.

Varma and Pathe (2013) studied the considerable variability in cultural and morphological characters of 22 isolates of *R. bataticola* obtained from soybean from different places of Madhya Pradesh.

Thirunarayanan *et al.,* (2017) conducted survey in different locations of Cuddalore district revealed the endemic nature of the root rot disease

incidence in sesame and studied the cultural characteristics of 7 isolates of *Macrophomina phaseolina.*

Satpathi and Gohel (2018a) studied cultural and morphological variability of different isolates of *M. phaseolina* collected from four location of Gujarat and they found considerable variation among the isolates of *M*. *phaseolin* on the basis of cultural characters shown by pathogen like colour of colony, topography of colony and margin of colony.

2.5 Morphological variability

In an experiment, Jain *et al.,* (1973) reported that the size of the sclerotia was found to be maximum in isolates of *Rhizoctonia bataticola* collected from soil and stem.

Linhai *et al*., (2011) observed rich variations in *M. phaseolina* morphological characteristics, including sclerotia quantity and sclerotium size in sesame. Prasad *et al*., (2011) reported differences among the isolates of *M. phaseolina* in terms of size and shape of sclerotia into safflower.

Ashraf *et al*., (2015) eight isolates produced large sized sclerotia (> 45µm), eleven produced medium sized (40–45 µm), and the remaining five isolates produced small sized sclerotia (< 40 µm) of maize.

Karibassappa *et al*., (2020) studied that size of sclerotia varied from 82.41 x 58.90 µm to 135.18 x 101.88 µm. Based on shape of sclerotia the isolates were classified in to three groups. irregular, round and ovoid groups in sesame crop.

2.6 Varietal Screening

Bartaria (1988) evaluated 15 varieties of sesame for resistance in the field condition and found out that the resistant varieties were BS-5-18-6(C) followed by BS-5-18-6(8). The dark brown seeded BS-5-18-6(C) was completely resistant against stem and root rot disease of sesame.

Dinakaran and Mohammed (2001) reported that susceptible varieties of sesame viz., TMV 3, Co 1 and VRI 1 recorded the maximum incidence of 66.7, 70.0 and 91.7 per cent disease respectively in Tamil Nadu.

John *et al*., (2005) screened 30 sesame cultivars against *Macrophomina* root rot and observed TLC-246, TLC-279 and TLC-289 as highly resistant to root rot.

Gupta *et al*., (2018) reported that varieties RT-46, RT-0125, MT-75, TKG-22 and Nirmala are tolerant to root & stem rot disease of sesame in Madhya Pradesh.

Farooq *et al.,* (2019) screened six commercial and 20 candidate line of sesame against *Macrophomina phaseolina* using sick plot method and pot experiment. Results show that none of the variety was found immune against root & stem rot disease in Pakistan.

2.7 Germplasm Screening

Thiyagu *et al*., (2007) conducted a screening programme in artificial (*in vitro*) and sick plot (*in vivo*) condition to identify the resistant genotype against Root rot disease of sesame caused by *Macrophomina phaseolina* (Tassi.) Goid. In sick plot condition, the genotypes were grown along the susceptible cultivars after every 4 rows of test entries. The genotypes namely ORM 7, ORM 14 and ORM 17 were identified as resistant to root rot which could be used as cultivars or used in hybridization programme.

Chaudhary *et al*., (2014) studied that Out of 27 entries evaluated against stem and root rot caused by *Macrophomina phaseolina*, only three entries *viz*., IC–205477, IC– 205506 and Krishna were identified as resistant.

Deepthi *et al.,* (2014) studied that among the evaluated sesame cultivars only one entry PKDS-91 was found moderately resistant to charcoal rot. Three entries i.e. OSC-366-I, SSD-2-I and OSC-79 were recorded as moderately susceptible.

Bedawy and Moharm (2019) studied Eighty six sesame genotypes were used for evaluating disease resistance, in two successive summer seasons 2017 and 2018, in the field. The combined data of disease incidence per cent obtained from both seasons showed that 13, 21, 38 and 14 genotypes were moderately resistant, Moderately Susceptible, Susceptible and Highly Susceptible, respectively.

Farooq *et al*., (2019) screened 20 germplasm lines of sesame against *M*. *phaseolina*. Resistance response of sesame germplasms was determined in pots and field under natural conditions.

2.8 Management of root & stem rot disease through fungicides

2.8.1 *In vivo* condition

Carbendazim was better among the fungicides even better than its combined effect with thiram in causing disease reduction. Carbendazim has been reported to be successful against the *Macrophomina* root rot of sesame. (Shukla and Singh,1973)

Chauhan (1988) Studied that treatments with carbendazim, quintozene and benomyl enhanced plant emergence and disease control in seedling disease of cotton caused due to *Rhizoctonia bataticola.*

Reznikov *et al*., (2016) In cultivars of Soybean NA8000 RG and Munasqa RR, the highest crop yield values were obtained with the Pyraclostrobin+ Thiophanate methyl mixture against charcoal rot disease.

Jyothi and Muhammad (2016) observed that seed treatment with tebuconazole (2g/kg of seed) recorded least disease incidence (8.70 %) with highest yield followed by seed treatment with tebuconazole (2g/kg) + carbendazim (1g/kg) recorded 15.66 % diseases incidence as compared to control which recorded 43.91 % diseases incidence.

Bashir *et al*., (2017) In field conditions, Nativo exhibited minimum Mean Disease Incidence (12.55%) whereas the interaction between treatments and days showed minimum of 14.95%, 12.82% and 9.90% disease incidence by Nativo as compared to all other treatments including control (66.86%, 77.57% and 87.22%) after day tenth, twenty and thirty in sesame against *M. phaseolina.*

Kulkarni *et al*., (2019) among the different treatments evaluated, T8 treatment (seed treatment with carbendazim (2 g/kg of seeds) followed by one foliar spray with carbendazim 0.1%) was found superior in managing the disease by recording lowest disease incidence (8.38%) with highest seed yield (619 kg/ha) in black gram.

2.8.2 *In vitro* condition

Lambhate *et al*., (2002) tested the efficacy of fungicides against *M. phaseolina,* root rot pathogen of cotton *In vitro* and reported that bavistin, ridomil M Z-72 and topsin-M at 0.1, 0.2 and 0.3 per cent showed cent per cent inhibition of mycelial growth of the fungus.

Bavistin (Carbendazim), Antracol (Propineb), Indofil M-45(Mancozeb+ Thiophanate-methyl) and Ridomil MZ (Mancozeb + Metalaxyl), applied at 300, 400, 500 and 1000 ppm, inhibit the mycelial growth of *Macrophomina phaseolina*, the causal agent of root & stem rot of sesame, in *In vitro* conditions using the poisoned food technique (Choudhary *et al.* 2004).

Khalikar *et al*., (2011) has conducted *in vitro* evaluation study into which seven fungicides were tested against the pathogen i.e. *Macrophomina phaseolina in vitro*. The highest inhibition (100%) of *M. phaseolina* was observed due to carbendazim (500 ppm), chlorothalonil (500 ppm), hexaconazol (500 ppm) and captan (2500 ppm) followed by mancozeb (2500 ppm) (94.39 %) and benomyl (1000 ppm) 93.4 % and rest of the treatments significantly inhibited colony growth over control.

Moradia (2011) was screened nine systemic fungicides at 250, 500, and 1000 ppm against *Macrophomina phaseolina* under *in vitro* conditions. All the fungicides were capable of inhibiting the growth of fungus at all the concentrations tried. Difenconazole (Score 25% EC), carboxin (Vitavex 75% W.P.) and carbendazim (12%) + mancozeb(63%) were found to be the best, which caused cent per cent inhibition of growth at all the concentrations tested.

Deepthi (2012) was tested the different fungicides against *M. phaseolina* and found out that penflufen and vitavex power gave 100% of inhibition of fungus in *In vitro* conditions.

Bashir *et al*., (2017) assessed six fungicides viz. Nativo, Score, Topsin-M, Mancozeb, Antracol and Topas against Charcoal rot disease of sesame caused by *Macrophomina phaseolina* (Tassi) Goid. with different concentrations. Nativo expressed minimum fungal colony growth (1.26 cm at 150 ppm concentration by disrupting the metabolism as well as by hampering the growth and development of pathogen.

Chaudhary *et al.,* (2017) tested ten fungicides against the pathogen *Macrophomina phaseolina In vitro* conditions causing Dry Root Rot of Soybean. The highest inhibition (100%) of *M. phaseolina* was observed due to Carbendazim 50% WP at different concentration (250, 500, 1000 ppm), Mancozeb 75% WP (1500, 2000, 2500 ppm), ridomil-MZ 72% WP (1000, 1500, 2000 ppm) and carbendazim 12% + mancozeb 63% (1500, 2000, 2500 ppm) followed by propiconazole at 250 ppm (87.21 %), 500 ppm (89.92 %) and 1000 ppm (92.64 %) and rest of the treatments significantly inhibited colony growth over control.

Maruti *et al.,* (2017) studied *In vitro* efficacy of non-systemic, systemic and combination fungicides against *R. bataticola* causing dry root rot of pigeon pea. Among contact fungicides tested, ziram recorded 100 per cent inhibition at all the concentrations (i.e., 0.1, 0.2 and 0.3 %) with the mean inhibition, whereas, mancozeb and thiram showed 100 per cent inhibition at 0.3 per cent concentration. Among systemic fungicides tested, tebuconazole showed complete inhibition at all the concentration (i.e., 0.05, 0.10 and 0.15), whereas, propiconazole showed 100 per cent inhibition of *R. bataticola* at 0.10 and 0.15 per cent concentration. Among combi products tested, carbendazim 12% + mancozeb 63% WP, trifloxystrobin 25% + tebuconazole 50% EC and carboxin 37.5% + thiram 37.5% WP showed cent per cent (100%) inhibition at all the concentrations (0.10%, 0.20% and 0.30 %).

Chapter -III

MATERIAL AND METHODS

MATERIAL AND METHODS

The following material and methods were used to “Studies on root & stem rot (*Macrophomina phaseolina*) (Tassi.) Goid. of sesame and its management through fungicides”.

3.1 Location of Work

The experiments were carried out in the laboratory of the Department of Plant Pathology at College of Agriculture, JNKVV, Jabalpur (M.P.) and field experiments were conducted in field of AICRP on Sesame and Niger, JNKVV, Jabalpur. Laboratory and glass house facilities were utilized during experimentation.

3.2 Collection of Diseased materials

Diseased plants of sesame showing typical symptoms of root and stem rot (*Macrophomina phaseolina*) (Tassi.) Goid. *i.e.* spindle shaped spots with light grey centres surrounded by brown margins, were collected from the experimental area of PC Unit, AICRP Sesame and Niger, JNKVV, Jabalpur. However, the infected portions were washed with tap water and dried with the help of blotter paper to remove the traces of water.

3.3 Cleaning and sterilization of glasswares

3.3.1 Glass wares

Corning and Borosil make glass wares were used throughout the course of study. Glassware’s (Petri dishes, flasks, test tubes and pipettes) were cleaned with detergent washed with tap water and then dipped into chromic acid solution, and finally rinsed with tap water glassware’s were sterilized in hot air oven at 180°C for two hours.

3.3.2 Metallic equipment

Metallic equipment like forceps, needle and cork borer were sterilized by dipping in alcohol and to red hot over flame of a spirit lamp.

3.4 Equipment's

Equipment's used during the course of investigation including hot air oven, autoclave, BOD incubator, laminar air flow, refrigerator, electric weighing balance, induction and compound microscope.

3.5 Chemicals

The chemicals were used of analytical and laboratory grades. Streptomycin sulphate was added to the medium to avoid bacterial contamination. The fungicides used into the experiment were at commercial formulation and were stored at 25ºC in the dark. Sodium hypochlorite 1% was used for surface sterilization of sesame seeds.

Table 3.1 list of commercial fungicides used into experiments

S. No.	Chemicals	Trade name	Company
1.	Tebuconazole 50%+ Trifloxystrobin 25% WG	Nativo	Bayer India.
2.	Azoxystrobin 23% SC	Mirador	ADAMA India Pvt. Ltd.
3.	Pyraclostrobin 5%+ Metiram 55% WG	Clutch	PI Industries, Ltd.
4.	Cymoxanil 8% + Mencozeb 64% WP	CMZ-72	Volkschem Crop Science Pvt. Ltd.
5.	Captan 70% + Hexaconazole 5% WP	Taqat	Rallis India Ltd.
6.	Carbendazim 12% + Mencozeb 63%	Saaf	UPL, Ltd.

3.6 Preparation of Culture Media

The Potato dextrose agar medium was used during the experiment and for isolation, maintenance of culture (Ainsworth, 1961). The composition of media are given below

Peeled and sliced potato : 200g.

Dextrose : 20g.

Agar Agar : 20g.

Distilled Water : 1000 ml.

For potato extraction, peeled and sliced potato was taken and boiled in 500 ml water. Then it was strained through muslin cloth. Agar Agar was washed with water and taken in 500 ml of water then heated until it melted fully. Melted Agar Agar was poured in potato extract and dextrose was added and the solution was finally makeup to one liter. The media was poured in conical flask and sterilized in an autoclave.

3.7 Isolation and Purification of pathogen

Infected portion were cut into pieces of 5-6 mm size and were surface sterilized with 0.1 percent sodium hypochloride (NaOCl) solution for one minute and followed by 3 washings with distilled water. The pieces were transferred on sterilized potato dextrose agar (PDA) medium by using forceps in petri dishes and incubated at 28±1ºC in BOD incubator to obtain mycelial growth. After 48 hours of incubation, the fungus give mycelial growth which are then purified after viewing it under light microscope.

