STUDIES ON *Macrophomina phaseolina* (Tassi) Goid

By

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B.Sc. (Agri.)

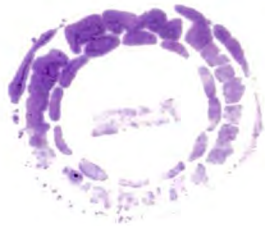
DISSERTATION

Submitted to the

Marathwada Krishi Vidyapeeth

in partial fulfillment of the requirement

for the degree of



MASTER OF SCIENCE

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IN

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2011

Affectionately
Dedicated
To My
Beloved Parents
and Research
Guide

CANDIDATE'S DECLARATION

I hereby declare that this dissertation or part

Thereof,

has not been previously submitted by me for

a degree of any other institution

or University.

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Date : 27/ 5 /2011

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CERTIFICATE-I

This is to certify that the dissertation entitled "**STUDIES ON *Macrophomina phaseolina* (Tassi) Goid**" submitted by **Mr. PATIL VIJAYSING DIWANSING** to the Marathwada Krishi Vidyapeeth, Parbhani in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE (Agriculture)** in subject of **PLANT PATHOLOGY** is record of original and bonafied research work carried out by him under my guidance and supervision. It is of sufficiently high standard to warrant its presentation for the award of the said degree.

I also certify that dissertation or part of has not been previously submitted by him for a degree of any university.

Place : **PARBHANI**
Date : 15 / 08 /2011

(K.D. Navgire)
Research Guide

CERTIFICATE-II

This is to certify that the dissertation entitled "**STUDIES ON *Macrophomina phaseolina* (Tassi) Goid**" submitted by **Mr. PATIL VIJAYSING DIWANSING** to the Marathwada Krishi Vidyapeeth, Parbhani in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **PLANT PATHOLOGY** has been approved by the student's advisory committee after oral examination in collaboration with the external examiner.

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ABBREVIATIONS

%	=	Per cent
@	=	At the rate
C.D	=	Critical difference
Cv.	=	Cultivar
DAI	=	Days after inoculation
Dia	=	Diameter
e.g.	=	Exampli Gratia
et al.	=	and others
etc.	=	Etceteras
Fig.	=	Figure(s)
ha	=	Hectare(s)
hrs	=	Hours
i.e.	=	That is
kg	=	Kilogram(s)
lbs	=	pounds
m	=	meter(s)
Max.	=	Maximum
Min.	=	Minimum
MKV	=	Marathwada krishi Vidyapeeth
Mm	=	millimeter
No.	=	Number(s)
^{0}C	=	degree Celsius
PDA	=	Potato dextrose agar
PDI	=	Percent disease incidence
Ppm	=	Parts per million
Rpm	=	revolution per minute
S.E	=	Standard error
Spp	=	Species
T	=	Treatment
viz.,	=	videlicet (namely)
w/v	=	weight per volume
WP	=	wettable powder

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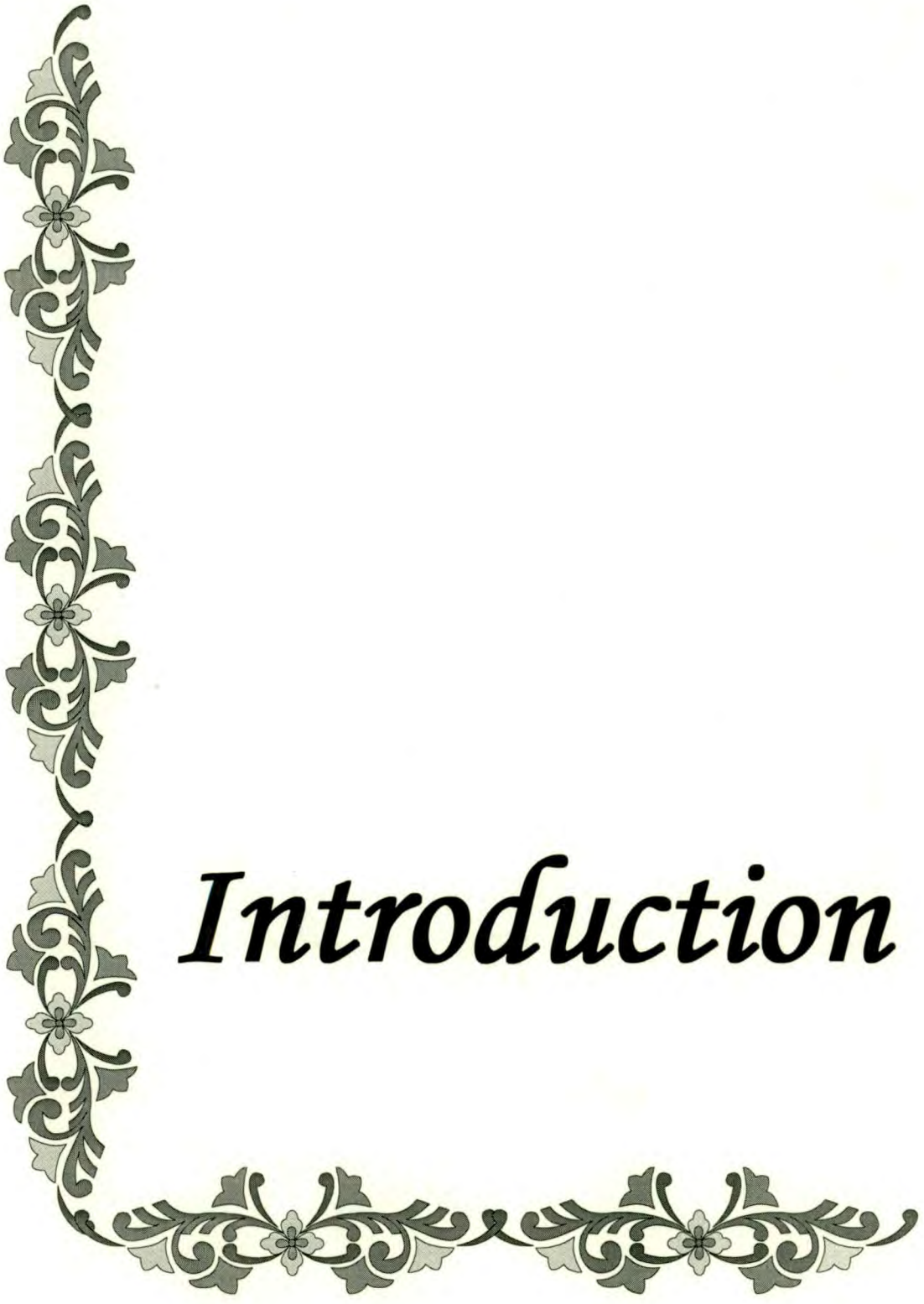
PLACE : PARBHANI
DATE : 15 / 08 / 2011

Patil V.D.



Introduction

CHAPTER-I

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. is one of the most damaging seed and soilborne fungus. The fungus can infect the root and lower stem of over 500 plant species and has a wide geographic distribution. Major hosts of *M. phaseolina* include, *Gossypium* spp., *Vigna mungo* L., *Vigna radiata* L.Wilczek. *Glycine max* L.Merril, *Carthamus tinctorius* L., *Sorghum bicolor* L.Monech Hepper, The symptoms due to *M. phaseolina* varies with the crop and environmental conditions and charcoal rot is an important disease symptoms during hot, dry weather or when unfavourable environmental conditions stress the plants.

In soybean charcoal rot is a disease of root and stem. Charcoal rot symptoms vary depending on the time of the year when the plant is infected. Usually charcoal rot is developed later in the season but it can cause a seedling disease since the roots of the plant can be infected anytime during the season. Infected seedlings shows a reddish brown discoloration at the soil line extending up to the stem that may turn dark brown to black. Foliage of infected seedlings can appear off-color or begin to dry out and turn brown. A twin-stemmed plant may develop if the fungus kills the growing point. Under cool and wet conditions young plants that are infected may survive but carry a latent infection that will express symptoms later in the season with hot, dry weather. In older soybean plants symptoms of charcoal rot include early maturation,

non-abscission of the leaves, chlorosis and failure to complete pod filling. Microsclerotia can be visible as black specks in the woody parts of the stems and roots. The microsclerotia are released into the soil as infected tissue decays (Bowers and Russin, 1999).

In black gram web blight caused by *M. phaseolina* is one of the most important foliar disease causing yield loss upto 30 per cent (Sati, 1998) *M. phaseolina* survives in soil as dormant mycelium, sclerotia and as saprophyte on organic matter which served as primary source of inoculums (Tiwari, 1993).

In cotton the symptoms observed on leaves are interveinal chlorosis followed by yellowing of leaves which turned to brown irregular spots. Extensive leaf shedding and brown lesions on stem are also observed. Number of bolls in infected plants are less than the healthy plants. It is a soil borne fungus thus control of diseases is difficult. (Agrios, 2004).

In mung bean several strains of *M. phaseolina* were reported to infect various plant parts producing diseases such as leaf blight, stalk rot, root rot, collar rot, blossom and fruit rot (Saksena, 1979). The pathogen mainly causes leaf and stem blight during the growth period. The disease appears mostly before flowering and at maturity stage. Black dot like pycnidia of *M. phaseolina* are formed on leaves and branches (Mahendra Pal, 1998). The affected plants show brownish spots on the younger leaves, these spots coalesces with each other and finally complete blighting of the leaves take place leading complete drying of the plant.

In safflower charcoal rot caused by *M.phaseolina* is characterized by gradual yellowing and drying of leaves, when the plants were mostly in their flowering stage and such plants are easily recognized from the distance. The yellowing start from the lower portion of the plant and gradually extend upwards, while the terminal leaves and flower buds remain green for some time. This was followed by wilting and finally the entire plant dried up. Affected plants could easily be pulled out of the soil. The collar region of the plant show grayish black discoloration which was often shrivelled and the plant often broke at that point. The top and lateral roots turn black and their barks easily sloughed off exposing the inner cortical tissues. Numerous black micro-sclerotial bodies could be seen on the affected region especially the cortical and pith tissues. (Singh and Bhowmik, 1979).

In sorghum charcoal rot caused by *M. phaseolina* is the most common and probably also the most important root and stalk rot disease of sorghum,which produced variety of symptoms associated with charcoal rot *viz*., root rot, stalk rots, lodging of plants, premature drying of stalks and poorly developed panicles with small inferior quality grains (Tarr 1962).

M. phaseolina (Tassi) Goid. [(syns. *M. phaseolina* (Maubl.) Ashby, *Rhizoctonia bataticola* (Taub.) Briton-Jones, *Sclerotium bataticola* Taub. and *Botryodiplodia phaseoli* (Maubl.) Thrium.)]. is a pathogen belonging to the phylum Deuteromycetes and class Coelomycetes. It is highly variable, with isolates differing in microsclerotial size and presence or absence of pycnidia. They are dark

to grayish to black with age. The pycnidia bear simple, rod-shaped conidiophores. Conidia are single celled, hyaline, and elliptic or oval.

Microsclerotia of *M. phaseolina* are jet black in color and smooth and round to oblong or irregular. Across isolates, microsclerotia appears vary on size and shape. Microsclerotia are formed from aggregates of hyphal cells joined by a melanin material.

M. phaseolina survives as microsclerotia in the soil and on infected plant debris. The microsclerotia serve as the primary source of inoculum and have been found to persist within the soil up to three years (Dhingra and Sinclair, 1977). The microsclerotia are black, spherical to oblong structures that are produced in the host tissue and released in to the soil as the infected plant decays. Seeds may also carry the fungus in the seed coat. Infested seeds do not germinate or produce seedlings.

Cultural management practices needs to must be implemented to minimize charcoal rot damage since there are no fungicides available for effective disease control. Crop rotation with non host is effective in some crop production systems. Rotation for three years may effectively reduce microsclerotia numbers and is useful for managing charcoal rot (White, 1999).

Taking into consideration the severity of *M. phaseolina* the isolation, variability and cross infectivity studies were carried out from different host *viz.*,cotton, blackgram, greengram, soyabean, safflower and sorghum. Studies on management through fungicides and bioagents were also conducted against *M. phaseolina.*

In present investigation *M. phaseolina* isolates from six different hosts were studied for cultural, morphological and physiological variability with following objectives.

Objectives:

1) To study variations among the different isolates of *Macrophomina phaseolina.*

A) Isolation , identification of the pathogen and pathogenicity test.

B) Morphological

i) Radial growth and sclerotial formation of isolates.

C) Nutritional study

i) Effect of different culture media.

ii) Effect of different carbon and nitrogen sources.

D) Physiological study

i) Effect of temperature and pH .

E) Management of the disease with fungicides and bioagent.

Review of Literature

CHAPTER-II

REVIEW OF LITERATURE

The ubiquitous fungus *Macrophomina phaseolina* (Tassi) Goid. with pycnidial stage of *Rhizoctoni bataticola* (Taub) Butl. have host range of over 500 plant species. *M. phaseolina* was a highly variable fungal pathogen responsible for economically significant damage to a wide variety of crops worldwide (Suriachandraselvan and Seetharaman, 2003). As a result, variations among its isolates in respect of morphological, cultural and pathological characters were frequently reported (Singh and Nene 1990; Upamanyu and Gupta, 2009). Several cultured media showed differential effects on the growth and cultural characteristics of different isolates of *M. phaseolina* infecting various host plants (Singh and Kaiser, 1994) and also on the same host plant (Dhingra and Sinclair, 1973, Das *et al.*2008). Literature on *M. phaseolina* in relation to the objectives under study is being revieved in the following paragraphs.

2.1 Host and Symptomatalogy

Uppal *et al.* (1936) reported that charcoal rot of sorghum was caused due to *M. phaseolina* in different sorghum growing states of India .

Singh *et al.* (1973) reported that *M. phaseolina* causing root rot disease in black gram was be most prominent among the fungi.

Meyer and Khare (1974) reported that under hot, dry environmental conditions, soybean (*Glycine max* L. Merril.) yield losses were significantly high due to root rot disease.

Sakuja (1974) recorded that infection of *R. bataticola* on mung bean cause leaf blight disease.

Saksena (1979) reported that *R. bataticola (M. phaseolina)* as the most wide spread destructive plant pathogen. *M.* phaseolina had received great attention all over the world, because earlier the pathogen was causing only root rot, stalk rot etc. Now it was known to attack different aerial plant parts to cause stalk rot, leaf spot and blights.

Anahosur and Patil (1983) found that charcoal rot of sorghum caused by *M. phaseolina* had become a serious menance specially after introduction of high yielding sorghum genotypes and had identified as destructive disease in post rainy season in Karnataka, Maharashtra, Gujarat and Andhra Pradesh.

Zote *et al.* (1983) reported that leaf blight caused by *M. phaseolina* was most destructive and widely distributed disease of mungbean in Maharashtra. The disease appeared at all stages of crop growth, causing root rot, collar rot, leaf blight or dieback.

Mayee and Datar (1986) during *kharif* 2004,conducted survey of cotton fields of four talukas in Aurangabad district and reported that leaf blight of cotton was caused due to due to *R. solani* Kuhn.

Dubey (1988) reported that aerial blight of soybean caused by R. *solani* Kuhn. was most menancing disease which caused heavy losses in yield in warm and humid parts of the country.

Yang *et al.* (1990a) reported *R. solani* as causal agent of foliar blight of soybean and its occurrence was favoured by prolonged period of high humidity and warm weather.

Mahendra Pal (1998) noted that *M. phaseolina* caused leaf and stem blight during the growth period. The disease appeared mostly before flower and at maturity stage. Black dot like pycnidia of *M. phaseolina* were found on leaves and branches.

Sharma *et al.* (2004) reported that *R. solani* Kuhn was polyphagous fungus and different types of symptoms were caused by forty five isolates of root rot and foliage blight collected from several locations of North India.

Govindappa *et al.* (2005) reported stem splitting symptoms in safflower induced by *M. phaseolina.* Stem spliting symptom was observed in 30 days old safflower plants as minute crack 2-3 cm above soil surface.

2.2 Size of sclerotia of *M. phaseolina* isolates

Jain *et al.* (1972) observed variations in sclerotial diameter of *R. bataticola* isolates obtained from soil and various part of infected black gram plants. The sclerotia from soil measured 79 x 63µm, from stem 73 x 63 µm, from seed 89 x 72 µm, from root 95 x 84 µm, from leaf 96 x 86 µm and from pod 93x 72 µm.

Ghosh and Sen (1973) isolated *R. bataticola* isolated from seven hosts on PDA. The isolates produced sclerotia of variable shapes and sizes; *Justica gendarussa* (65.6-114.0 µm), *Glycine max* (45.2-81.6 µm), *Cicer arientum* (98.1-119.2 µm), *Corchorus capsularis* (40.2-85.0 µm), *Kochia scoparia* (52.6-80.1 µm), *Arachis hypogea* (110-114 µm) and *Cajanus cajan (*89.1-116.3 µm).

Byadgi and Hegde (1985) recorded variation in sclerotial diameter of *R. bataticola* isolates from various hosts. Bean isolates

measured 74.05 µm, bengal gram 80.25 µm, cowpea 60.89 µm, soybean 72.15 µm, sorghum 78.41 µm, glyricidia 101.51 µm. It was noted that sclerotia at maturity were globose to irregular and brown to black in color.

Olaya and Abawi (1996) reported that *M. phaseolina* can grow and produce large amount of microsclerotia under relatively low water potential allowing disease to be recognized as favouring drought.

Shekhar *et al.* (2006) obtained seven isolates of *M. phaseolina* causing charcoal rot of maize from different agro-ecological zones of India. Hyderabad isolate produced highest number of sclerotia of bigger size (95.7 µm), whereas Coimbatore isolate produced minimum number of sclerotia with smaller size (66.9 µm).

2.3 Pathogenicity test

Bekesi *et al.* (1970) reported that *M. phaseolina* from Hungary on sunflower plant causes sudden wilting of plant after pollination for the first time.

Singh and Bhowmik (1979) reported numerous black micro-sclerotial bodies on the affected region specially the cortical and pith tissue of safflower plant. Repeatedly isolation from affected plants, yielded a fungus which culture was identified as *R. bataticola.* Infected plants did not showed pycnidial bodies and were not formed in culture tubes.

Hooda and Grower (1988) obtained fourteen isolates of *M. phaseolina* from different plant species and observed isolates producing abundant sclerotia in culture were more pathogenic than isolates producing fever sclerotia.

Muthusamy and Marriappan (1991) studied the diseases of economic importance in soybean were root rot (*M.phaseolina*), collar rot (*Sclerotium rolfsii)*, anthracnose (*Colletotricum truncatum*) and bacterial pustules (*Pseudomonas* and *Xanthomonas spp.*). Among these, losses upto 77 per cent were reported due to *M.phaseolina.*

Prameela Devi and Singh (1998) obtained *M. phaseolina* isolates from black gram and green gram from 11 different localities and categorized as highly virulent (MP-2, MP-3), moderately virulent (MP-1, MP-4 and MP-6) and weakly virulent (MP-5) on the basis of disease intensity of black gram and green gram.

Kale (1999) isolated *R. bataticola* from infected leaf sample by using tissue isolation method and pathogenicity was proved on three weeks old mungbean plant.

Mehetre (2000) studied pathogenicity of the fungus *M. phaseolina* in sick soil in earthen pots. Seedlings of safflower variety Tara exhibited typical symptoms within 21 to 30 days of the crop growth.

Patil *et al.*(2005) recorded that initial inoculums in the soil determines the severity of soil-borne pathogen. Incorporation of *Sclerotium rolfsii*, first and *R. bataticola* after 10 days resulted in 68 per cent mortality with 6.19 per cent rate of multiplication.

Satraj *et al.* (2005) the different inoculum level of wilt fungus *Furarum oxysporum f.sp., ciceri* and root rot fungus *M. phaseolina* and *R. solani* caused significant reduction in various parameter of chickpea such as plant weight, pollen fertility, number of nodules, water absorption capability, nitrate reductase activity and chlorophyll content.

Patil *et al.* (2005) reported that the higher rate of multiplication was observed in *R. bataticola* at 75 g / kg soil i.e. 5.34 times and in 100g/kg soil. It was 5.28 times indicates that with the higher inoculums level rate of multiplication was reduced.

Tiwari and Khare (2008) reported that hyphal penetration of host tissue from epidermal wall to pith occurred within 48 hour of inoculation of *Rhizoctonia* on mung bean var., Pusa Baisakhi. Penetration of epidermal wall was observed through infection peg from wart like spongy microsclerotia type cushion. Direct penetration through hyphae inside the epidermal wall was also observed. Intra cellular penetration was more than intercellular penetration through the epidermal cellwall up to the vascular bundle and pith. Sclerotial initiation was observed in dead host tissue. Cellular injury was observed as browning of host cell.