The cultures are then purified by using Hyphal tip technique (Rangaswami, 1972). The hyphal tip from margins of grown colonies were cut with the help of sterilized 5 mm cork borer and transferred to PDA containing petri dish, and are further maintained on PDA slants and incubated at 28±1ºC for further studies. On the basis of morphological characters as described by Ashby (1927) and Goidanich (1947) of mycelium and sclerotia, the isolate were identified as *M. phaseolina.*

3.8 Multiplication of the pathogen

The soil inoculums of *(M. phaseolina)* was prepared on Sand maize meal medium (1 part partial broken maize grains + 3 part sand + distilled water to moisten the media). Conical flasks of 500 ml capacity were filled with 200 g of sand maize meal medium and plugged with cotton. The flasks were sterilized in autoclave at two successive days at 121ºC for 30 minutes at 1.05 kg per square cm pressure. After sterilization the flasks were inoculated with 5 mm discs of virulent culture *(M. phaseolina).* The inoculated flasks were incubated at room temperature for 30 days to get profuse fungal growth.

3.9 Pathogenicity test

Sick pot soil method (Salunkhe and Deshpande, 2014) was used to prove the pathogenicity of *M. phaseolina.* The culture was mixed in the sterilized soil/sand mixture (1:1). Seeds of susceptible variety VRI 1 were surface sterilized in 2.0% sodium hypochlorite for 5 minutes. After that 15 seeds were sown in each pot at 2 cm depth after 15 days of inoculating the culture. Seedlings showing typical symptoms were pulled out and the pathogen was re-isolated on PDA medium. The culture was then compared to previous isolated culture and the pathogenicity (Koch postulates) was proved.

3.10 Cultural and Morphological Variability among the *Macrophomina phaseolina* (*Rhizoctonia bataticola*) isolates

The root and stem rot diseased sample of sesame were collected from three different states *viz.,* Madhya Pradesh, Rajasthan and Tamil Nadu. The pathogen was isolated and these isolates were then further used for variability studies. The mycelial discs of 5 mm diameter was cut from the edge of a three day old culture and transferred aseptically to 90mm petri dish containing 20 ml PDA. These plates were incubated at 28±1ºC into BOD incubator. Each treatment was replicated thrice.

Table 3.2 Isolates of *M. phaseolina* collected from different sesame growing states

S. No.	Location of Pathogen	State name	Isolate code
1.	Jabalpur	Madhya Pradesh	Mp 1
2.	Mandor	Rajasthan	Mp 2
3.	Vridhachalam	Tamil Nadu	Mp 3

3.10.1 Preparation of Slides

A loop full amount of pure culture was taken into glass slides from four positions of culture plate. The culture was stained with 0.1% lactophenol and observations on different morphological characteristics were taken and recorded for each of the isolate.

3.10.2 Cultural variability

The colonies of isolates were characterized for various cultural characters at 72 h after incubation. Seven day old culture were used to record texture, colour, type of margins and presence or absence of aerial mycelium into the cultures**.**

3.10.3 Morphological Variability

The slides of different isolates were prepared from 10 days old culture medium for study of morphological characters *viz.,* size of sclerotia, colour of sclerotia and shape of sclerotia were recorded after 10 days of incubation. The morphological characters of different isolates of *M. phaseolina* including shape of sclerotia, size of sclerotia (µm) / microscopic field of 10x were measured. The photomicrographs were also taken by using camera attachment binocular microscope to show the typical morphology of sclerotia of the isolates. For measuring sclerotial size, slides from seven days old pure cultures of *M. phaseolina* isolates were prepared.

3.11 Reaction of sesame cultivar against root and stem rot infection under field condition

The experiment was conducted using eight sesame varieties which were sown in the field at 17/07/2019 in the field condition. The incidence of stem and root rot was recorded at pre- flowering and capsule formation stage.

3.11.1 Calculation of per cent disease incidence

Per cent disease incidence at the

- Pre flowering stage
- Capsule formation stage will be recorded on different varieties of sesame crop grown under field condition.

  The disease incidence was calculated as per following formula (Vidhyasekaran and Muthimilan, 1995)

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plant}}{\text{Total number of plants}} \times 100$$

The per cent disease incidence was evaluated and the reaction was categorized according to modified disease scale adopted by Dinakaran *et al*., (1995) as under,

Table 3.3 Disease reaction of root & stem rot

Per cent incidence	Category
No incidence	Immune
1-10% incidence	Resistant
11-25% incidence	Moderately Resistant
26-50% incidence	Moderately susceptible
51-70% incidence	Susceptible
More than 70% incidence	Highly Susceptible

3.11.2 Evaluation of sesame genotypes against root and stem rot

200 sesame genotypes DBR (Dark Brown Replication) were screened under field condition.

3.12 Management of root & stem rot of sesame through fungicides

3.12.1 *In vitro* evaluation of chemical fungicides against *M. phaseolina*

The poisoned food techniques (Nene and Thapliyal, 1982) was followed to evaluate the efficacy of fungicides. Six fungicides (Tebuconazole + Trifloxystrobin, Azoxystrobin, Pyraclostrobin + Metiram, Cymoxanil + Mencozeb, Captan + Hexaconazole and Carbendazim + Mencozeb) were tested in laboratory against *M. phaseolina* at a concentration of 500 ppm, 1000 ppm and 1500 ppm respectively with three replications were kept for each fungicides.

Molten sterilized potato dextrose agar was used as medium and required quantity of each fungicide was added separately so as to get a requisite concentration of that fungicide. The fungicides were thoroughly mixed by stirring and about 20 ml poisoned medium was poured to each of the 90 mm petri dishes and allowed for solidification. The actively growing periphery of the seven days old culture of *M. phaseolina* was carefully cut

using a cork borer and transferred aseptically to the centre of each petridish containing the poisoned solid medium. Suitable control was maintained by growing the cultures on PDA without fungicides. The plates were then incubated at 28 ± 1ºC in BOD incubator and observations were recorded after 192 hours of inoculation. The per cent growth inhibitions under the influences of different fungicides were calculated on the basis of control.

Per cent inhibition of mycelial growth was calculated by the following formula given by Vincent, (1947)

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

Where as

C = Diameter of fungus colony (mm) in control plate

T = Diameter of fungus colony (mm) in treated plate

Experimental Details:

Design : CRD (Combined Randomized Design)

No. of Replication : 3 (Three)

No. of Treatments : 6

T1 : Tebuconazole 50%+ Trifloxystrobin 25% WG

T2 : Azoxystrobin 23% SC

T3 : Pyraclostrobin 5%+ Metiram 55% WG

T4 : Cymoxanil 8%+ Mencozeb 64% WP

T5 : Captan 70% + Hexaconazole 5% WP

T6 : Carbendazim 12% + Mencozeb 63%

Concentration : 500, 1000 and 1500 ppm

3.12.2 *In vivo* evaluation of fungicides

This test was conducted to evaluate the most effective fungicide under field condition, in which various fungicides was used as foliar spray with recommended dose.

Location

In the experimental area of PC Unit, AICRP Sesame and Niger, JNKVV, Jabalpur (Madhya Pradesh) *Kharif* season -2019

Experimental Details:

Design : RBD (Randomized Block -Design)

No. of Replication : 3

No. of Treatment : 6

Plot Size : 2.4 × 3 m

Plant to plant spacing : 10-12 cm

Row to row spacing : 30 cm

Variety : VRI1

Date of sowing : 19 /07/ 2019

Table 3.4 Details of the Treatments includes

S. No.	Mode of Application	Treatments
T1	Spraying at capsule initiation and second spray after 15 days interval.	Tebuconazole 50% + Trifloxystrobin 25% @ 0.5g/l
T2	First spray at capsule initiation and second spray after 15 days interval.	Azoxystrobin @ 1ml/l
T3	First spray at capsule initiation and second spray after 15 days interval.	Pyraclostrobin + Metiram @ 3g/l
T4	First spray at capsule initiation and second spray after 15 days interval.	Cymoxanil + Mencozeb @ 2g/l
T5	First spray at capsule initiation and second spray after 15 days interval.	Captan + Hexaconazole @ 2g/l
T6	First spray at capsule initiation and second spray after 15 days interval.	Carbendazim + Mencozeb @ 2.5g/l
T7	Untreated check	

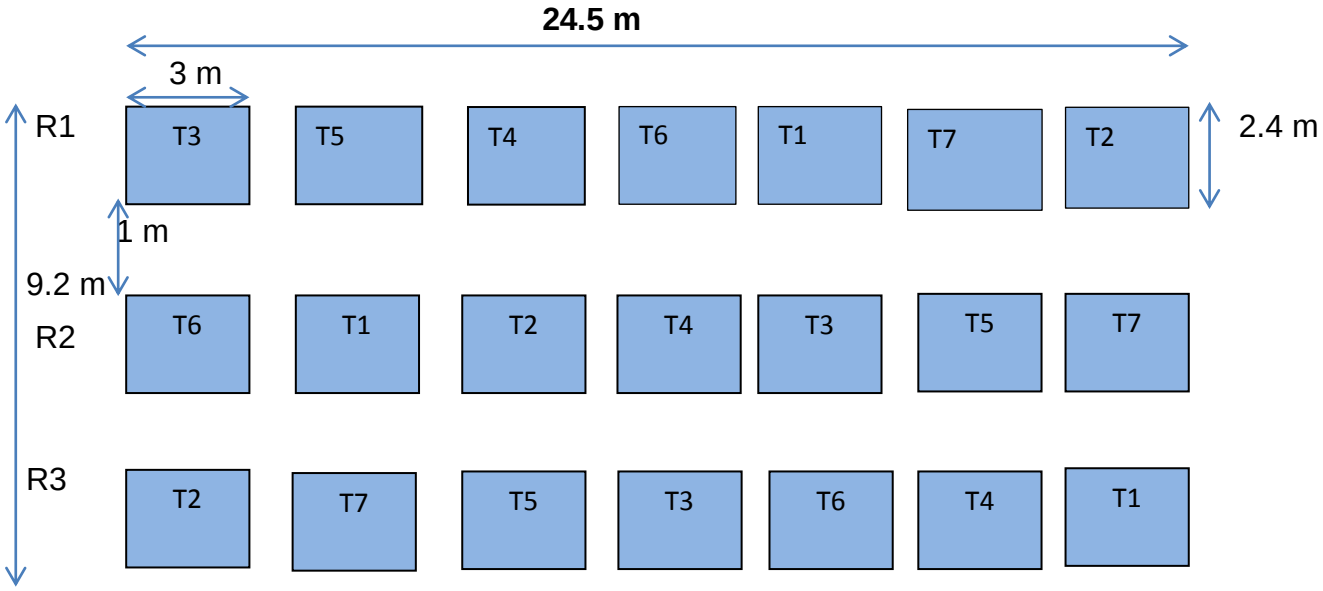
3.12.2.1 Observation recorded

Per cent disease incidence

Per cent disease incidence will be recorded of sesame crop grown under field condition and for the effect of different fungicides in field condition

Grain yield (g/plot)

The grain yield g/plot was recorded after threshing, cleaning and drying up to moisture 9% and then weighed the yield with the of electronic balance.



Layout of Experiment

3.13 Statistical analysis

Year and season of Experiment: *Kharif* 2019

(i) Analysais of variance- Randomized Block Design :

The data were subjected to statistical analysis after tabulation. The data of field experiments were analyzed following Randomized Block Design (Cochran and Cox, 1957).

ANOVA for Randomized Block Design

Sources of variance	Degree of Freedom	Sum of Squares (SS)	Mean sum of Squares (MSS)	F-Calculated	Table value 5%
Replications	(r-1)	SSR	SSR/r-1=a	a/c	
Treatments	(t-1)	SST	SST/t-1=b	b/c	F at 5% (t-1), (r-1) (t-1)
Error	(r-1) (t-1)	SSE	SSE/(r-1)(t-1)=c	-	-
Total	(r×t-1)	-	-	-	-

Where,

r = number of replications

t = number of treatments

SSR = Replication mean sum of square

SST = Treatment mean sum of square

SSE = Error mean sum of square

d.f = Degree of freedom

The significance among different treatment means was judged by critical difference (CD) at 5% level of significance for comparison among the treatments, for which the marginal means of each treatment was considered. The following formula was used for various estimations.

(1) $SEm\pm = \sqrt{EMS/r}$

(2) $\text{Critical difference (CD)} = \text{SEm} \times \sqrt{2} \times \text{t at } 0.05$

Where,

Ems = Error mean sum of square

t ='t' value at 5 % level at error d.f.

r = number of replications

SEm± = standard error of any treatment mean\

CD = Critical Difference

(ii) Completely Randomized Design (CRD)

The statistical analysis of the data of all the laboratory experiments were done following Completely Randomized Design.

ANOVA for CRD

S. No.	Source of Variation	Degree of Freedom	Sum of Squares (SS)	Mean sum of Squares (MSS)	F-Calculated	Table value 5%
1.	Treatment	(t-1)				
2.	Error	t(r-1)				
	Total	rt-1				

SEm± = √EMS/r

SEd± = √2EMS/r

CD = SEd± × t at 5%

Where,

r = No. of replications

ESM = Error mean square

SEm± = Standard error of treatment mean

SEd± = Standard error of difference to two treatment mean

CD = Critical difference to two treatments mean

3.14 Meteorological data

The weather condition during the course of studies from July, 2019 presented in the table

Table 3.5 Meteorological data during the crop season of 2019-20 at Jabalpur

Month	Week of Observation	SMW	Temperature		Rain fall (mm)	No. of rainy Days	Relative Humidity	
			Max.	Min.			Morning	Evening
July	1JULY-7JULY	27.0	29.9	23.1	178.8	3	94	87
July	8JULY-14JULY	28.0	31.4	25.0	59.8	1	82	64
July	15JULY-21JULY	29.0	34.9	25.0	24.4	2	82	59
July	22JULY-28JULY	30.0	32.3	24.0	48.0	3	88	74
Aug.	29JULY-4AUG	31.0	29.6	23.9	47.6	4	93	81
Aug.	5AUG-11AUG	32.0	29.9	23.5	210.0	4	92	81
Aug.	12AUG-18AUG	33.0	28.6	22.9	302.1	4	91	83
Aug.	19AUG-25AUG	34.0	29.2	22.5	212.9	7	97	83
Sept.	26AUG-1SEPT	35.0	30.9	23.4	57.2	4	93	75
Sept.	2SEPT-8SEPT	36.0	31.6	23.4	185.0	5	94	79
Sept.	9SEPT-15SEPT	37.0	28.5	23.4	101.4	5	93	83
Sept.	16SEPT-22SEPT	38.0	31.7	22.7	53.1	3	91	78
Sept.	23SEPT-29SEPT	39.0	29.5	22.1	77.6	4	93	78
Oct.	30SEPT-6OCT	40.0	30.1	21.0	14.3	2	90	66
Oct.	7OCT-13OCT	41.0	30.3	18.0	0.0	0	91	66

Source:- Meteorological Observatory, College of Agriculture, JNKVV, Jabalpur (M.P.)

Chapter -IV

RESULTS

RESULTS

The results of the experiment conducted during the course of investigation on “Studies on root and stem rot (*Macrophomina phaseolina)* (Tassi.) Goid. of sesame and its management through fungicides” are presented here under,

4.1 Collection of diseased plant samples

The plant samples showing the symptoms of (Plate 1) root and stem rot were collected from All India Coordinated research Project on Sesame and Niger JNKVV, Jabalpur (M.P.). The symptoms of disease on plant are visible from the time of emergence. At the time of seedling stage, pathogen causes dark and irregular lesions on epicotyls and hypocotyls of seedlings and sometimes the symptoms may causes the death of seedlings. In case of adult plants symptoms of the disease were sudden wilting of growing plant mainly after the flowering stage, stem portion near the ground level show dark brown and dark lesion which extend upward and rupture the stem. Root portion can be seen having black sclerotia sprinkled over the surface of root.

4.2 Isolation, purification and identification of the *M. phaseolina* (*Rhizoctonia bataticola*)

Fresh diseased parts of sesame plants showing disease symptoms were used for the isolation of the pathogen. The pathogen isolation was done in petri dishes on potato dextrose agar medium. The surface sterilized diseased bits produces white to brown cottony growth of fungus after 48 hours of incubation at 28 ± 1 ºC temperature in BOD incubator. The uniform colonies originating from diseased bits were separated, purified by sub-culturing with single hyphal tip method and were maintained on potato dextrose agar slants. The fungus produced abundant aerial mycelium in the culture plate with sclerotia imbedded within the hyphae on the agar surface. The colony of the pathogen start to became grey and darkened with age due to formation of black sclerotia. Numerous dark brown to black colour sclerotia can be seen on the reverse side of the culture plate. The culture was identified as *M. phaseolina* (Plate 2) on the basis of following characteristics:-

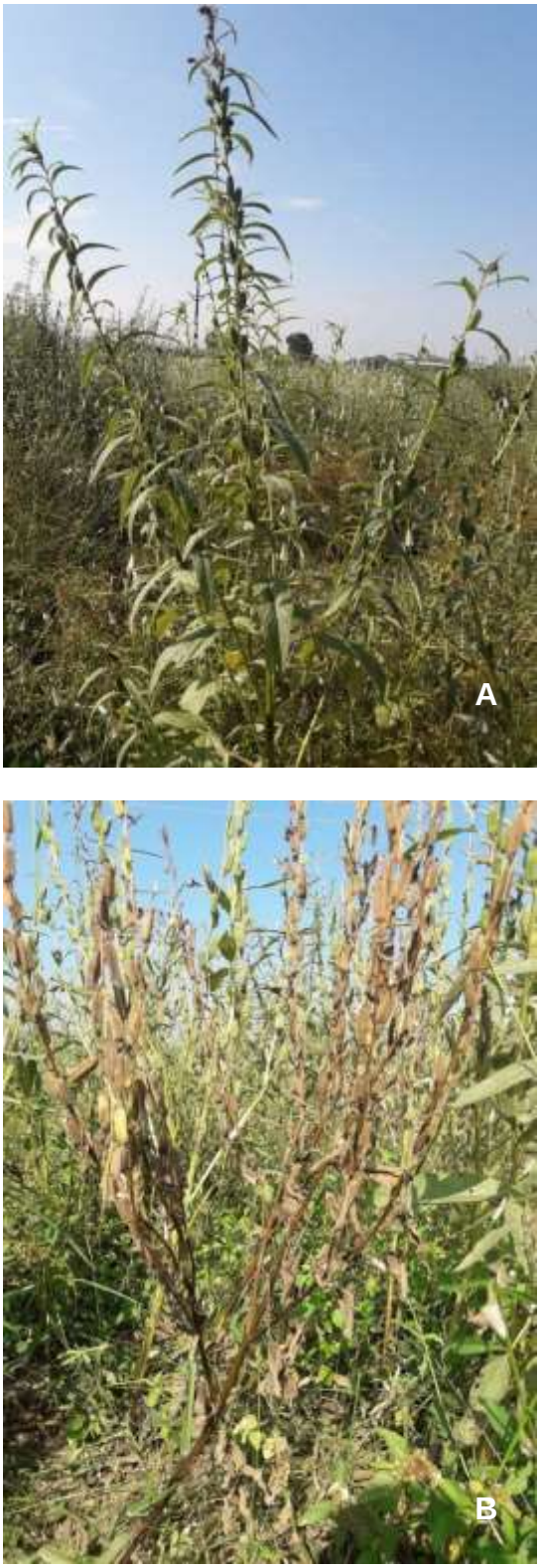


Plate 1 Showing: A. Healthy plant of sesame

B. Sesame Plant showing symptom of root and stem rot

4.2.1 Hyphae

Hyphae were septate, initially hyaline and vacuolated turning to a honey or black colour. The vegetative mycelium was characterized by the formation of monilid or barrel-shaped cells and the formation of septum near the branching of the mycelium. Branching occurs at right angle to parent hyphae, but branching at acute angles is also common. (Plate 2-C)

4.2.2 Sclerotia

Sclerotia were formed from the aggregation of hyphae with 50 to 200 individual cells coupled by a melanin pigment. At first the sclerotia were colourless but afterwards they turned black, hard and shiny. The sclerotia measures from 62.93-129.36 µm x 46.93-111.28 µm. The characteristics were identical to the characters of *M. phaseolina*. (Plate 2-D)

4.3 Pathogenicity test

Pathogenicity of the culture of *M. phaseolina* was tested by growing sesame plants in pots containing pathogen infested soil. Soil maize meal medium was prepared by using sand 2 parts and partially shredded maize 1 part proportion. The media was suitably moistened and transferred in 250 ml conical flasks which were sterilized at 1.05 kg per square cm for 30 minutes into autoclave. The prepared sterilized media were inoculated with *M. phaseolina* cultures and incubated at 28 ± 1ºC for 10 days. The sand maize meal media inoculated with *M. phaseolina* were added to soil at 20 g per kg soil and mixed thoroughly. Ten healthy seeds of sesame (VRI1) were sown in each pot. The results of the study indicated that pathogen caused seed rot, pre emergence and post emergence mortality. The plants in inoculated pots showed typical symptoms of the disease while plants in uninoculated pots remained healthy. The fungus was reisolated from infected plant portions and various characters like colour of colony, hyphal and sclerotial structure was found to be identical to the previous cultures. (Plate2-E&F)

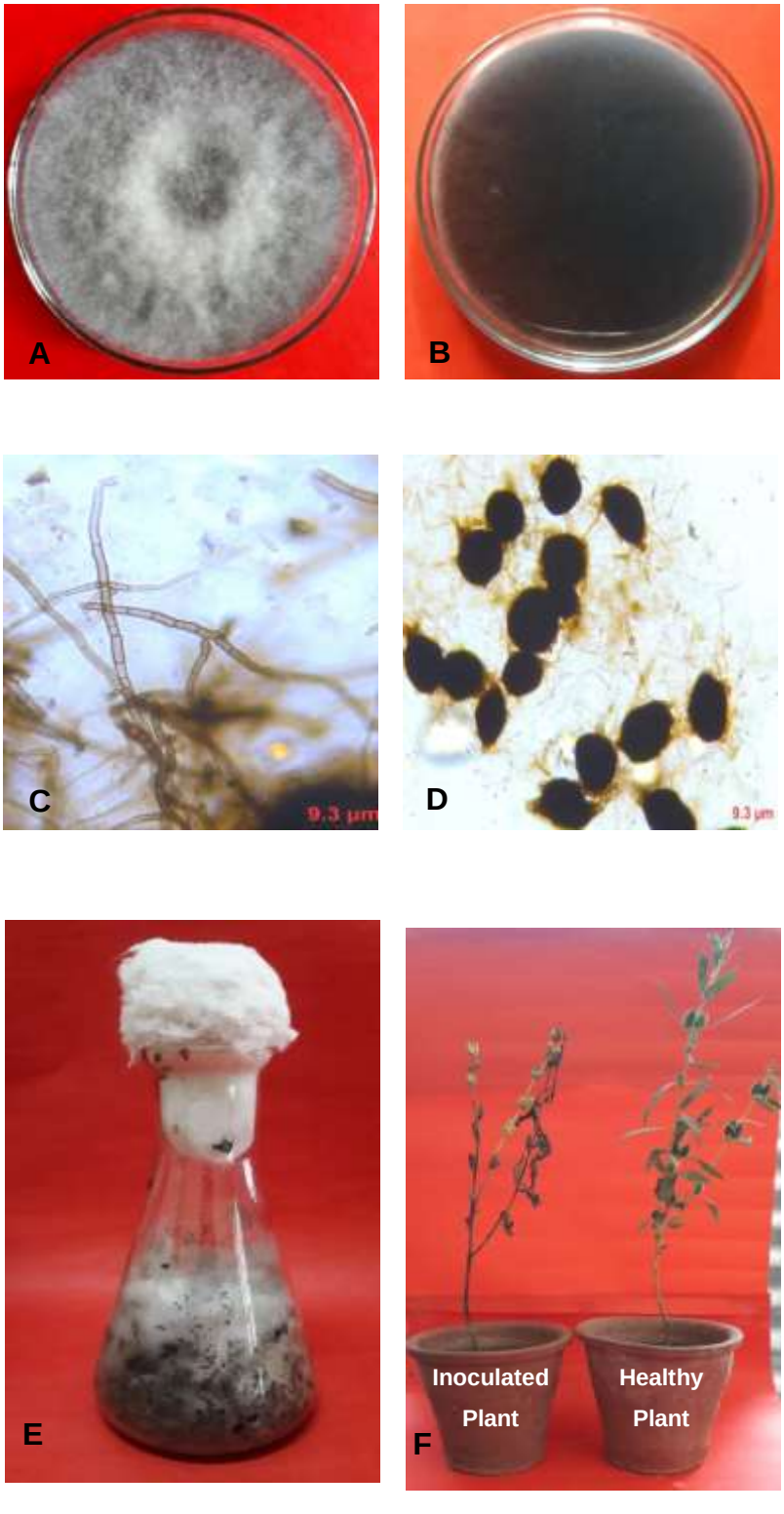


Plate 2 Showing:-

A & B. Pure culture of *M. phaseolina*
C & D. Hyphal & Sclerotial structure of *M. phaseolina*
E. Mass multiplication of *M. phaseolina*
F. Proving pathogenicity of *M. phaseolina*

4.4 Studies of variability in *Macrophomina phaseolina (Rhizoctonia bataticola)*

4.4.1 Cultural variability in *Macrophomina phaseolina (Rhizoctonia bataticola)*

Variability in the cultural characteristics of three isolates of *M. phaseolina* was studied on potato dextrose agar medium. The colony growth rate, colony colour, colony texture and type of margin of the colony were studied.