2.4 Host range and cross infectivity test

Kannaiyan and Prasad (1978a) reported that rice isolates (sheath blight) does not seriously attack *Sorghum vulgare, S. helpensis* and *S. sudanensis* but maize, fingermillet, pearl millet and *Echinochloa colona* var. *frumentosa* were highly succeptible to *M. phaseolina.*

Nayak *et al.* (1979) reported *Cynodon dactylon, Setaria glauca, Paspalum flavidum, P. scrobiculatum, Echinocola colona , E.crusgali, Eriochloa procera, Ergrostis pilosa, Panicum repens, Leptochloa chinensis, Paspalum distinctum, Cyprus rotundus, C.radiatus, C.iria, Chloris spp.* and *Digitaria spp.* as collateral hosts of *R. solani.*

Mishra and Sinha (1982) found that *R. bataticola* causing wilt in linseed was cross pathogenic to six collateral hosts *viz., Sorghum*

halpense, Cynodon dactylon, Vigna sinensis, Cajanus cajan, Cyprus rotundus, Panicum atrosanguincum.

Kaswate (2002) studied the host range of *R. bataticola* isolates obtained from eight different host crops and recorded that pigeon pea and chick pea isolates were pathogenic to green gram, cotton, safflower and nonpathogenic to cluster bean and okra.

Salunkhe (2007) showed that *R. bataticola* cotton isolate was pathogenic to black gram, cowpea and sunflower while green gram, soybean, sorghum, sesame and cluster bean were non infected.

2.5 Compatibility among isolates of *M. phaseolina*

Raut and Ingle (1990) studied the compatibility of thirteen different isolates of *R. bataticola* and found that seven combinations of *R. bataticola* isolates *viz.,* okra-chili, okra-sesame, okra-sorghum, chili-sorghum, cotton-green gram, cotton-sorghum and green gram-cluster bean were non compatible with each other on PDA.

Manici *et al.* (1995) reported that the pathogenicity of 24 isolates of *M. phaseolina* from sunflower was tested on maize, sorghum, soybean, safflower, sunflower, sugar beet, kenaf and melon seedlings. The isolates were very pathogenic on soybean, moderately pathogenic on sunflower, safflower, sorghum and melon, midely pathogenic on sugar beet and kenaf and non pathogenic on maize using *in vitro* seedling inoculation assay.

Eight combinations *viz.,* okra-cluster bean, chilli-soybean, cowpea- green gram, cowpea- cluster bean, cotton- cluster bean, cotton-sesame, cotton-soybean and green gram-sesame were nearly compatible

and colonies of the remaining combinations showed complete compatible reactions.

2.6 Nutritional study

2.6.1 Effect of different culture media on growth and sclerotial formation

Sahi *et al.* (1992) reported maximum growth of *M. phaseolina* on potato dextrose agar medium.

Singh and Kaiser (1994) reported abundant growth of *M. phaseolina* on potato dextrose agar medium.

Surianchandraselvan and Seetharaman (2003) tested five culture media for mycelial growth and sclerotial production of 25 geographical isolates of *M. phaseolina* causing charcoal rot of sunflower. Among the media tested, PDA supported the best growth and sclerotial production of isolates of the pathogen requiring at lowest period for the latter.

Sharma *et al.* (2004) reported that *M. phaseolina* from pearl millet, sesame, horse gram and moth bean crops showed the marked variation in cultural characters. The pearl millet, sesame and moth bean isolates exhibited maximum growth on PDA medium, while horse gram isolate showed maximum growth on malt extract agar medium.

Bainade *et al.* (2005) recorded color, colonization, structure and production of sclerotia by *M. phaseolina* on eleven different solid media. The highest growth of *M. phaseolina* was observed on mung leaf extract medium followed by soybean seed medium, potato dextrose agar medium, peptone dextrose, rose bengal medium and Richard's media.

Upamanyu (2009) recorded that *R. solani* on eighteen isolates of french bean supported maximum growth on potato dextrose broth followed by Richard's medium while Czapek's dox medium supported the less growth.

Salunkhe (2009) reported that during nutritional study growth of fungus on all six media was found significantly different from each other. PDA was found best medium for growth and sclerotial production of *R. bataticola* isolates over other media. All isolates showed fast mycelial growth on PDA followed by Czapek's dox, Richard's ,oat meal and peptone agar while mycelial growth on malt extract medium was poor, although on this medium no sclerotial production was observed in soybean isolates on peptone agar medium.

2.6.2 Effect of carbon and nitrogen sources

Shanmungam and Govindswamy (1973) reported that among the different carbon sources significantly highest growth of *M. phaseolina* was obtained in dextrose followed by maltose and fructose. As well as among different nitrogen sources tested asparagine was significantly superior to other nitrogen sources for maximum growth of *M.phaseolina.*

Diaz Franco (1984) reported that the best carbohydrate source for growth of *M. phaseolina* were dextrose, fructose, sucrose and galactose. Sclerotia were formed most rapidly on sucrose followed by dextrose.

Khune *et al.* (1993) studied the effect of nitrogen sources *viz.*, urea, potassium nitrate, ammonium sulphate, sodium nitrate and ammonium nitrate on the growth of *M.phaseolina.* Ammonium nitrate

gave the highest mean colony diameter and highest sclerotial formation. Potassium nitrate at the higher concentration supported luxuriant growth as well as high sclerotial formation of *M. phaseolina.*

Lakhpale *et al.* (1995) studied the effect of different carbon and nitrogen sources on growth and sclerotial formation of paddy isolates of *R. solani.* They reported that, out of six carbon sources (starch, manitol, galactose, lactose, sorbitol and dextrose), dextrose was the best source of carbon which promoted good growth and abundant sclerotial formation than lactose and galactose. It was also reportednoted that among nitrogen sources tested, asparagines was best for growth and sclerotial formation of *R. solani* isolates followed by sodium nitrate, potassium nitrate and ammonium nitrate.

Upamanyu (2009) reported that the glucose among carbon sources and ammonium nitrate and ammonium tartrate as well as potassium nitrate among nitrogen sources yielded maximum growth of *R. solani.*

2.7 Physiological study

2.7.1 Effect of temperature and pH

Byadgi and Hegde (1988) observed that sporophytic activity of *R. bataticola* in soil was maximum at 30^0C at which fungus colonized 76.60 per cent of the segment buried. Colonization was also good at 35^0C, but decreased considerably above 35^0C and below 30^0C. Minimum colonization of 6.67 per cent was noticed at 15^0C.

Kousik *et al.* (1995) reported that isolates representing 11 anastomosis groups (AGS) of *M.phaseolina* among which AG-1 IB and AG-5 were more virulent on soyabean leaves at 20, 25 and 30^0C than

isolates of AG-1 IC and AG-4. Maximum numbers of infection cushions were formed on soybean leaves although some caused minimal severity. Isolates of AG-1 IA formed significantly more infection cushion and caused greater disease severity than AG-1 IB and other isolates at 35^0C. Maximum seedling infection based on per cent area of hypocotyls region covered by lesions occurred at 25^0C for AG-1 (IA, IB, and IC), AG-4 and AG-5 caused greater seedling infection at 20^0C than at 25^0C and 30^0C. Other AGS cause minimal damage to seedling.

Bainade *et al.* (2005) studied the effect of temperature and pH on *M.phaseolina* isolate of mung bean blight. They reported that optimum temperature 35^0C favoured the maximum growth and sclerotial production. Among the pH levels, neutral i.e. pH 7 recorded 89.6mm growth followed by pH 8 (61.33 mm).

Jha and Sharma (2005) reported 30-35^0C as optimum temperature for growth and sclerotial production of *R. bataticola* with decreased mycelial growth at 40^0C or above and also observed that pH 7 as optimum for growth and sclerotial formation of pathogen.

Salunkhe (2009) reported that all *R. bataticola* isolates were able to grow at temperature range from 20 to 40^0C. Maximum growth was observed at 35^0C and minimum growth was observed at 20^0C. Mycelial growth rate increased as temperature lended from 20 to 35^0C. The maximum sclerotial production in most of isolate was recorded at 35^0C and almost no sclerotial formation at 20^0C and 40^0C as well as the effect of pH on growth of *R. bataticola* isolate was studied at pH range 5 to 9. He also reported that there was no significant difference in radial

growth in isolates at pH range 5 to 9 but at pH 7.0 growth of all isolate was comparatively superior.

2.8 Efficacy of fungicides and bioagents against *M. phaseolina*

Raut and Bhombe (1983) found that among systemic fungicides, Benlate T and among non systemic fungicides Diathane Z-78 proved most effective and eliminated the seed infection of 92 and 79 per cent respectively by *M. phaseolina* in sunflower.

Pall *et al.* (1990) reported that the best fungicide being MBC Carbendezim followed by Thiram against *M. phaseolina.*

Devi and Singh (1997) found that Carbendazim and Thiophanate methyl at 0.2 per cent were most effective growth inhibitors of *M. phaseolina* causing seedling mortality of black gram.

Monga and Ray (1997) reported that Thiophanate methyl and Celest completely inhibited *R. solani* at lowest tested concentration of 50 ppm. In case of Captan and Kitazin complete inhibition was noted at 500 ppm Dodine and Sulphur were less toxic to *R. solani* and maximum inhibition at 1000 ppm.

Yadav *et al.* (2000) reported that cotton seed treated with Carbendazim @ 0.2 per cent reduced root rot caused by *R. solani* to the extent of 74.4 per cent.

Sethuraman *et al.* (2001) reported that seed treatment + soil application of *T.viride* at 4g/kg and *P. fluorescens* at 10 g/kg either applied indivisually or combined (talc based) (4.16, 4.59 and 2.59 per cent incidence) and seed treatment + soil application of *T.viride* (gypsum based) recorded significantly less incidence of root rot (4.71 per cent in black gram than the control (14.04 per cent).

Jahagirdar *et al.* (2001) reported that in charcoal rot of sorghum caused by *M. phaseolina* seed treatment with *T. viride* 4g/kg of seed recorded average charcoal rot incidence of 17.7 per cent, 11.8 per cent and 10 per cent and *P. fluorescence* recorded 23.4 per cent, 13.1 per cent and 9.1 per cent per cent in cultivars M-31, GRS-1 and 9-13 respectively.