Colony growth

Three isolates of *M. phaseolina* (*Rhizoctonia bataticola*) were studied. There was no significant difference in the colony growth rate of all isolate at 72 hours after inoculation at 28 ± 1ºC. The maximum colony growth 90 mm was observed in the all isolates. (Table 4.1 & Plate 3)

Colony colour

Three isolates of *M. phaseolina* (*Rhizoctonia bataticola)*, were studied for colony colour. Based upon visual observation of colony colour the cultures were observed 2 types of colour into the colonies. The isolate Mp 3 was observed dark black colour colony, while isolate Mp 1 and Mp 2 were observed blackish grey colour colony. (Table 4.1 & Plate 3)

Colony texture

The isolate of *M. phaseolina* (*Rhizoctonia bataticola)* were observed 2 types of colony texture *viz.,* appressed and fluffy growth and suppressed and dense growth. Isolate Mp 1 and Mp 2 were observed appressed and fluffy growth and isolate Mp 3 was observed suppressed and dense growth into the culture medium. (Table 4.1 & Plate 3)

Types of margin

The isolates of *M. phaseolina (Rhizoctonia bataticola)* produces mainly 2 types of margins were seen, irregular and scattered and luxuriant and uniform. Out of 3 *M. phaseolina* isolates Mp 1 and Mp 2 were observed irregular and scattered margins while isolate Mp 3 was observed luxuriant and uniform margins into the culture medium. (Table 4.1 & Plate 3)

Table 4.1 Variability in cultural characteristics of *Macrophomina phaseolina (Rhizoctonia bataticola)* isolates

S. No.	Isolate code	Colony texture	Colony colour	Type of margin	Colony Growth (mm)
1.	Mp 1 (Madhya Pradesh)	Appressed and fluffy growth	Grey	Irregular and scattered margins	90.0
2.	Mp 2 (Rajasthan)	Appressed and fluffy growth	Grey	Irregular and scattered margins	90.0
3.	Mp 3 (Tamil Nadu)	Suppressed and dense growth	Black	Luxuriant and uniform margins	90.0

4.4.2 Morphological variability of *Macrophomina phaseolina (Rhizoctonia bataticola)*

Variability in the morphological characteristics of three isolates of *M. phaseolina (Rhizoctonia bataticola)* were studied on potato dextrose agar medium. The morphological characters of different isolates shape of sclerotia, size of sclerotia per microscopic field of 10x were measured.

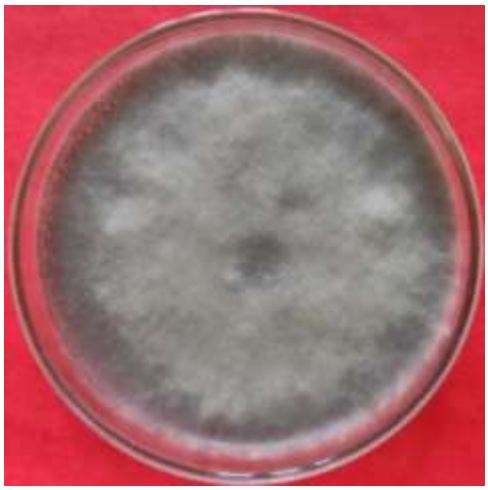
Sclerotial size & Shape

On the basis of microscopic observations the size of sclerotia varied from 57 µm x 55 µm to 97 µm x 89 µm was observed (Table 4.2 & Plate 4) and the shape of sclerotia varies from circular to irregular. Among the isolate Mp 1 and Mp 3 have irregular shape sclerotia while the Isolate Mp 2 produce circular shapes sclerotia and all the isolates were produced black colour sclerotia.

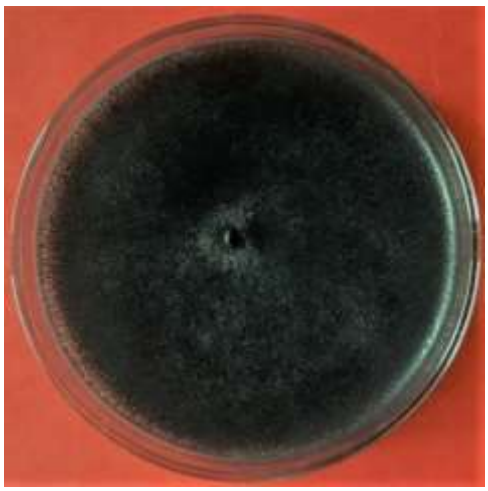
Table 4.2 Variability in morphological characteristics of *Macrophomina phaseolina (Rhizoctonia bataticola)* isolates

S. No.	Isolate code	Sclerotia			
		Length x Width (µm)		Shape	Colour
		Length (µm)	Width (µm)		
1	Mp 1 (Madhya Pradesh)	97	89	Irregular	Black
2	Mp 2 (Rajasthan)	57	55	Circular	Black
3	Mp 3 (Tamil Nadu)	71	66	Irregular	Black

Mp 1 (Jabalpur, Madhya Pradesh)



Mp 2 (Mandor, Rajasthan)



Mp 3 (Vridhachalam, Tamil Nadu)

Plate 3 Showing: Cultural variability of different *M. phaseolina* (*Rhizoctonia bataticola*) isolates

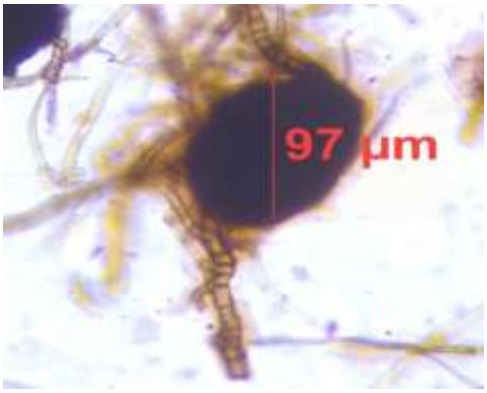
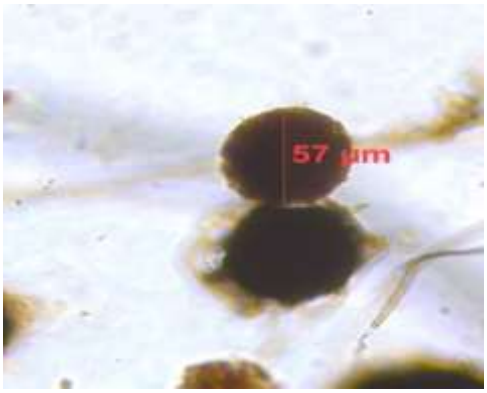
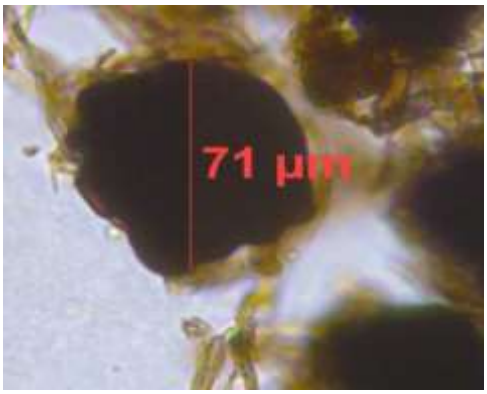
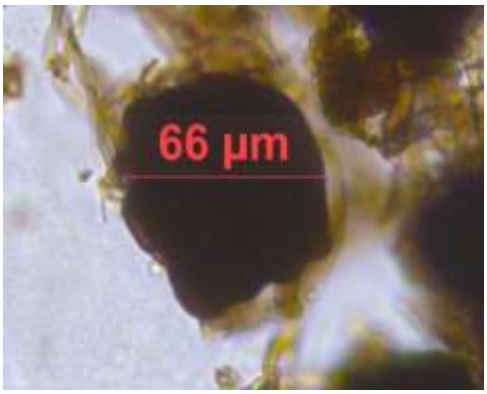
Isolate of *R. bataticola*	Length (µm)	Width (µm)
Mp 1 (Madhya Pradesh)		
Mp 2 (Rajasthan)		
Mp 3 (Tamil Nadu)		

Plate 4 Showing: Sclerotial characters of *M. phaseolina (Rhizoctonia bataticola)* isolates.

4.5 Reaction of sesame cultivars against root & stem rot (*M. phaseolina*) infection under natural condition

Eight sesame cultivars were evaluated under field condition. The per cent disease incidence was recorded at pre flowering and capsule formation stage. Data presented in the table 4.3 & Fig 4.1 showed, that sesame cultivars GT10 (8.0%), RT346 (8.0%) and TKG306 (9.66%) were found resistant against root & stem rot infection. However VRI 1 (Susceptible check) have found maximum disease incidence (28.00%).

Table 4.3 Reaction of sesame cultivars against root & stem rot (*M. phaseolina*) infection under natural condition

S. No.	Varieties	Disease Incidence (%)	
		Pre flowering stage*	Capsule formation stage*
1	GT10	3.00	8.00
2	TKG22	8.00	10.33
3	TKG308	5.00	11.66
4	RT351	9.00	24.00
5	TKG306	7.00	9.66
6	RT346	3.00	8.00
7	RAMA	3.00	10.00
8	VRI1	9.00	28.00
	SE(m)±	0.248	0.436
	CD at 5%	0.760	1.337

*Mean of three replication

4.6 Reaction of sesame germplasm against root & stem rot of sesame

Screening of 200 genotypes against root and stem rot disease incidence were presented in (Table 4.4 and plate 5) revealed that on screening of 200 germplasm lines (DBR); 0 (zero) lines were found immune (disease scale 0), (40) forty lines were found resistant (disease scale 1), (45) forty five lines were found moderately resistant (disease scale 2), (92) ninety two lines were found moderately susceptible (disease scale 3), (14) fourteen lines were found susceptible (disease scale 4) and nine (9) lines were found highly susceptible (disease scale 5).

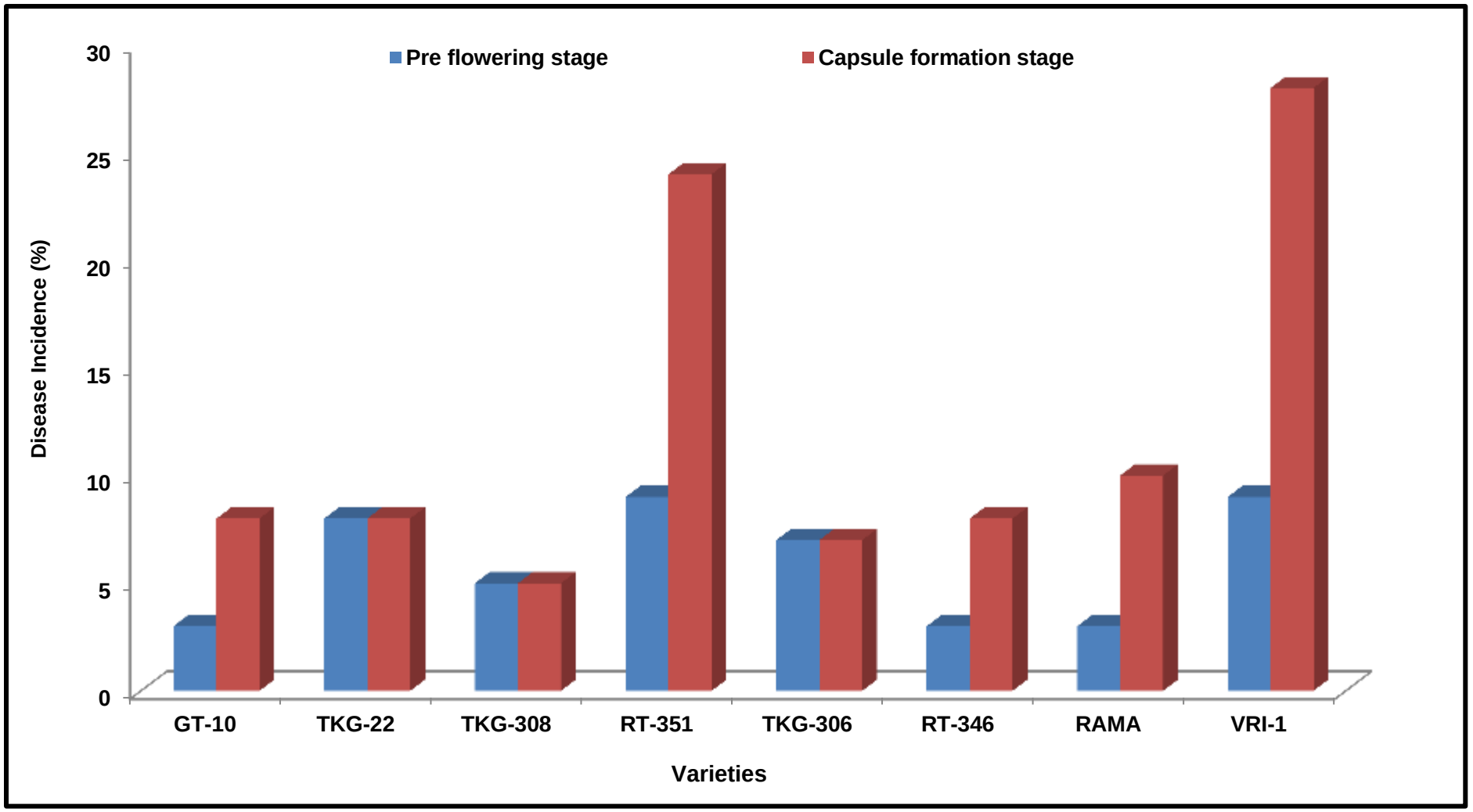


Fig. 4.1 Evaluation of Sesame Varieties against Root and Stem rot (*M. phaseolina*) under natural condition

Table 4.4 Screening of Sesame germplasm against root and stem rot caused by *M. phaseolina* in field under natural condition.

S. No.	Name of Entries	Disease Incidence (%)	Reaction
1.	SI- 3237	53.33	Susceptible
2	IC-131607	46.66	Moderately susceptible
3	SI-3179	46.66	Moderately susceptible
4	SI-3231	20.00	Moderately resistant
5	EC-33507	13.33	Moderately resistant
6	IS-321	6.66	Resistant
7	SI-1156	20.00	Moderately resistant
8	EC-335011-A	80.00	Highly susceptible
9	EC-334990	20.00	Moderately resistant
10	EC-334989	33.33	Moderately susceptible
11	ICA-14146-A	20.00	Moderately resistant
12	BC-303427	53.33	Susceptible
13	IS-665	6.66	Resistant
14	SI-3234	20.00	Moderately resistant
15	EC-334280	33.33	Moderately susceptible
16	S-0182-1	33.33	Moderately susceptible
17	IS-475	73.33	Highly susceptible
18	EC-334983	46.66	Moderately susceptible
19	KIS-375	46.66	Moderately susceptible
20	Agra- Balik	33.33	Moderately susceptible
21	IS-100-8	40.00	Moderately susceptible
22	SI-1679	6.66	Resistant
23	SI-76-1	20.00	Moderately resistant
24	EC-334984	46.66	Moderately susceptible
25	SP-1144	46.66	Moderately susceptible
26	EC-334950-I	73.33	Highly susceptible
27	EC-335010	13.33	Moderately resistant
28	EC-334001	53.33	Susceptible
29	EC-334979	33.33	Moderately susceptible
30	KIS-398	53.33	Susceptible
31.	EC-334977	73.33	Highly susceptible
32.	KIC-1634-B	73.33	Highly susceptible
33.	EC-334973	53.33	Susceptible
34.	EC-178-2	46.66	Moderately susceptible
35.	SI-1516	80.00	Highly susceptible
36.	IS-728	73.33	Highly susceptible
37.	EC-384985-I	40.00	Moderately susceptible
38.	EC-334994	13.33	Moderately resistant
39.	EC-334974	40.00	Moderately susceptible
40.	EC-349	40.00	Moderately susceptible

S. No.	Name of Entries	Disease Incidence (%)	Reaction
41.	IS-461-1-84	6.66	Resistant
42.	EC-334999	46.66	Moderately susceptible
43.	NIC-7905	60.00	Susceptible
44.	SI-1687	20.00	Moderately resistant
45.	EC-3340998	26.66	Moderately susceptible
46.	SI-1033	13.33	Moderately resistant
47.	EC-665	33.33	Moderately susceptible
48.	SI-1225	40.00	Moderately susceptible
49.	IS-366	73.33	Highly susceptible
50.	EC-33962	46.66	Moderately susceptible
51.	IS-723	26.66	Moderately susceptible
52.	SI-253	20.00	Moderately resistant
53.	S-0388	6.66	Resistant
54.	ES-75-2-84	40.00	Moderately susceptible
55.	ES-334966	6.66	Resistant
56.	ES-81	26.66	Moderately susceptible
57.	IC-199443	26.66	Moderately susceptible
58.	EC-334995	33.33	Moderately susceptible
59.	EC-3349997	33.33	Moderately susceptible
60.	KMR-1	53.33	Susceptible
61.	ES-62	26.66	Moderately susceptible
62.	SI-2192	20.00	Moderately resistant
63.	IS-17	6.66	Resistant
64.	IS-722-2-84	33.33	Moderately susceptible
65.	IS-3179	20.00	Moderately resistant
66.	IS-446-1-64	33.33	Moderately susceptible
67.	IS-393-1	46.66	Moderately susceptible
68.	EC-303440-B	26.66	Moderately susceptible
69.	IS-461-1-84-I	20.00	Moderately resistant
70.	EC-335005	40.00	Moderately susceptible
71.	NIC-163-88	20.00	Moderately resistant
72.	SI-995	13.33	Moderately resistant
73.	SI-1345	20.00	Moderately resistant
74.	SI-63	46.66	Moderately susceptible
75.	EC-334993	6.66	Resistant
76.	SI-2008	26.66	Moderately susceptible
77.	NIC-8288	53.33	Susceptible
78.	EC-334971	53.33	Susceptible
79.	EC-310439	66.66	Susceptible
80.	SI-7192	40.00	Moderately susceptible

S. No.	Name of Entries	Disease Incidence (%)	Reaction
81.	SI-3263	33.33	Moderately susceptible
82.	SI-712	46.66	Moderately susceptible
83.	SI-29973	53.33	Susceptible
84.	IS-3079-1	46.66	Moderately susceptible
85.	SI-1146	60.00	Susceptible
86.	EC-335004	33.33	Moderately susceptible
87.	EC-335000	33.33	Moderately susceptible
88.	SI-2174-1	33.33	Moderately susceptible
89.	ES-150-1	53.33	Susceptible
90.	EC-334992	33.33	Moderately susceptible
91.	ES-42	40.00	Moderately susceptible
92.	SI-3168	26.66	Moderately susceptible
93.	SI-37	6.66	Resistant
94.	SI-1850	13.33	Moderately resistant
95.	ES-1162	33.33	Moderately susceptible
96.	NIC-8348	40.00	Moderately susceptible
97.	G-7	26.66	Moderately susceptible
98.	G-52	6.66	Resistant
99.	NIC-9985	20.00	Moderately resistant
100.	TSS-4	13.33	Moderately resistant
101.	Nitamtpur-1	33.33	Moderately susceptible
102.	NCC-16272	6.66	Resistant
103.	SI-3178	26.66	Moderately susceptible
104.	IC-14329	33.33	Moderately susceptible
105.	RJS-123	33.33	Moderately susceptible
106.	IS-145	6.66	Resistant
107.	G-23	6.66	Resistant
108.	TMV	13.33	Moderately resistant
109.	NIC-9066	6.66	Resistant
110.	SI-2039	13.33	Moderately resistant
111.	IS-372	6.66	Resistant
112.	S-0028	6.66	Resistant
113.	EC-334973	26.66	Moderately susceptible
114.	SI-2225-B	6.66	Resistant
115.	ES-37	6.66	Resistant
116.	IS-503	13.33	Moderately resistant
117.	IS-101-2-A	40.00	Moderately susceptible
118.	IS-717	6.66	Resistant
119.	IC-204670	6.66	Resistant
120.	78-326	13.33	Moderately resistant

S. No.	Name of Entries	Disease Incidence (%)	Reaction
121.	T-4	33.33	Moderately susceptible
122.	G-25	40.00	Moderately susceptible
123.	SI-2138-2	46.66	Moderately susceptible
124.	NIC-162662	6.66	Resistant
125.	NI-3181	33.33	Moderately susceptible
126.	S-0140	26.66	Moderately susceptible
127.	G-11	40.00	Moderately susceptible
128.	RJS-62	26.66	Moderately susceptible
129.	S-0598	6.66	Resistant
130.	SI-2123	6.66	Resistant
131.	EC-334960	6.66	Resistant
132.	IS-451	26.66	Moderately susceptible
133.	Sulean-21	6.66	Resistant
134.	EC-334999-I	33.33	Moderately susceptible
135.	G-45	26.66	Moderately susceptible
136.	G-36	6.66	Resistant
137.	EC-334997	33.33	Moderately susceptible
138.	ES-75-4-84	46.66	Moderately susceptible
139.	G-9	20.00	Moderately resistant
140.	1610-2	20.00	Moderately resistant
141.	G-14	26.66	Moderately susceptible
142.	IS-364-I	26.66	Moderately susceptible
143.	G-48	20.00	Moderately resistant
144.	NIC-17304-B	33.33	Moderately susceptible
145.	KIS-282	13.33	Moderately resistant
146.	G-12	13.33	Moderately resistant
147.	IS=641-1-84	46.66	Moderately susceptible
148.	EC-33498	6.66	Resistant
149.	G-37	20.00	Moderately resistant
150.	G-41	26.66	Moderately susceptible
151.	IS-972	40.00	Moderately susceptible
152.	EC-303442	6.66	Resistant
153.	EC-334957	33.33	Moderately susceptible
154.	RJS-77	6.66	Resistant
155.	EC-334961-I	6.66	Resistant
156.	G-43	6.66	Resistant
157.	S-0481	6.66	Resistant
158.	ES-75	6.66	Resistant
159.	S-0273	6.66	Resistant
160.	G-3	6.66	Resistant

S. No.	Name of Entries	Disease Incidence (%)	Reaction
161.	Tilo/hana	20.00	Moderately resistant
162.	EC-335001-A	6.66	Resistant
163.	S-003116	26.66	Moderately susceptible
164.	NIC-16114-B	6.66	Resistant
165.	BS-61	33.33	Moderately susceptible
166.	EC-334927	6.66	Resistant
167.	NIC-161848	26.66	Moderately susceptible
168.	EC-164966	13.33	Moderately resistant
169.	KMR-90	26.66	Moderately susceptible
170.	Ledguda	26.66	Moderately susceptible
171.	EC-335003	13.33	Moderately resistant
172.	TIC-74	26.66	Moderately susceptible
173.	Anand Local	13.33	Moderately resistant
174.	IS-722-2-84-I	20.00	Moderately resistant
175.	S-0606	33.33	Moderately susceptible
176.	IS-653-A	53.33	Susceptible
177.	NIC-17477-I	20.00	Moderately resistant
178.	12-Jun	46.66	Moderately susceptible
179.	S-0449	26.66	Moderately susceptible
180.	NIL-16426	26.66	Moderately susceptible
181.	S-0539	20.00	Moderately resistant
182.	G-10	40.00	Moderately susceptible
183.	SI-1687-I	13.33	Moderately resistant
184.	IC-14331	13.33	Moderately resistant
185.	Oct-81	20.00	Moderately resistant
186.	EC-182832	20.00	Moderately resistant
187.	G-18	26.66	Moderately susceptible
188.	NIC-8033	33.33	Moderately susceptible
189.	SI-805	33.33	Moderately susceptible
190.	G-2	13.33	Moderately resistant
191.	G-6	40.00	Moderately susceptible
192.	RJS-185	40.00	Moderately susceptible
193.	NIC-8282	73.33	Highly susceptible
194.	IS-387	46.66	Moderately susceptible
195.	G-13	40.00	Moderately susceptible
196.	EC-334955	26.66	Moderately susceptible
197.	NCR/82/NO/Bo/NS	13.33	Moderately resistant
198.	IS-205-I	6.66	Resistant
199.	NIC-8062	6.66	Resistant
200.	G-8	13.33	Moderately resistant
SC	VRI 1	80.00	Highly Susceptible

Plate 5 Showing:

A. Sesame cultivars screening against root and stem rot disease in Natural conditions

B. Germplasms screening of sesame against root and stem rot disease at natural conditions

Table 4.5 Screening of sesame germplasms against root & stem rot

Reaction	No. of Entries	Entries
Immune	0	NIL
Resistant	40	IS-321, SI-1679, IS-461-1-54, IS-17,EC-334993,G-52,G-23, SI-2225-B,IS-717, S-0598, Sulean-21,G-36,EC-33498, RJS-77, G-43, S-0481, G-3, NIC-16114-B, EC-334927IS-665, S-0388, ES-334966, SI-37, NCC-16272, IS-145, NIC-9065, IS-372,S-0028, ES-37, IC-204870, NIC-162662, SI-2123, EC-334960, EC-303442,EC-334961-I, ES-75, S-0273,EC-335001-A, IS-205-I, NIC-8062
Moderately Resistant	45	SI-3231, EC-33507, SI-1156,EC-334990, ICA-14146-A, SI-3234,SI-76-1, EC-335010, EC-334994,SI-1687, SI-1033, SI-253, SI-1033,SI-253, SI-2192, IS-3179, IS-461-1-84-1,NIC-163-88, SI-995, SI-1850, NIC-9985, TSS-4, SI-2039, IS-503, 78-326, G-9, 1610-2, G-48, KIS-282, G-12, G-37,Tilo / hana, EC-164966, EC-335003, Anand local, IS-722-2-84-I,NIC-17477-I, S-0539, SI-1687-I, IC-14331, Oct-81, EC-182832, G-2, NCR/82/NO/BO/NS, G-8
Moderately susceptible	92	IC-131607, SI-3179, EC-334989,EC-334280, S-0182-1, EC334983, KIS-375, Agra-Balik, IS-100-8, EC-334984, SP-1144, EC-334979, EC-178-2, EC-384985-1, EC-334974, SI-349, EC-354999, EC-3340998, EC-665,SI-1225, EC-33962, IS-723, ES-75-2-84, ES-81, IC-199443, EC-334995,EC- 3349997,ES-62,SI-2192,IS-722-2-84, IS-446-1-64, IS-393-1,EC-303440-B, EC-335005, SI-63,SI-2008, SI-7192, SI-3263, SI-712,IS-3079-1, EC-335004, EC-335000,SI-2174-1, EC-334992, ES-42, SI-3168,ES-1162, NIC-8348, G-7, Nitamtpur-1, SI-3178, IC-14329, RJS-123,EC-334973, IS-101-7-A, T-4, G-25,SS-2135-2, NI-3181, S-0140, G-11,RJS-62, IS-451, EC-334999-1, G-45,EC-334997, ES-75-4-84, G-14, IS-364-1,NIC-17304-B, IS-641-1-84, G-41,IS-972, EC-334957, S-003116, BS-61, NIC-161848, KMR-90, Ledguda,TIC-74, S-0606, 12-JUN, S-0449,NIL-16426, G-10, G-18, NIC-8033,SI-805, G-6, RJS-185, IS-387, G-13,EC-334955
Susceptible	14	SI-3237, BC-303427, EC-334001,KIS-398, EC-334973, NIC-7905,KMR-1, NIC-8288, EC334971,EC-310439, SI-29973, SI-3079-1,ES-150-1,IS-653-A
Highly Susceptible	9	NIC-8282, EC-335011-A, IS-475, EC-334950-1, EC-334977, KIC-1634-B, SI-1516, IS-728, IS-366

4.7 Effect of different fungicides against *Macrophomina phaseolina*

4.7.1 *In vitro* evaluation of fungicides against *Macrophomina phaseolina* (Tassi.) Goid. for management of root & stem rot

With the object to find out the best fungicides to manage *M. phaseolina*, six fungicides at three concentrations were evaluated under *in vitro* conditions. The per cent inhibition for each fungicides was calculated and presented in the Table 4.6.