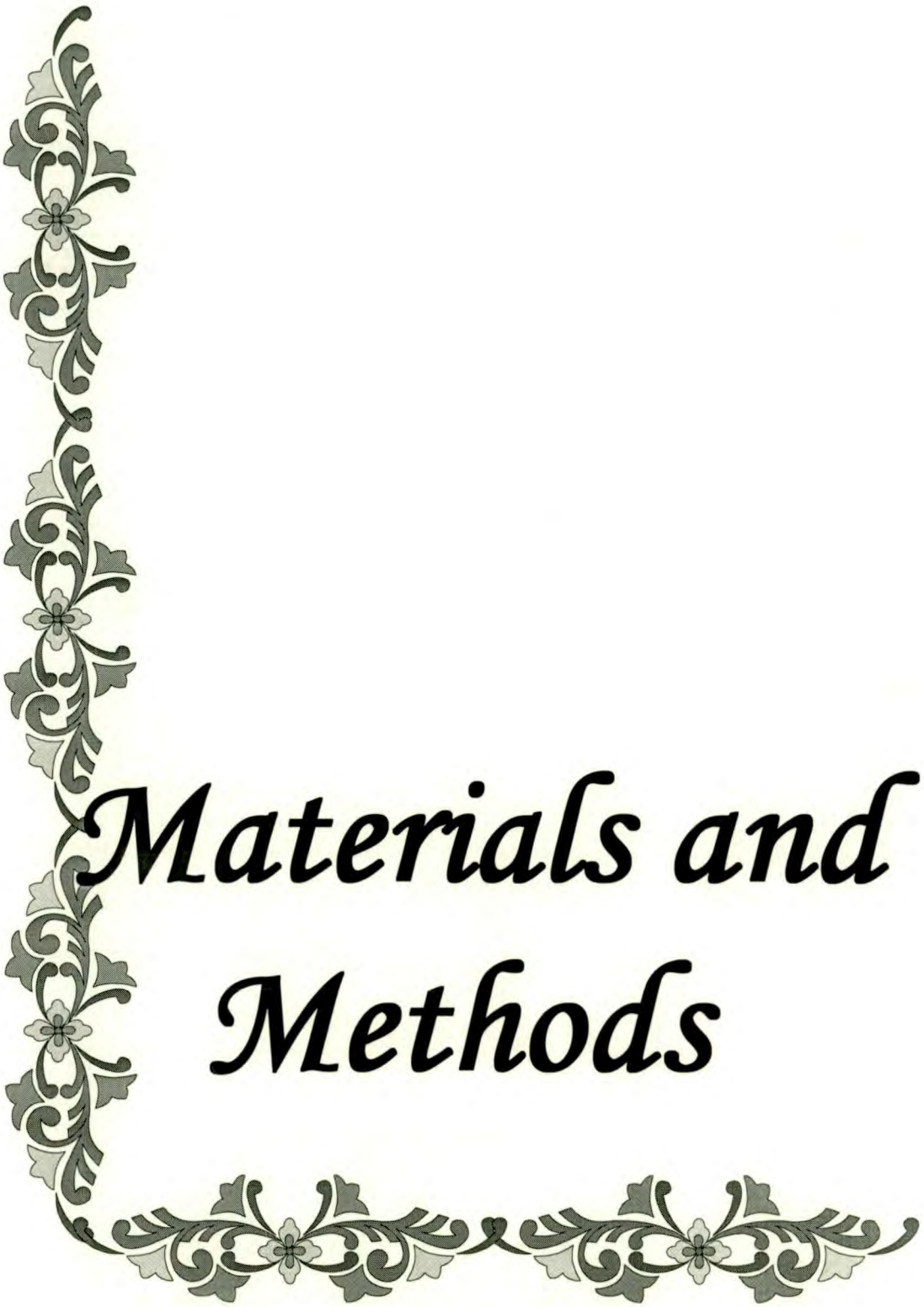
Mohanbabu and Seetharama (2002) reported that seed treatment with talc based formulation of *T. viride* (4g/kg) + *P. fluorescence* (5g/kg) recorded the germination (83.6 per cent) shoot length 28.12 cm, root length (18.35 cm), dry matter production (9.77 g) and vigour index (3501.04) which was highest as compared to the control.

Singh *et al.* (2003) studied the efficacy of antagonists against *M. phaseolina* causing roor rot of black gram and reported that among six fungicide tested as seed treatment Carboxin (2g/kg seed) reduced 64.51 per cent disease incidence followed by cotton. Carbendazim (2g/kg seed) (60.55 per cent) against root rot. Soil application of *T.viride* biomass (500 g/kg soil) proved most effect which gave 72.08 per cent disease control, followed by *Gliocladium virens* which exhibited 64.94 per cent disease control.

Vaish and Sinha (2003) evaluated *Trichoderma* species and eight systemic fungicide *viz.,* Contaf, Tilt, Validomycin, Pulsor, Folicur, Anvil, Quintal and Swing with four concentrations against *R. solani* causing sheath blight of rice. They reported that antagonists *T.virens* fungicides Contaf, Pulsor, and Anvil were highly effective in inhibiting the mycelial growth of *R. solani.*

Suryawanshi *et al.* (2008) evaluated (*invitro* and *invivo*) seven fungicides against *Macrophomina* blight of mungbean and reported all the test fungicides as effective against the test pathogen. In *invitro* studies all the fungicides inhibited mycelia growth of pathogen and per cent inhibition was ranged from 71.90 to 94.18. Field studies indicated that all the fungicides significantly reduced the disease intensity with increased seed yield over untreated control. However Carbendazim (0.05 per cent) was found most effective with least disease intensity (10.67 per cent), highest disease control (58.45 per cent) and increased seed yield (429.67 kg/ha). This was followed by Benomyl (0.15 per cent) and Mancozeb (0.2 per cent) with disease intensity of 16.46 and 17.11 per cent, respectively.

Gore *et al.* (2008) reported that in mungbean blight maximum per cent disease control was achieved with Carbendazim followed by Benomyl with minimum per cent disease. Minimum control was observed in copper oxychloride and Captan. Highest yield was obtained in Carbendazim @ 0.15 per cent. The lowest yield in control.



Materials and Methods

CHAPTER-III

MATERIALS AND METHODS

3.1 MATERIALS

Following materials were used for the experimentation:

3.1.1 Collection of diseased samples

The samples of cotton (*Gossypium* spp.), green gram (*Vigna radiata* L.Wilczek), black gram (*Vigna mungo* L.Hepper), soybean (*Glycine max* L.Merril), safflower (*Carthmus tinctorius*) and sorghum (*Sorghum bicolor* L.Monech) showing the symptoms of root/collar rot were collected from respective research experimental farm of Marathwada Krishi Vidyapeeth Parbhani.

3.1.2 Glasswares and equipments

Petri plates, flasks, test tubes, measuring cylinder, pipettes, cork borer, weighing balance, incubators and freezer, autoclave, hot air oven were made available from Department of Plant Pathology, M.K.V. Parbhani.

3.1.3 Culture media Used

The cultural studies were carried out using various media *viz.,* potato dextrose agar, Czapeck's dox, Richard's, malt extract, peptone agar etc.For preparation of culture media, ingredients were and double distillation water made available from Department of Plant Pathology, M.K.V. Parbhani.

3.2 METHODS

3.2.1 Sterilization of glassware and other materials

Glasswares were sterilized in hot air oven at 180^0C for 1 hour. Cultur media were sterilized in autoclave at 1.05 kg/cm^2 for 15 minutes, soil was sterilized using 10 percent formalin solution.

3.2.2 Preparation of culture media

Potato dextrose agar was used for isolation and maintenance of *M. phaseolina* isolates.

PDA was prepared as sliced pieces of peeled potatoes(200g) were first boiled in 500ml distilled water till cooked. Potato extract thus obtained was strained through double layered muslin cloth. In another 500 ml water, agar-agar (20g) and dextrose (20g) were dissolved and to this potato extract was mixed. Finally the volume of medium was adjusted to one liter by adding distilled water. The medium was distributed in sterile glass conical flasks and tubes to one third capacity, plugged with cotton and sterilized in autoclave at 1.05 kg/cm^2 for 15 minutes. To prepare slants, PDA tubes were kept in slanting position till the medium was solidified.

3.2.3 Isolation and purification of culture.

Isolation of the test pathogen was undertaken in laminar air flow chamber under aseptic conditions. The chamber was disinfected with spirit before use. UV light was kept on up to 20 minutes before starting the work in chamber. After that UV light was switched off.

Melted autoclaved PDA (45^0C) was poured in sterilized petri plates (20 ml/plate) and allowed to solidify. Just before pouring of

PDA, small quantity of streptomycin was added to PDA to avoid bacterial contamination.

From infected root/ stem, small bits of 2 mm diameter were cut with flame sterilized and cooled scalpel. These bits were surface sterilized with 0.1 per cent mercury chloride ($HgCl_2$) solution for one minute and then washed with three changes of sterilized distilled water to remove traces of $HgCl_2$.Sterilized bits were dried around the flame of spirit lamp and then placed on solid PDA medium in plates. Four bits per plate at equidistance was placed. The plates were incubated at room temperature (27 ± 2^0C) till colonies of the test fungus grew around the infected bits. After confirmation of identity, the fungus was transferred on PDA slants.

The isolates of *M. phaseolina* were purified by hyphal tip method. For this, small amount of culture was transferred on solid PDA in plates. After 24-48 hour of incubation, the plates were examined under stereoscopic microscope from reverse side. The tips of growing hyphae /mycelium were marked with ink. These marked bits along with small block of agar agar transferred on PDA with flame sterilized inoculating needle. The purified cultures were maintained at 20^0C in refrigerator. Sub-culturing was done at 20-30 day interval.

3.3 Morphological study

3.3.1 Growth and sclerotial formation of *M. phaseolina* isolates

Autoclaved PDA was poured in the plates and on solidified medium the *M .phaseolina* isolates was inoculated separately. After seven days of incubation 5 mm fungal disc were cut with the help of flame sterilized cooled cork borer. Three discs of each isolate were then

transferred on solidified PDA plates, one disc per plate and these plates were incubated at room temperature (27 ± 2^0C). Radial growth of each isolate was recorded on 3rd, 5th and 7th day of incubation. The colonies were measured in two marked direction at right angle to each other, passing through the centre of colony and average colony diameter was worked out. Sclerotial formation and colony characters were recorded on 5th day of inoculation.

3.3.2 Measurement of sclerotia size

Olympus research microscope was used to measure the sclerotial diameter. Value of one part of ocular micrometer was calibrated under low magnification (10x **x** 15x) of microscope and then diameter of 50 randomly selected sclerotia of each isolate was measured. Finally mean sclerotial diameter of each isolate was worked out. Shape of each isolate was also recorded.

3.4 Pathogenicity test

3.4.1 Preparation of mass culture

Sorghum : sand medium was used for mass multiplication of the test pathogen. It was prepared by mixing 500 g sorghum and 200 g dry sand with 500 ml distilled water in 2000 ml capacity conical flask and autoclaved at 1.05 kg/cm^2 for 30 minutes, for two consecutive days. Autoclaved and cooled sorghum sand medium was then inoculated with pure culture of *M.phaseolina* isolates separately. The inoculated flasks were incubated at room temperature for two weeks and used for experiment.