As the concentration of fungicides increased from 500 to 1500 ppm there was reduction in mycelial growth of *M. phaseolina* as compared to control.

At 500 ppm, no growth was observed in Tebuconazole 50% +Trifloxystrobin 25%. Comparatively less growth was observed at Carbendazim + Mencozeb (15.16 mm). Maximum growth was observed at Azoxystrobin (56.33 mm) at 500 ppm (Fig 4.2 & Plate 6).

At 1000 ppm, no growth was observed in Tebuconazole 50% +Trifloxystrobin 25%. The growth of *M. phaseolina* was completely inhibited by Captan + Hexaconazole and Carbendazim + Mencozeb at 1000 and 1500 ppm. Maximum growth was observed into Azoxystrobin (53.33 mm) at 1000 ppm.

At 1500 ppm, the maximum growth was seen into Azoxystrobin (47.83 mm), followed by Pyraclostrobin + Metiram (25.33 mm) growth. The maximum growth of *M. phaseolina* was recorded into control (90.00 mm).

All fungicides were superior over control at 500 ppm, 1000 ppm and 1500 ppm. Complete inhibition of mycelial growth was recorded by T1 (Tebuconazole 50% +Trifloxystrobin 25%) at all the three concentrations taken. However T6 (Carbendazim + Mencozeb) and T5 (Captan+ Hexaconazole) exhibited complete inhibition at 1000 ppm. At 1500 ppm T3 (Pyraclostrobin + Metiram) and T4 (Cymoxanil + Mencozeb) inhibited more than 50% growth of *M. phaseolina* while T2 (Azoxystrobin) showed less than 50% inhibition of growth at all used concentrations. (Fig 4.3)

Table 4.6 *In vitro* evaluation of fungicides against *Macrophomina phaseolina* (Tassi.) Goid. for management of root & stem rot

S. No.	Fungicides	Radial Growth (mm)*			Average growth (mm)	Inhibition (%)*			Average Inhibition %
		Concentration (ppm)				Concentration (ppm)			
		500	1000	1500		500	1000	1500	
1.	Tebuconazole 50% + Trifloxystrobin 25%	0.00	0.00	0.00	0.00	100	100	100	100
2.	Azoxystrobin	56.33	53.33	47.83	52.49	37.41	40.74	46.85	41.66
3.	Pyraclostrobin + Metiram	49.16	40.83	25.33	38.44	45.37	54.63	71.85	57.28
4.	Cymoxanil + Mencozeb	23.00	14.66	7.83	15.16	74.44	83.71	91.3	83.15
5.	Captan + Hexaconazole	20.00	0.00	0.00	6.66	77.77	100	100	92.59
6.	Carbendazim + Mencozeb	15.16	0.00	0.00	5.05	83.31	100	100	94.43
7.	Control	90.00	90.00	90.00	90	0.00	0.00	0.00	
8.	SE(m)± CD at 5%	0.393 1.205	0.450 1.378	0.378 1.158					

*Mean of Three Replications

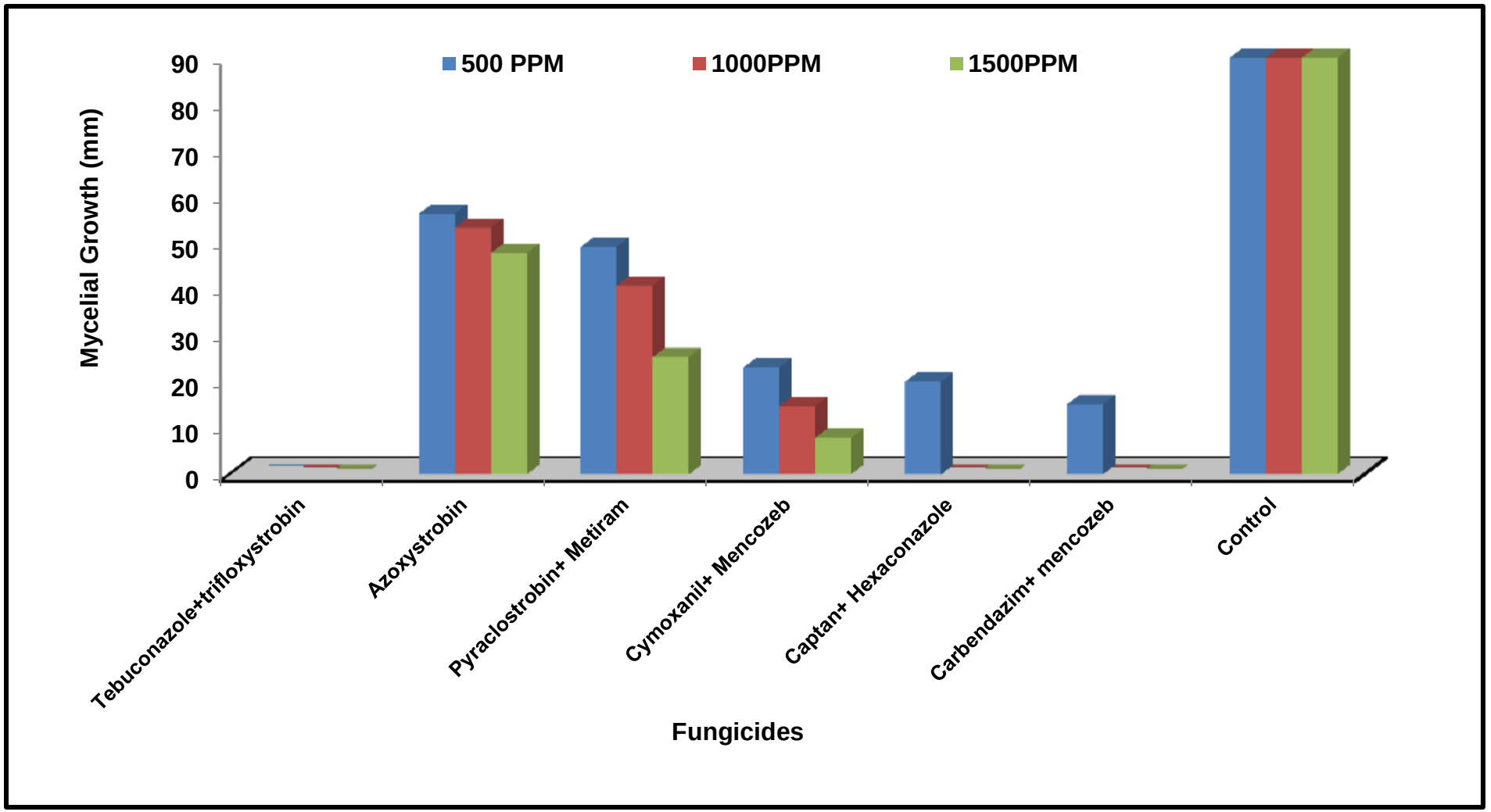


Fig. 4.2: Mycelial growth of *Macrophomina Phaseolina* at different concentrations of Fungicides

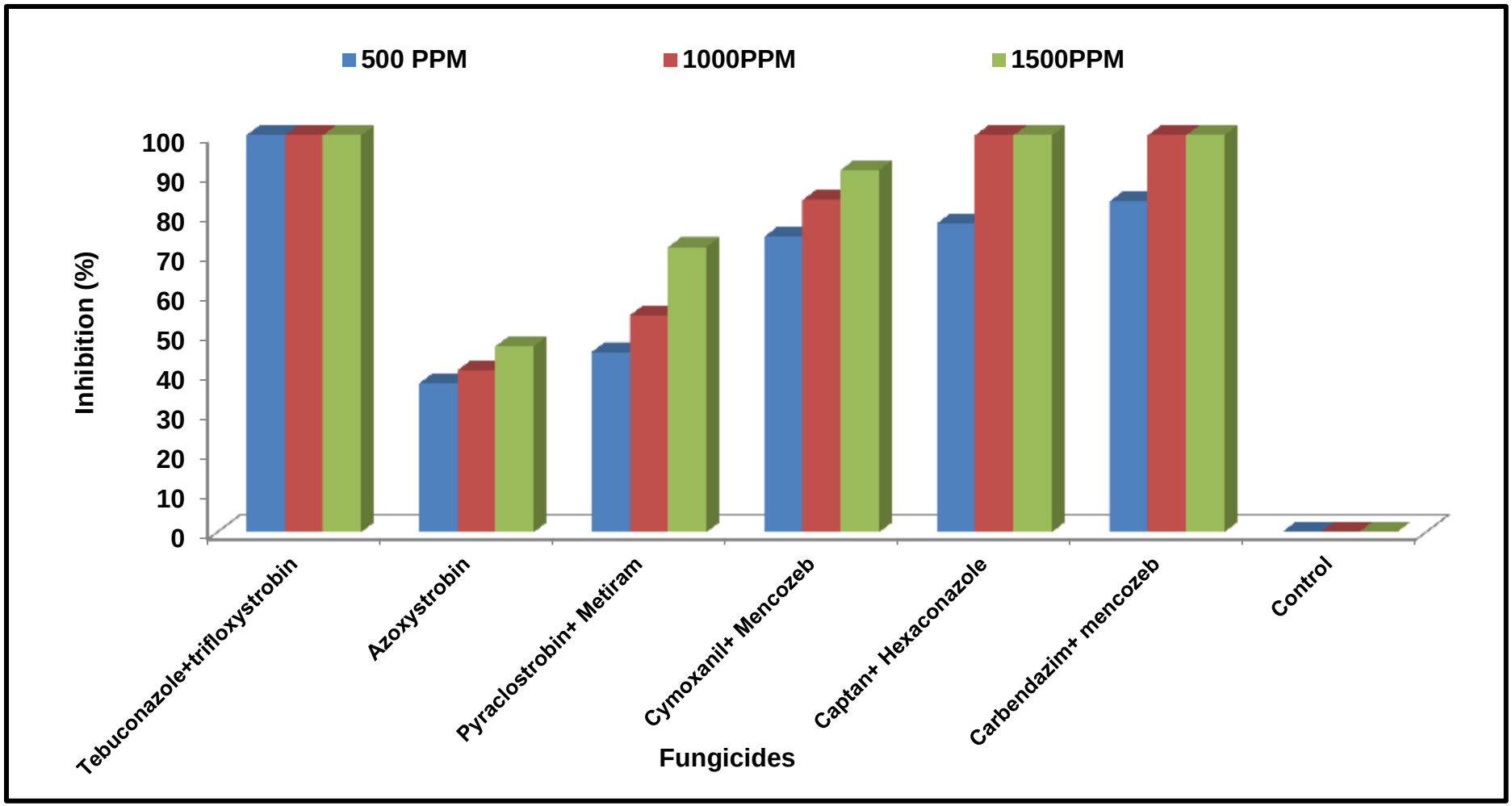


Fig. 4.3 : Percent inhibition of *Macrophomina Phaseolina* at different concentrations of Fungicides

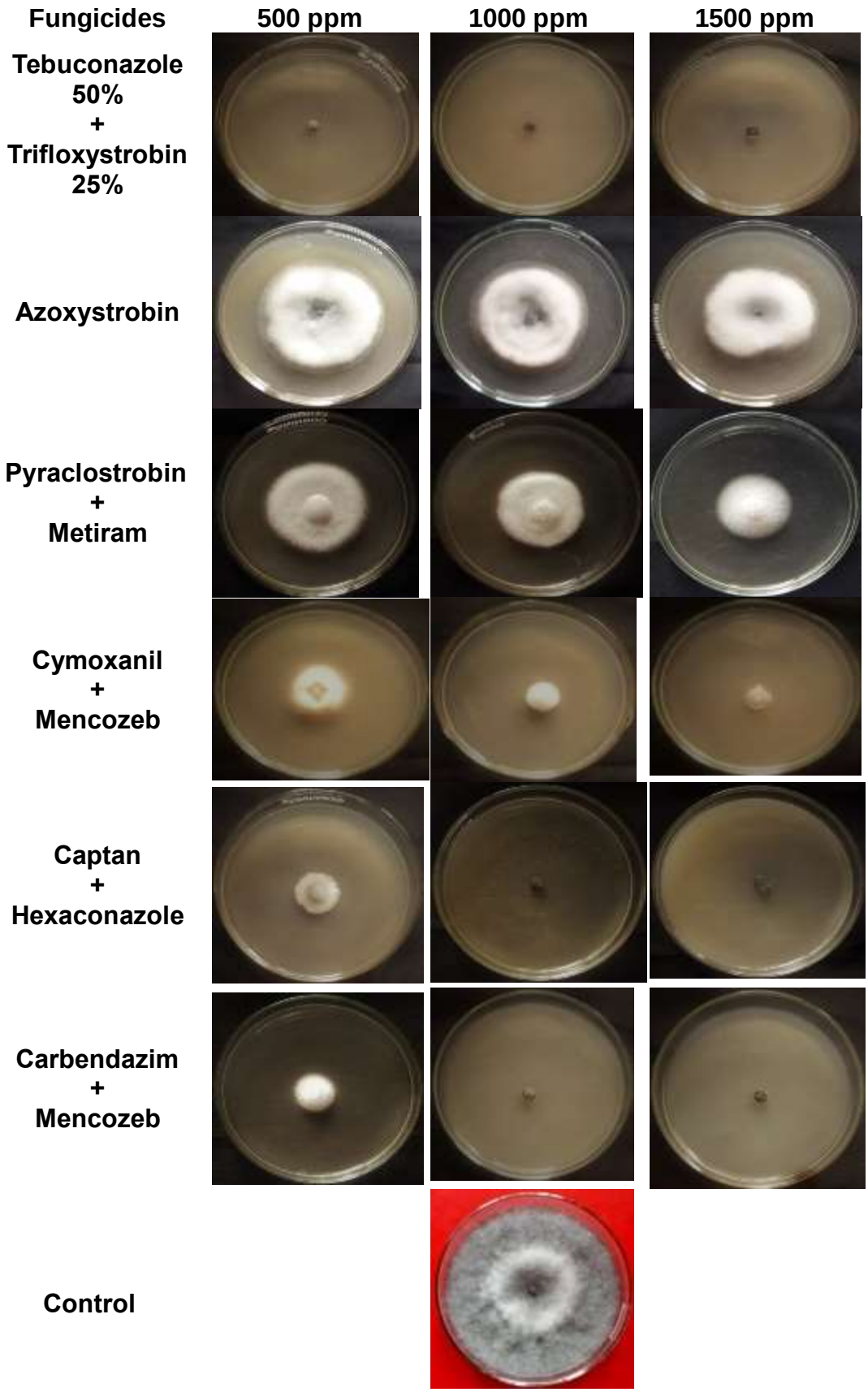


Plate 6 Showing: *In vitro* evaluation of fungicides against *M. phaseolina*

4.7.2 *In vivo* evaluation of fungicides against *Macrophomina phaseolina* (Tassi.) Goid. for management of root & stem rot