3.4.2 Preparation of sick soil and pathogenicity test.

The earthen pots (25cm) were disinfected with 5 percent formalin solution and soil was sterilized with 10 per cent formalin solution.

Two weeks old mass culture of *M. phaseolina* isolates were mixed separately in soil in 1:10 proportion (one part of inoculum and ten parts of sterilized soil) and pots were filled before four days of sowing the seeds. These pots were kept wet and in moist conditions. The pots containing sterilized soil without inoculum served as control. Before sowing, the seeds were surface sterilized with 4 percent sodium hypochlorite solution for 2 minutes followed by three washing with sterilized distilled water. Such seeds were used for sowing of each isolate with three replications. Observations were recorded up to 30 DAS. In case of sorghum, observations recorded at 60-65 DAS.

Percent rotted or wilted seedlings in the artificial sick soil was calculated using the formula given by Hooda and Grover (1982).

$$\text{`Percent seedling rotted/wilted} = \frac{\text{No. of infected seedlings in inoculated soil}}{\text{No. of seedlings in uninoculated soil}} \times 100$$

3.5 Host range and cross infectivity of *M. phaseolina* isolates

In sterilized soil mass culture of *M.phaseolina* isolate was mixed thoroughly in 1:10 proportion (one part of inoculum to 10 parts of sterilized soil) and filled in disinfected earthen pots. These pots were then incubated for four days under wet and moist condition. Surface

sterilized seeds of cotton, green gram, black gram, soybean, safflower and sorghum were sown in cross inoculation manner. The respective fungus cultures were added to the base of each host for making the plants more amenable to root rot infection. Three replications for each isolate were mintained.

The observations on mortality, seedling rotted/ wilted were recorded at seven day interval and finally after 30 days.

3.6 Compatibility among isolates of *M.phaseolina*

Compatibility among the isolates of *M. phaseolina* from six hosts *viz.*, cotton, green gram, black gram, soybean, safflower and sorghum was studied on PDA. Autoclaved PDA was poured in sterilized plates under aseptic conditions and allowed to solidify. Fungal disc of 5 mm size of seven days old culture of two isolates were placed on PDA medium opposite to each other, 10 mm away from the rim of plate. For each isolates combination, three replications were maintained. The plates were incubated at 27 ± 2^{0}C and compatibility (fusion of colonies) among the isolates was observed upto 7 days after incubation.

The observations were recorded as compatible (complete merging of colonies of two isolates), almost compatible (gap of 0.1 to 0.3 mm between colonies of two isolates) and non compatible where the isolates showed wide inhibition zone between colonies.

3.7 Nutritional Study

3.7.1 Effect of culture media

Potato dextrose agar, Czapek's dox agar, Richard's agar, peptone agar media and malt extract agar were used to find out the best medium for the growth and sclerotial formation of *M. phaseolina* isolates.

Twenty ml of medium was poured in each sterilized Petri plate and culture was grown on it. Five mm discs of fungus were cut with the help of sterilized and cooled cork borer from the margin of seven days old culture of *M. phaseolina* grown on PDA. One disc of the culture was placed in inverted position in centre of each Petri plate. Three replications were maintained for each isolate. Inoculated plates were incubated at room temperature (27 ± 2^0C).

The mycelial growth was measured on 3rd, 5th, and 7th days after inoculation. The observations on the time required for the initiation of sclerotia was recorded. The number of sclerotia per microscopic field was counted. Accordingly each isolate was grouped as : + = 10-20, ++ = 21-30 and +++ = above 30 sclerotia per microscopic field (Das ,1988) in respect of sclerotial number.

Experiment details:

Design: CRD

Replication: Four

Treatments : 5 culture media

T_1 : Potato dextrose agar

T_2 : Czapek's dox agar

T_3 : Richard's agar

T_4 : Peptone agar

T_5 : Malt extract agar

3.7.2 Effect of different carbon sources

Czapek's dox was used as basic medium for nutritional study. Sucrose which is carbon source of medium was substituted by different carbon sources *viz.,* dextrose, maltose, manitol, starch, cellulose on the basis of molecular weight. These modified media were autoclaved and poured in sterilized plates at 45^0C. After solidification, 5mm disc of each isolate of *M. phaseolina* was kept at centre of medium separately for each isolate three replication were maintained. The inoculated plates were incubated at room temperature upto 7 days. Observations on radial growth and sclerotia formation were recorded on 3^{rd}, 5^{th} and 7^{th} days of inoculation. Original Czapek's dox medium with sucrose was used as control.

Experiment details:

Design : CRD

Replication: Four

Treatments: 5 carbon sources

T_1 : Dextrose

T_2 : Maltose

T_3 : Manitol

T_4 : Starch

T_5 : Cellulose

3.7.3 Effect of nitrogen sources

Sodium nitrate, a source of nitrogen of Czapek's dox medium was substituted by ammonium nitrate on the basis of molecular weight. Observations from replicated experiments on radial growth and sclerotial formation of *M. phaseolina* isolates were recorded on this

modified media after 3rd, 5th and 7th days of inoculation Original medium with sodium nitrate served as control.

3.8 Physiological study

3.8.1 Effect of temperature

Sterilized Petri plates poured with autoclaved PDA were placed at 20, 25, 30, 35 and 40^{0}C in an incubator to get medium acclimatized to that particular changed temperature prior to inoculation. After 24 hrs, poured Petri plates were inoculated centrally each with 5 mm disc from the margin of seven days old culture of the different *M. phaseolina* isolates. Inoculated plates were re-incubated at the respective temperature, with three replications for each isolate. The observations on mycelia growth and selerotial formation were recorded on 3rd, 5th and 7th days after inoculation.

Experiment details:

Design : CRD

Replication : Four

Treatments : 5 temperatures (^{0}C)

T_1 : 20

T_2 : 25

T_3 : 30

T_4 : 35

T_5 : 40

3.8.2 Effect of pH

The initial pH of potato dextrose agar was adjusted at 5, 6, 7, 8, 9 with the help of 0.1N HCl or 0.1N NaOH prior to autoclaving.

Sterilized Petri plates were poured with this media after solidification of medium; the plates were inoculated with 5 mm disc of mycelial culture of *M. phaseolina* isolated separately and for each isolates three replication were made. Inoculated plates were incubated at 27± 2^0C temperature observation on for mycelial growth and sclerotia formation were recorded on 3rd, 5th and 7th days after inoculation.

Experiment details:

Design : CRD

Replication : Four

Treatments : 5 pH levels

T_1 : 5

T_2 : 6

T_3 : 7

T_4 : 8

T_5 : 9

3.9 Effect of fungicides and bioagents on *M.phaseolina.*

Pot culture experiment

A pot culture experiment was planned and conducted in screen house to study the efficacy of seven fungicides and two bioagents as a seed treatment against *M. phaseolina* applying CRD and the treatments were replicated thrice.

Experimental Details:

Design : CRD

Replication : Three

Treatments : 10 (7 fungicides + 2 bioagents + control)

T_1 : Carbendazim (0.1 %)

T_2 : Propiconazole (0.1 %)

T_3 : Hexaconazole (0.1 %)

T_4 : Iprodione (0.2 %)

T_5 : Thiram 75% WP (0.3 %)

T_6 : Mancozeb (0.025 %)

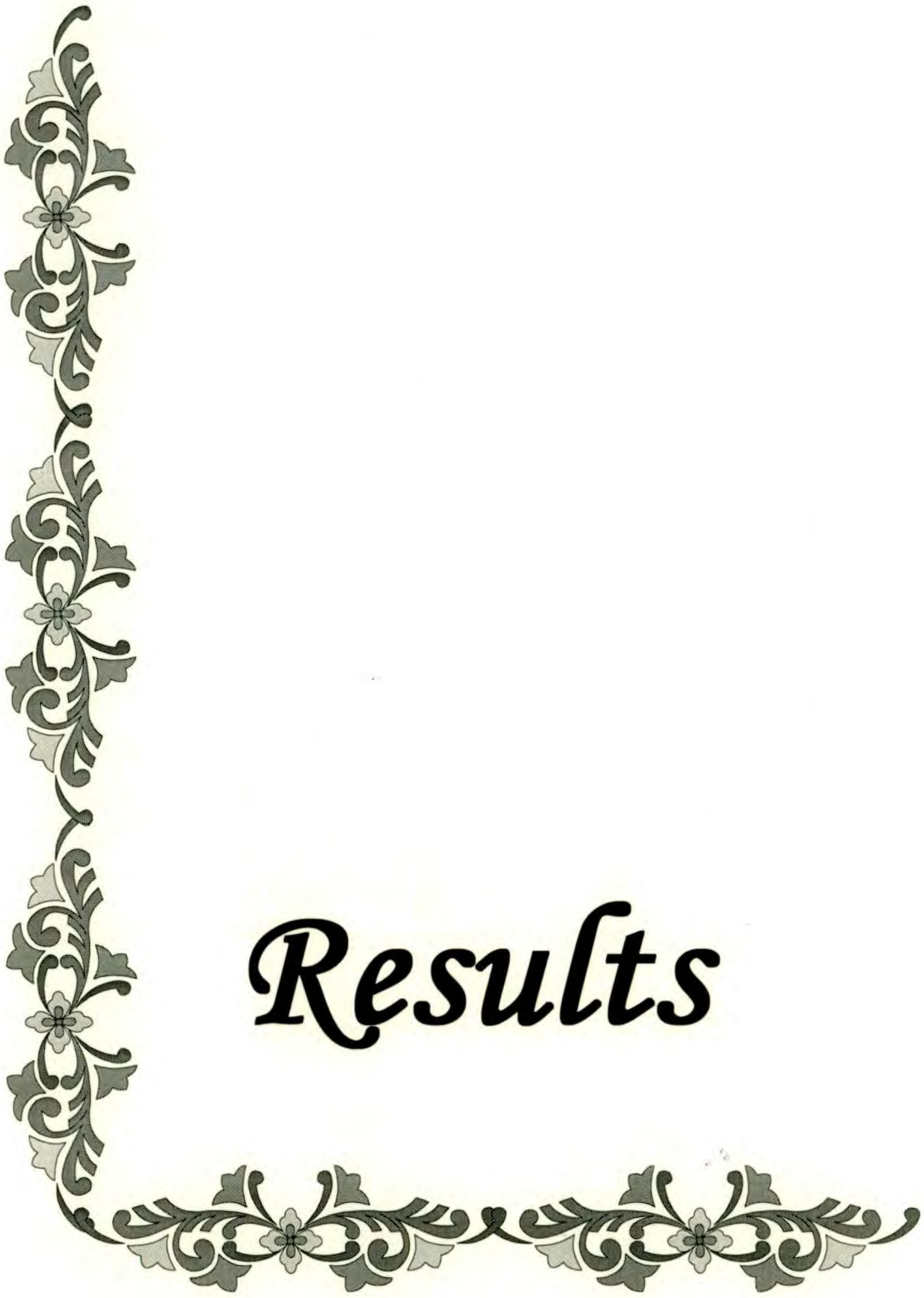
T_7 : SAAF (Carbendazim+ Mancozeb) (0.2 %)

T_8 : *T.viride* (5 g/kg)

T_9 :*P. fluroscence* (5g/kg)

T_{10} : Control

Pot experiment were conducted under screen house conditions in completely randomized block design to study the efficacy of fungicides and bioagents against root/collar rot disease. Sterilized soil was used in pots. Mass multiplied cultures of *M. phaseolina* was incorporate in pots containing sterilized soil @ 25 g/kg soil to make them sick before 72 hrs of sowing. Fifteen treated seeds were sown in each inoculated pots. Observations on germination and root/collar rot were recorded from each pot up to 40 days from sowing. Percent rot incidence and disease control were finally calculated on the basis of control sets.



Results

CHAPTER-IV

RESULTS

Soil borne sclerotial fungus *Macrophomina phaseolina* (Tassi) Goid is causing root and stem rots in number of hosts from different plant species. It differ in their morphological, cultural and physiological characters.

4.1. Collection, isolation, purification and identification of the pathogen

4.1.1. Isolation

Isolation of the fungus from the samples of six hosts collected from the field of University farm were done in Petri dishes using potato dextrose agar medium. The surface sterilized root bits of respective host yielded the fungus after 48 hours of incubation at 27 $\pm$ 2°C. The uniform colonies originating from diseased bits were separated, purified and identified (Table1). Results (Table 1) revealed that *M. phaseolina* was the major cause associated with root rot / charcoal rot in all the host crops tested and its percentage was ranged from 60.00 to 95.00.Other fungi found associated in traces were *Fusarium, Aspergillus,* and *Alternaria* species.

Table 1 Association of *Macrophomina phaseolina* (Tassi) Goid isolates with root rot /charcoal rot diseased specimens of various host crops.

Host crops	Locations	Sample collected	No. of infected bit kept for isolation	No. of bits yielded *M. phaseolina*	Other fungi associated	Association of *M. phaseolina* (%)
Cotton	Cotton Research Scheme	Root rot	20	12	-----	60.00
Green gram	Seed Testing Research Unit	Root rot	20	16	*Fusarium* spp.	80.00
Black Gram	Vegetable field	Root rot	20	17	*Fusarium* spp.	85.00
Soybean	AICRP on Soybean	Root rot	20	18	*Sclerotium rolfsii*	90.00
Safflower	AICRP on Safflower	Root rot	20	17	*Fusarium* spp., *Aspergillus* spp.	85.00
Sorghum	Sorghum Research Station	Charcoal rot	20	19	*Fusarium* spp.	95.00

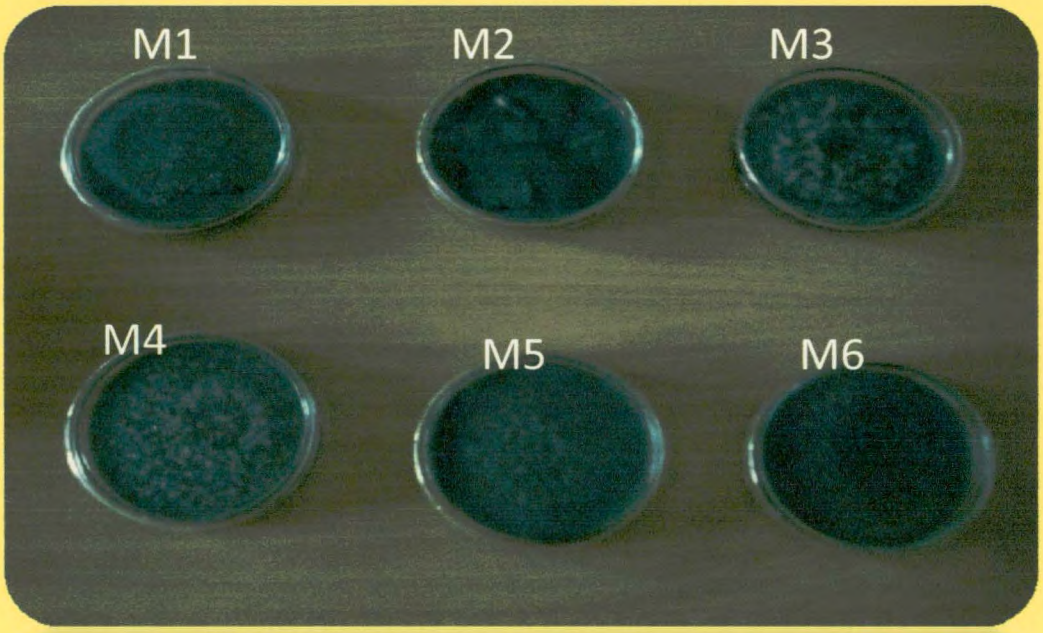
4.1.2. Purification and identification

The pure culture was obtained using single hyphal tip method. The pure culture thus obtained was identified as *Macrophomina phaseolina* on the basis of morphological characters described by CMI publication.Purified cultures of the fungus were maintained on PDA slants for further investigations.

4.2. Morphological study

The mycelial growth varied from sub-aerial to aerial and showed right angle or acute angle branching pattern. In majority of isolates; *M. phaseolina* color turned from whitish to blackish, dull white and finally dark black with age due to sclerotial formation. The colony color varied from greenish black to charcoal black, although from reverse side all isolated were blackish (Table 2). The colonies of all isolates of *M. phaseolina* appeared circular on PDA (Plate I). Some colonies were profuse only in centre but most of the showed profuse and compact growth.

PLATE I



M1-COTTON, M2-GREEN GRAM, M3-BLACK GRAM,
M4-SOYBEAN, M5-SAFFLOWER, M6-SORGHUM

Pure culture of *M. phaseolina*

Table 2 Morphological characteristics of *M. phaseolina* isolates obtained from various hosts crops.

Character	Cotton	Green gram	Black gram	Soybean	Safflower	Sorghum
Colony color (upper and lower)	Initially whitish black turn into black	Light Black to black	Light black turn into black	Initially whitish black turn into black	Light Black to black	Light black
Colony appearance	Profuse and compact	Profuse on upper side	Profuse and compact	Profuse and compact	Profuse in center	Profuse in center
Mycelium	Aerial	Aerial	Sub-aerial	Aerial	Sub-aerial	Sub-aerial
Branching pattern	Right angle	Right angle	Right angle	Acute angle	Right angle	Right angle
Margin of colony	Circular	Circular	Circular	Circular	Circular	Circular
Growth	Fast	Very fast	Medium fast	Slow	Fast	Medium fast
Sclerotial formation	Abundant	Medium	Medium	Medium	Abundant	Abundant

4.2.1 Radial growth and sclerotial formation

Among the six isolates of *M. phaseolina* green grami solates showed slow growth, black gram and sorghum isolates showed medium fast growth; while cotton, soybean and safflower isolates showed very fast growth on PDA . On seventh day of incubation all isolates showed full growth (90 mm).

Number of sclerotia also varied with the isolates. Cotton, safflower and sorghum showed more vigorous growth and abundant sclerotial formation. While in green gram, black gram and soybean isolates had moderate rate of sclerotial formation (Table 2).

4.2.2 Size of sclerotia

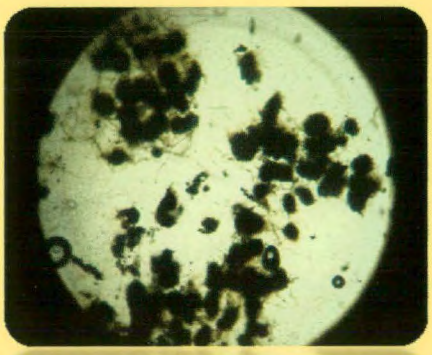
The size and shape of sclerotia in different isolates was also found to vary with host crops (Table 3,Plate II & III). The cotton isolate showed largest sclerotia diameter (105.26 µm). Whereas in soybean it was 47.57 µm.

Table 3 Shape and size of sclerotia of *M.phaseolina* isolates.

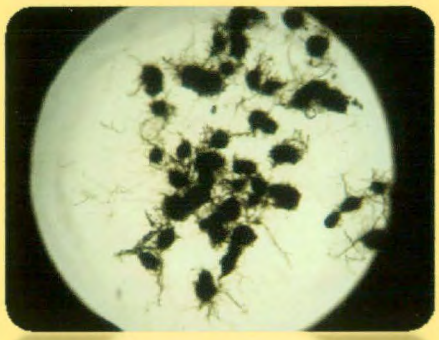
Isolates from	Sclerotia shape	Mean sclerotia size (µm)
Cotton	Round, oblong, irregular	105.26
Green gram	Round, irregular	73.97
Black gram	Round, oblong, irregular	79.17
Soybean	Round, irregular	47.57
Safflower	Round, irregular	85.17
Sorghum	Round, irregular	88.64