In the field trial conducted for the management of root and stem rot disease of sesame, all the treatments were found to be superior over control. Table 4.7 & Fig 4.4 indicated that among the treatments, T1 (Foliar spray of Trifloxystrobin + Tebuconazole @ 0.5 g/l at capsule initiation and second spray after 15 days interval) recorded the minimum disease incidence of 22.4% and the maximum yield of 617.33 kg/ha., followed by T6 (Foliar spraying of Carbendazim + Mancozeb @ 2.5 g/l at capsule initiation and second spray after 15 days interval) which recorded the disease incidence of 27.1% and the yield of 582.7 kg/ha. The maximum disease incidence of 48.9% and the minimum yield of 428 kg/ha was observed in the control followed by T2 (foliar spraying of Azoxystrobin @ 1.0 ml/l at capsule initiation and second spray after 15 days interval which recorded 37.3% disease incidence and yield of 494.0 kg/ ha.

Table 4.7 Field performances of fungicides on root & stem rot

S. No.	Treatments	*Disease Incidence (%)	*Yield (kg/ha)
T_1	Spraying of Tebuconazole 50%+ Trifloxystrobin 25%@ 0.5 g/l at capsule initiation and second spray after 15 days interval	22.4	617.33
T_2	Spraying of Azoxystrobin @ 1.0 ml/l at capsule initiation and second spray after 15 days interval	37.3	494.00
T_3	Spraying of Pyraclostrobin + Metiram @ 3.0 g/l at capsule initiation and second spray after 15 days interval	34.9	524.33
T_4	Spraying of Cymoxanil + Mancozeb @ 2.0 g/l at capsule initiation and second spray after 15 days interval	32.7	556.66
T_5	Spraying of Captan + Hexaconazole @ 2.0 g/l at capsule initiation and second spray after 15 days interval	30.6	566.66
T_6	Spraying of Carbendazim + Mancozeb @ 2.5 g/l at capsule initiation and second spray after 15 days interval	27.1	582.7
T_7	Untreated check	48.9	428
CD at 5 %		2.051	2.23
SE(m)±		0.658	0.716

*Mean of Three Replication

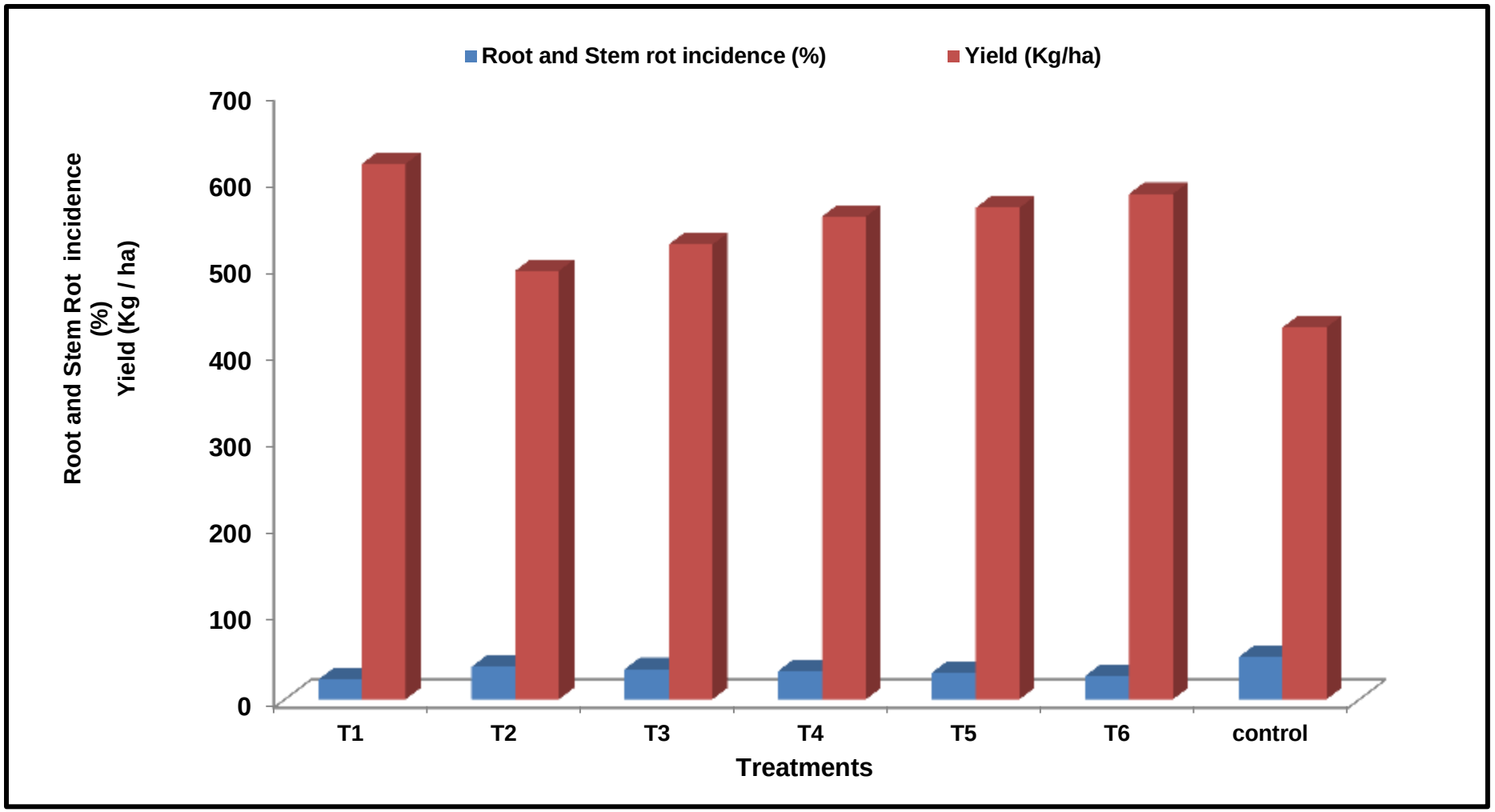


Fig 4.4 Field performance of various fungicides against root & stem rot *M. phaseolina* (Yield Kg/ha)

Chapter -V

DISCUSSION

DISCUSSION

Root rot & stem rot caused by *Macrophomina phaseolina* has become serious disease causing annual yield losses up to 67 per cent. The pathogen is soil & seed borne in nature and survives in the form of sclerotia. Root & stem rot caused by *Macrophomina phaseolina* (Tassi.) Goid. [Sclerotial stage-*Rhizoctonia bataticola* (Taub.) Butler] is one of the most important diseases of sesame in India (Chattopadhyay and Sastry, 1998). The disease is becoming severe in sesame growing areas due to increased level of inoculum in soil. Area and production of sesame is declining day by day in the traditional sesame growing areas due to severe biotic stresses such as Root & stem rot, Phyllody, Bacterial blight, Fusarium wilt, Powdery mildew, Alternaria leaf spot and Cercospora leaf spot. In case of presence of variability of different isolates of *M. phaseolina* the results suggested the prevalence of high degree of variability in the cultural and morphological variables in *M. phaseolina* isolates. Chemical management is feasible for effective management of the root & stem rot. Source of resistance are also important for breeding programme.

5.1 Studies of cultural variability of *Macrophomina phaseolina (Rhizoctonia bataticola)*

The culture of *Macrophomina phaseolina (Rhizoctonia bataticola)* isolated from different samples collected from different states showed that different types of cultural and morphological characters were observed.

5.1.1 Colony growth of *Macrophomina phaseolina (Rhizoctonia bataticola)*

There was no difference in the colony growth rate recorded among the isolates of *M. phaseolina* (*Rhizoctonia bataticola*) at 72 hours after inoculation at 28 ± 1ºC. the maximum colony growth 90 mm were observed in the all isolates. (Table 4.1 & Plate 3)

The results are in agreement with Varma & Pathe (2013) who did not observe any difference among 22 isolates of *Rhizoctonia bataticola* in the colony growth on potato dextrose agar medium.

5.1.2 Colour of colony of *Macrophomina phaseolina (Rhizoctonia bataticola)*

Based upon the studies on colony colour of *M. phaseolina* isolates the isolates produces 2 type of colour which was dark black and blackish grey colour. From the three isolates Mp 1 (Madhya Pradesh) and Mp 2 (Rajasthan) isolates produces blackish grey colour into the colony and Mp 3 (Tamil Nadu) isolate produce dark black colour into the colony. The result satisfy the findings of Shekhar *et al.,* (2006) who gave same results after studying cultural characters of seven isolates of *Macrophomina phaseolina* collected from different geographical regions of India. Mohanapriya *et al*., (2017) also reported that in all the 10 isolates of *M. phaseolina* different type of colony colour was shown on Potato Dextrose Agar (PDA) medium.

5.1.3 Texture of culture of *Macrophomina phaseolina (Rhizoctonia bataticola)*

Based upon the studies on colony texture the isolate of *M. phaseolina* produce 2 types of culture texture *viz,* Appressed and fluffy growth and suppressed and dense growth. Isolate Mp 1 (Madhya Pradesh) and Mp 2 (Rajasthan) produced Appressed and fluffy growth and isolate Mp 3 (Tamil Nadu) give suppressed and dense growth into the culture medium. The result findings satisfy the result found by Satpathi and Gohel (2018a) who get the same result on PDA medium. They found out that isolates *M. phaseolina* of different geographical region has different topography of colony *Viz*., Fluffy, Dense uniform and circular and Flat and dense. Similar results were also shown by Verma and Pathe (2013) who classified growth type of colony into Fluffy and submerged. Out of 22 isolates of *Rhizoctonia bataticola*, sixteen isolates found to show fluffy growth and six isolates shows submerged growth.

5.1.4 Types of margin into isolates of *Macrophomina phaseolina* (*Rhizoctonia bataticola*)

The isolates of *M. phaseolina* produce mainly 2 types of margins. Irregular and scattered and Luxuriant and uniform. Out of 3 *M. phaseolina* isolates Mp 1 (Madhya Pradesh) and Mp 2 (Rajasthan) produces irregular and

scattered margins while isolate Mp 3 (Tamil Nadu) produces luxuriant and uniform margins into the culture medium. The same results were observed by Satpathi and Gohel (2018a) ,they found that *M. phaseolina* show four different types of margin *i.e.* Irregular and scattered, Luxuriant Uniform, Dense Uniform and dense irregular on PDA medium.

5.2 Morphological variability of *Macrophomina phaseolina (Rhizoctonia bataticola)*

5.2.1 Sclerotial Size & Shape

On the basis of microscopic observations the size of sclerotia varied from 57-97 µm x 55-89 µm was observed. The isolates of various geographical regions give different types of variation into the size and shape of sclerotia. In three isolates of *M. phaseolina* the shape of sclerotia varies from circular to irregular. Among which isolate Mp 1 (Madhya Pradesh) and Mp 3 (Tamil Nadu) have irregular shape sclerotia while the Isolate Mp 2 (Rajasthan) produce circular shapes sclerotia.

Same results were also given by Sobti and Sharma (1992) reported sclerotial size of 60µm-165µm x 57µm-114 µm. Prasad *et al*., (2011) they also reported difference among the isolates of *M. phaseolina* in terms of size of sclerotia. Ashraf *et al*., (2015) studied 24 isolates of *M. phaseolina* from four districts of Punjab (Pakistan) and found variability into the sclerotial size of different isolates.

Mandal *et al.,*(1998) conclude that they also find variation into the shapes of sclerotia into the isolates of *M. phaseolina*. Wagan *et al.,*(2018) conclude the same result into which they studies 32 isolates of *M. phaseolina* and found variation into the shape of the sclerotia in terms of various type of shape ovoid, Irregular and round type of sclerotia.

5.2.2 Sclerotia colour

On the basis of microscopic observations all the sclerotia of different isolates show black colour. The findings were in agreement with the findings of Karibasappa *et al*., (2020) who observe no any colour difference into the colour of sclerotia.