PLATE II

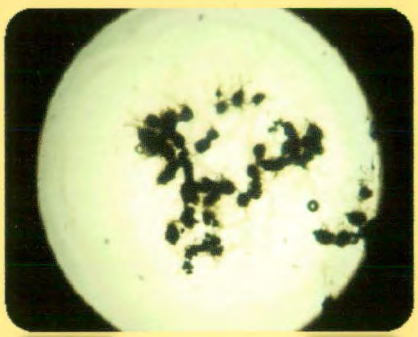
M1



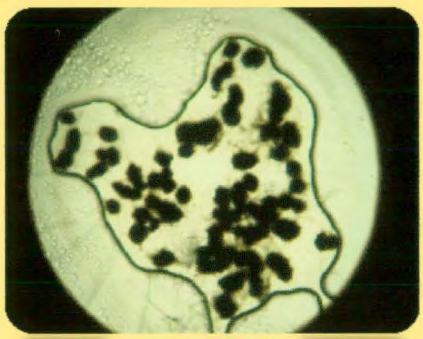
M2



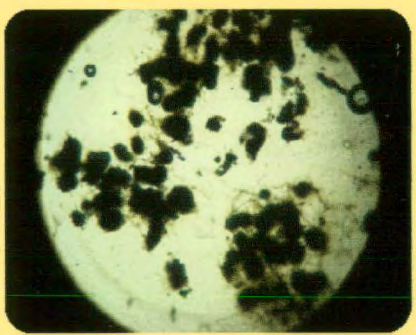
M3



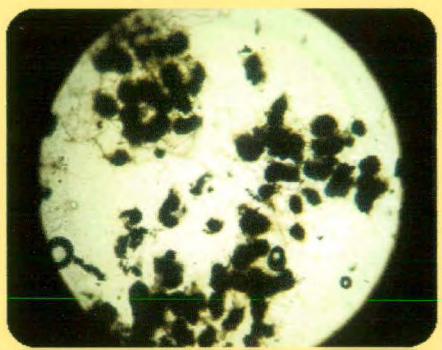
M4



M5



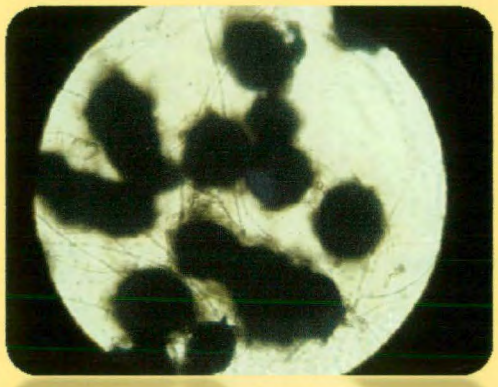
M6



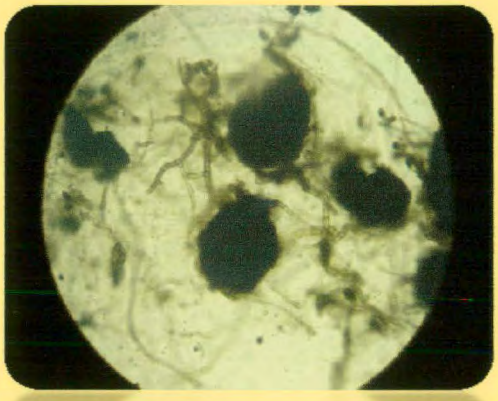
Microscopic photographs of *M.phaseolina* isolates at 10x X 10x

PLATE III

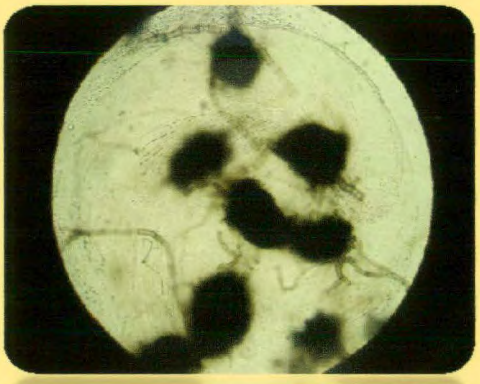
M1



M2



M3



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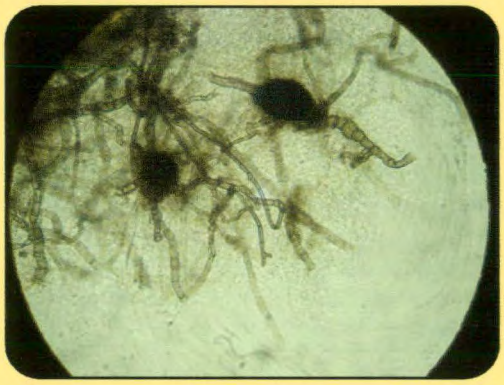

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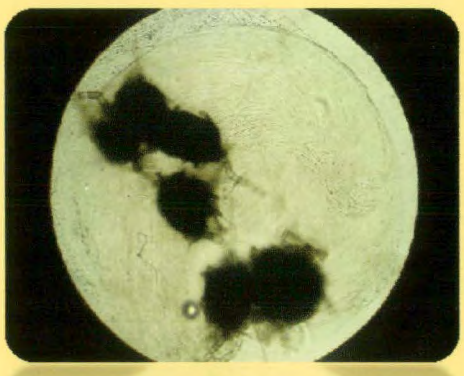

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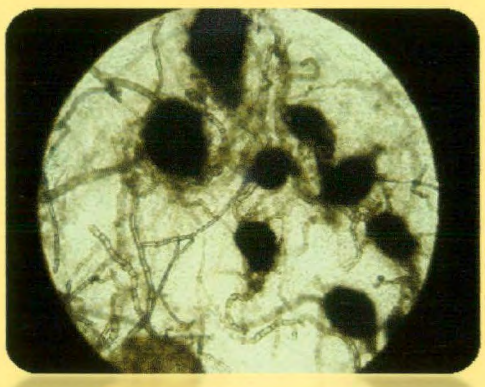


Microscopic photographs of *M.phaseolina* isolates at 10x X 40x

4.3 Pathogenicity test

The pathogenicity test was conducted under glasshouse on cotton, black gram, green gram, soybean, safflower and sorghum by sick soil technique.

Results (Table 4) revealed that seedling mortality was started from 15th day after germination. In sorghum symptoms observed on 60 days after sowing. Susceptibility was determined on the basis of percent rotted/wilted seedlings per pots. The data indicated that all isolates caused infection to host plants tested. However, highest infection (90%) was recorded in cotton and safflower, whereas least infection was recorded in green gram (60%).

Table 4 Pathogenicity test of different isolates of *M. phaseolina*

Isolates from	***No. of seeds sown**	***Seedlings rotted/wilted**	**Percent seedling rotted/wilted**
Cotton	10	9	90
Green gram	10	6	60
Black gram	10	7	70
Soybean	10	8	80
Safflower	10	9	90
Sorghum	10	7	70
Control	10	-	-

*Average of three replications

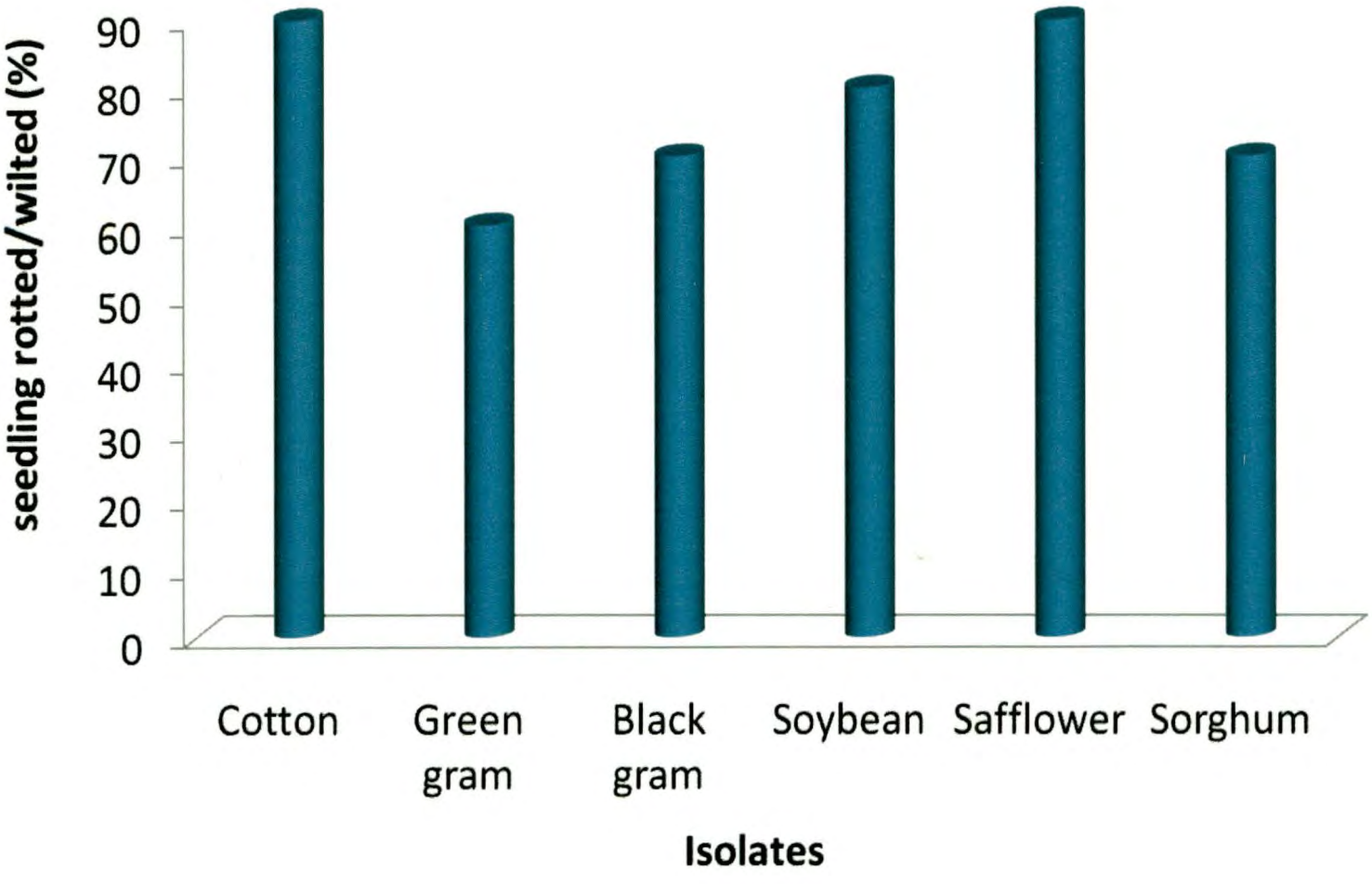
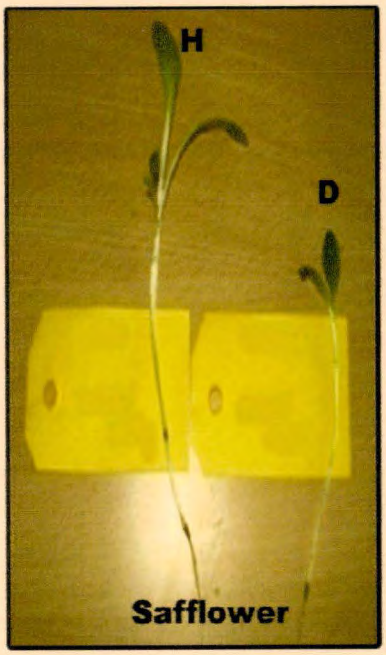


Fig. 1. Pathogenicity test of *M. phaseolina* isolates

PLATE IV



Pathogenicity test of *M.phaseolina*

4.4 Host range and cross infectivity of *M. phaseolina*

The host range and cross infectivity study was conducted under glasshouse and results are presented in Table 5Results revealed that cotton isolate was pathogenic to black gram and safflower; while green gram, soybean and sorghum seedling were remained non-infected.