5.3 Evaluation of sesame cultivars against root & stem rot *(M. phaseolina)* under natural field conditions.

Eight tested cultivars of sesame perform differently under field condition against root & stem rot disease into which the per cent disease incidence were recorded at pre flowering and capsule formation stage. Sesame cultivars reacted differently at stages of disease development the sesame cultivars variety GT10, RT346, TKG306 was found resistant against root and stem rot disease. While susceptible check VRI1 gave has highest disease incidence at both pre flowering and capsule initiation stages.

The same results were also found by Singh *et al*., (1989) and Satpathi and Gohel (2018b) who screened twenty sesame varieties/germplasm along with susceptible check (GT2) against root & stem rot disease under field condition.

5.3.1 Evaluation of sesame germplasm against root & stem rot (*M. phaseolina*)

Screening of 200 genotypes (DBR) against root & stem rot disease incidence were presented in Table 4.4 & plate 6 revealed that; 0 (zero) lines was found immune (disease scale 0), (40) forty lines found resistant (disease scale 1) , (45) forty five lines found moderately resistant (disease scale 2) , (92) ninety two lines found moderately susceptible (disease scale 3), (14) fourteen lines found susceptible (disease scale 4) and nine (9) lines found highly susceptible (disease scale 5).

The results were favored by the findings of Chaudhary *et al.,* (2014) who evaluated 27 entries against stem and root rot caused by *Macrophomina phaseolina*, only three entries *viz*., IC–205477, IC– 205506 and Krishna were identified as resistant.

Similar observations were also made by John *et al*., (2005) and Gupta *et al*., (2020) who evaluated a large number of genotypes against pathogen *M. phaseolina* under natural conditions.

5.4. Evaluation of fungicides against *Macrophomina phaseolina* (Tassi.) Goid. for management of root & stem rot

5.4.1 *In vitro* evaluation of fungicides against *Macrophomina phaseolina* (Tassi.) Goid. for management of root & stem rot

Table 4.6, Fig. 4.2, 4.3 & Plate 6 indicated that all fungicides were superior over control at 500 ppm, 1000 ppm and 1500 ppm and complete inhibition of mycelial growth was recorded by Tebuconazole 50% + Trifloxystrobin 25 % at all the three concentrations taken (100% inhibition) which is followed by Carbendazim + Mencozeb which gave (94.43%) of average inhibition at all three concentrations taken. Minimum average inhibition was given by Azoxystrobin (41.66%) at all concentrations taken.

The current studies satisfy the findings of Bashir *et al*., (2017) who evaluated six fungicides against *M. phaseolina* causing root & stem rot of sesame and the mean colony growth of all treatments expressed that Nativo (Tebuconazole 50% + Trifloxystrobin 25 %) exhibited minimum colony growth of pathogen. The findings of the current research are in line with the studies of Kumar *et al.*, (2016) who assessed Trifloxystrobin 25% + Tebuconazole 50% (Nativo) @ 5, 10, 15, and 25 ppm against *Macrophomina phaseolina* and observed that Nativo expressed significant reduction in colony growth as compared to other fungicides.

5.4.2 *In vivo* evaluation of fungicides for management of root & stem rot

In the field trial conducted for the management of root & stem rot disease of sesame, all the treatments were found to be superior over control. Table 4.7 & Fig. 4.4 indicated that among the treatments, T1 (foliar spray of Tebuconazole 50% + Trifloxystrobin 25% @ 0.5 g/l at capsule initiation and second spray after 15 days interval) recorded the minimum disease incidence 22.4% and the maximum yield of 617.33 kg/ha, followed by T6 (Spraying of Carbendazim + Mancozeb @ 2.5 g/l at capsule initiation and second spray after 15 days interval) having disease incidence of 27.1% and yield of 582.7 kg/ha. T2 (Spraying of Azoxystrobin @ 1.0 ml/l at capsule initiation and

second spray after 15 days interval) recorded the maximum disease incidence 37.3% and the yield of 494 kg/ha.

The current studies were also in favour with the findings of Bashir *et al*.,2017 who found that in field conditions, Nativo (Tebuconazole 50% + Trifloxystrobin 25%) exhibited minimum mean disease incidence (12.55%) whereas the interaction between treatments and days showed minimum of 14.95%, 12.82% and 9.90% disease incidence by Nativo as compared to all other treatments including control (66.86%, 77.57% and 87.22%) after day tenth, twenty and thirty. It was concluded that Nativo (Tebuconazole 50% + Trifloxystrobin 25%) inhibiting the growth of *M. phaseolina* causal organism of root and stem rot of sesame into field conditions.

Chapter-VI

SUMMERY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

SUMMERY, CONCLUSION AND SUGGESTIONS

FOR FURTHER WORK

6.1 Summary

The cultural and morphological characteristics of the three isolates were studied, the cultural characters of the isolates colony growth, colony texture, colony colour and colony margin type studied. Into which all the isolates show variability into the characters of the isolates. In case of colony colour isolate Mp 1 (Jabalpur, Madhya Pradesh) and Isolate Mp 2 (Mandor, Rajasthan) show grey coloured colony was observed but isolate Mp 3 (Vridhachalam, Tamil Nadu) show dark black colour colony. In case of colony texture two type of colony texture was seen, Appressed and fluffy growth and suppressed and dense growth. In case of Mp 1 (Jabalpur) and Mp 2 (Mandor) isolate , appressed and fluffy growth types of growth was observed while in case of isolate Mp 3 (Vridhachalam) suppressed and dense growth was seen. All the isolates of *M. phaseolina* do not show any kind of significant difference in colony growth. Maximum radial growth (90.00 mm) was observed in all three isolates of *M. phaseolina*. On the other hand in case of type of margin of isolates 2 different types of margins were observed that is irregular and scattered and luxuriant and uniform. Into which the isolates Mp 1 (Jabalpur) and Mp 2 (Mandor) show irregular and scattered margins while Mp 3 (Vridhachalam) show luxuriant and uniform margins.

In case of morphological variability all the isolates sclerotia colour was found black. In case of size of sclerotia Mp 1 (Jabalpur, Madhya Pradesh) isolate sclerotia measures 97 x 89 µm (length x Width); Isolate Mp 2 (Mandor, Rajasthan) sclerotia measures 57 x 55 µm; Isolate Mp 3 (Vridhachalam, Tamil Nadu) sclerotia measures 71 x 66 µm. The shape of the sclerotia were differ and isolate Mp 1 (Jabalpur, Madhya Pradesh) and Mp 3 (Vridhachalam, Tamil Nadu) show irregular type of sclerotia and isolate Mp 2 (Mandor, Rajasthan) show round types of sclerotia also there were no difference among the colour of the sclerotia all three isolates show produces black colour of sclerotia.

Eight sesame cultivars was evaluated under natural field condition for their performance against root & stem rot disease caused by *M. phaseolina* at pre flowering stage and capsule formation stage. Susceptible check VRI1 was shown the maximum disease incidence at pre flowering stage and capsule formation stage while minimum disease incidence were seen into variety GT10, TKG306 and RT346.

200 DBR germplasm lines were also evaluated against the root & stem rot disease under field conditions. Among the germplasm, zero lines found immune, forty lines found resistant (1-10%), forty five lines found moderately resistant (11-25%), Ninety Two lines found moderately susceptible (26-50%), Fourteen lines found susceptible (51-70%), Nine lines found highly susceptible (more than 70%). The maximum disease incidence was found into the susceptible check (VRI1 80%).

Six systemic fungicides were evaluated at *In vitro* condition by using poison food techniques at three different concentration 500 ppm, 1000 ppm and 1500 ppm. Into which T1 (Tebuconazole 50% + Trifloxystrobin 25%), gave 100% inhibition against test fungus (*M. phaseolina*) at all concentration (500, 1000 and 1500ppm) followed by T6 (Carbendazim + Mencozeb), give 94.43% of average inhibition, followed by T5 (Captan + Hexaconazole), give 92.59% of average inhibition, followed by T4 (Cymoxanil + mencozeb) gave 83.15% average inhibition, followed by T5 (Pyraclostrobin + Metiram) gave 57.28% of average inhibition. Least mycelial inhibition was given by T2 (Azoxystrobin), give only 41.66%.

In the *In vivo* fungicidal trial conducted for the management of root & stem rot disease of sesame. Among the treatments, T1 (Spraying of Tebuconazole 50%+ Trifloxystrobin 25% @ 0.5 g/l) recorded the minimum disease incidence 22.4% and the maximum yield of 617.33 kg/ha. followed by T6 (Spraying of Carbendazim + Mancozeb @ 2.5 g/l) having disease incidence of 27.1% and yield of 582.7 kg/ha. while maximum disease incidence per cent (48.9%) and minimum yield (428 kg/ha.) was found in control.

6.2 Conclusions

- The isolates of *Macrophomina phaseolina* (Tassi.) Goid. which is collected from different geographical region of India. The cultural and morphological variability was seen among the isolates of *M. phaseolina* which show the considerable variation into the isolates of *M. phaseolina.*
- Out of eight varieties evaluated at field condition against root & stem rot variety GT10, TKG306 and RT346 perform good at pre flowering stage and capsule formation stage while VRI1 the susceptible check having highest disease incidence.
- Out of 200 germplasm lines (DBR) were also evaluated against the root & stem rot disease under field conditions into which zero lines found immune, forty lines found resistant, forty five lines found moderately resistant, ninety two lines found moderately susceptible, fourteen lines found susceptible, nine lines found highly susceptible. The maximum disease incidence was found into the susceptible check VRI1.
- Out of Six systemic fungicides were evaluated at *In vitro* condition by using poisoned food techniques at three different concentration 500 ppm, 1000 ppm and 1500 ppm. Tebuconazole 50% + Trifloxystrobin 25% gave 100% inhibition, followed by Carbendazim + Mencozeb, give 94.43% inhibition. Least mycelial inhibition was given by Azoxystrobin, give only 41.66% of average mycelial inhibition at all three concentrations taken.
- In the *In vivo* fungicidal trial conducted for the management of root & stem rot disease of sesame, Among the treatments, T1 (Spraying of Tebuconazole 50%+ Trifloxystrobin 25%@ 0.5 g/l) recorded the minimum disease incidence 22.4% and the maximum yield of 617.33 kg/ha., (This technology should be used by farmers for the management of root & stem rot disease). followed by T6 (Spraying of Carbendazim + Mancozeb@ 2.5 g/l) having disease incidence of 27.1% and yield of 582.7 kg/ha. The maximum disease incidence of 48.9% and the minimum yield of 428 kg/ha was observed in the control.

6.3 Suggestions for Further Work

- Survey to record prevalence and severity of *M. phaseolina* into different parts of Madhya Pradesh state.
- Influence of different weather parameters for the distribution and development of the root & stem rot disease of sesame.
- Effect of weather parameters on initiation development and progression of disease.
- Collection and study of more *M. phaseolina* isolates from different geographical region of India.
- Under field condition those germplasm should screened against root & stem rot found resistant should be screened next year again against root & stem rot for the use of breeding programme purpose.
- Field Screening of more number of fungicides, botanicals and bio agents and their integration need to be studied.
- Screening under sick plot to confirm their screening.

Chapter-VII

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APPENDICES

APPENDICES

Evaluation of sesame varieties against *M. phaseolina* under natural conditions.

Per cent disease incidence (At Pre- Flowering Stage)

Source of variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	2	2.083			
Treatment	7	140.667	20.095	108.903	0.00000
Error	14	2.583	0.185		
Total	23	145.333			

Evaluation of sesame varieties against *M. phaseolina* under natural conditions.

Per cent disease incidence (At Capsule Formation Stage)

Source of variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	2	1.333			
Treatment	7	1,194.625	170.661	298.656	0.00000
Error	14	8.000	0.571		
Total	23	1,203.958			

Effect of Fungicides against *M. phaseolina* under *In vitro* conditions

Mycelial growth (At 500 ppm)

Source of variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	6	16,972.310	2,828.718	6,092.624	0.00000
Error	14	6.500	0.464		
Total	20	16,978.810			

Effect of Fungicides against *M. phaseolina* under *In vitro* conditions

Mycelial growth (At 1000 ppm)

Source of variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	6	21,537.310	3,589.552	5,912.203	0.00000
Error	14	8.500	0.607		
Total	20	21,545.810			

Effect of Fungicides against *M. phaseolina* under *In vitro* conditions

Mycelial growth (At 1500 ppm)

Source of variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Treatment	6	20,741.643	3,456.940	8,066.194	0.00000
Error	14	6.000	0.429		
Total	20	20,747.643			

Effect of Fungicides against *M. phaseolina* under *In vivo* conditions

(Per cent Stem and Root rot incidence)

Source of variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	2	0.349			
Treatment	6	1,316.21	219.368	168.837	0.00000
Error	12	15.592	1.299		
Total	20	1,332.15			

Effect of Fungicides against *M. phaseolina* under *In vivo* conditions

(Yield Kg/ha)

Source of variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	2	4.3			
Treatment	6	71,049.27	11,841.55	7,704.14	0.00000
Error	12	18.444	1.537		
Total	20	71,072.02			

CURRICULUM VITAE

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For the partial fulfillment of the master's degree programme, she was allotted a research problem on "**Studies on root & stem rot (*Macrophomina phaseolina)* (Tassi) Goid. of sesame and its management through fungicides**" which was successfully conducted by her and being submitted in the form of the thesis.