Table 5 Host range and cross infectivity of *M. phaseolina* isolates

Isolates from	**Host**					
	Cotton	**Green gram**	**Black gram**	**Soybean**	**Safflower**	**Sorghum**
Cotton	+	-	+	-	+	-
Green gram	-	+	+	+	+	+
Black gram	+	+	+	+	+	+
Soybean	-	+	+	+	+	+
Safflower	+	+	+	+	+	+
Sorghum	-	+	+	+	+	+

+ = Highly infected

- = Non infected

Green gram isolate caused infection to black gram, soybean, sorghum and safflower, while cotton remained non-infected.

The sorghum isolate caused infection to black gram, green gram, safflower and soybean but cotton seedling remained non-infected.

The safflower and black gram isolates were most aggressive among the test isolates and infected to all the hosts tested.It was also

found that safflower and black gram were susceptible host infected by all isolates of *M. phaseolina*.

4.5 Compatibility among isolates of *M. phaseolina*

The data of compatibility among isolates was presented in (Table 6 and Plate IV). Indicated that two combinations of *M. phaseolina viz.*, cotton-black gram and cotton-sorghum were non-compatible with each other.Only one combination *viz.*, cotton-soybean, indicated almost near compatible reaction. All other remaining combinations merged with each other and thus showed complete compatibility.

Table 6 Compatibility among isolates of *M. phaseolina*.

Isolates from	**Host**					
	Cotton	**Green gram**	**Black gram**	**Soybean**	**Safflower**	**Sorghum**
Cotton	C	C	NC	AC	C	NC
Green gram	C	C	C	C	C	C
Black gram	NC	C	C	C	C	C
Soybean	AC	C	C	C	C	C
Safflower	C	C	C	C	C	C
Sorghum	NC	C	C	C	C	C

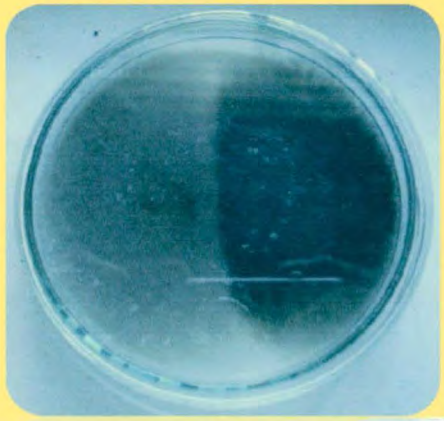
Compatible (C) = Complete merging of colonies of two isolates

Almost compatible (AC) = Gap of 0.1-0.3 mm between colonies of two isolates

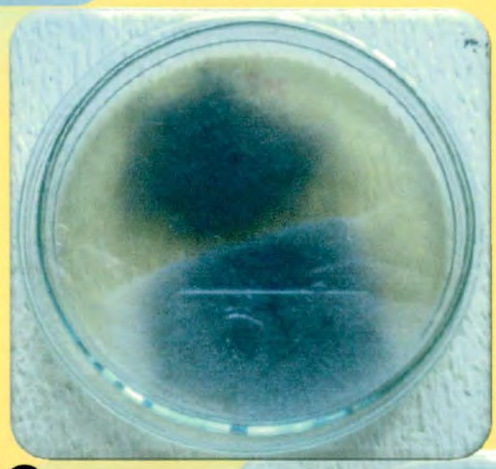
Non compatible (NC) = Wide inhibition zone between colonies of two isolate

PLATE V

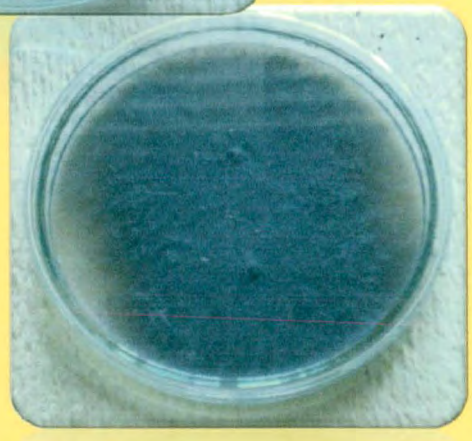
AC



NC



C



AC-Almost compatible
C-Compatible
NC-Non compatible

Compatibility among isolates of *M. phaseolina*

4.6 Nutritional study

4.6.1 Effect of culture media

Table 7 Effect of culture media on growth (mm) and sclerotial production of *Macrophomina phaseolina* isolates.

Isolates from	Cotton			Green gram			Black gram		
Media	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**
Potato dextrose agar	52.56 (+++)	89.70 (+++)	89.70 (+++)	39.66 (+++)	80.00 (+++)	89.70 (+++)	50.56 (+++)	80.33 (+++)	89.36 (+++)
Czapek's dox agar	39.33 (N)	75.43 (++)	89.96 (++)	32.00 (N)	76.33 (++)	89.99 (++)	29.33 (N)	68.36 (+)	89.70 (+)
Richard's agar	34.33 (++)	63.33 (+++)	89.96 (+++)	29.33 (++)	68.33 (+++)	89.93 (+++)	26.00 (++)	61.33 (+++)	75.36 (+++)
Peptone agar	26.17 (N)	54.66 (++)	74.33 (++)	23.63 (N)	49.79 (++)	62.66 (++)	21.02 (N)	49.18 (++)	59.00 (+++)
Malt extract agar	18.50 (++)	39.31 (+++)	66.66 (+++)	20.00 (+)	53.33 (++)	79.13 (+++)	20.50 (+++)	43.25 (+++)	75.32 (+++)
F- test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE m ±	0.56	0.55	0.33	0.51	0.60	0.40	0.61	0.60	0.53
CD at 0.01%	1.65	1.44	1.06	1.60	1.89	1.28	1.92	1.90	1.68

Table 7 contd....

	Soybean			Safflower			Sorghum		
Media	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**
Potato dextrose agar	59.33 (++)	80.10 (+++)	89.93 (+++)	54.96 (+++)	74.40 (+++)	90.00 (+++)	51.33 (+++)	81.66 (+++)	89.70 (+++)
Czapek's dox	50.35 (N)	78.66 (N)	89.96 (+)	33.00 (N)	70.33 (+)	8.25 (++)	38.33 (N)	76.66 (+)	89.96 (++)
Richard's	48.33 (++)	78.33 (+++)	89.96 (+++)	33.35 (++)	57.30 (+++)	89.96 (+++)	43.23 (++)	76.00 (+++)	89.99 (+++)
Peptone agar	28.11 (N)	53.34 (N)	60.16 (N)	24.35 (N)	71.00 (+++)	74.37 (++)	19.00 (N)	38.86 (++)	56.86 (+++)
Malt extract	22.66 (+)	50.33 (++)	73.00 (+++)	20.70 (+)	53.37 (++)	82.03 (+++)	28.83 (+)	54.33 (++)	76.00 (+++)
F- test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE m $\pm$	0.54	0.72	0.36	0.57	0.65	0.40	0.57	0.50	0.28
CD at 0.01%	1.70	2.29	1.15	1.80	2.05	1.28	1.82	1.60	0.89

Sclerotial formation

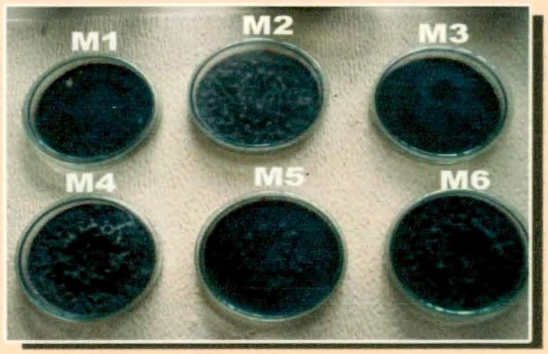
N: No formation

+: Rare (10-20 sclerotia per microscopic field)

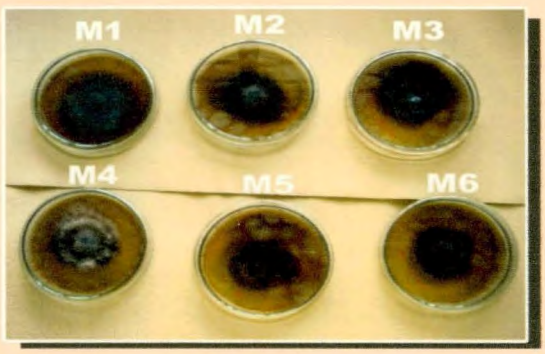
++: Medium (21-30 sclerotia per microscopic field)

+++: Abundant (above 30 sclerotia per microscopic field)

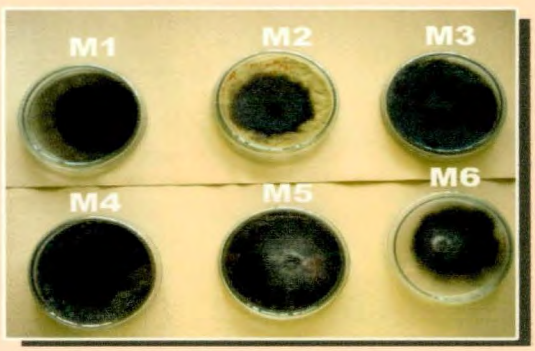
PLATE VI



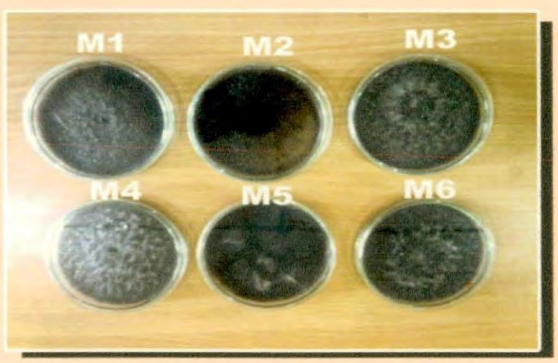
Potato Dextrose Agar



Czapek's Dox



Richard's



Peptone agar

Growth of *M. phaseolina* on different culture media (7DAI)

Results (Table 7) revealed that all the five culture media tested encouraged better growth and sclerotial production of *M. phaseolina* isolates tested. Among the media, PDA was found to be the best growth and sclerotial production of *M. phaseolina* over other media. All isolates showed abundant growth on PDA followed by Czapek's dox, Richard's and peptone agar media while mycelial growth on malt extract media was very slow.

Data represented in Table 7 indicated that sclerotial production was abundant on PDA followed by Richards and malt extract, while peptone agar medium showed moderate mycelial growth and sclerotial production in soybean isolate. In all isolates mycelial growth of *M. phaseolina* on malt extract was poor.

4.5.2 Effect of carbon sources

Carbon being a major structural and functional element for the fungus growth, number of carbon sources were tested to see the suitable carbon source for the growth of *M. phaseolina* of different isolates and results are presented in Table 8, Plate VII and Fig.3.

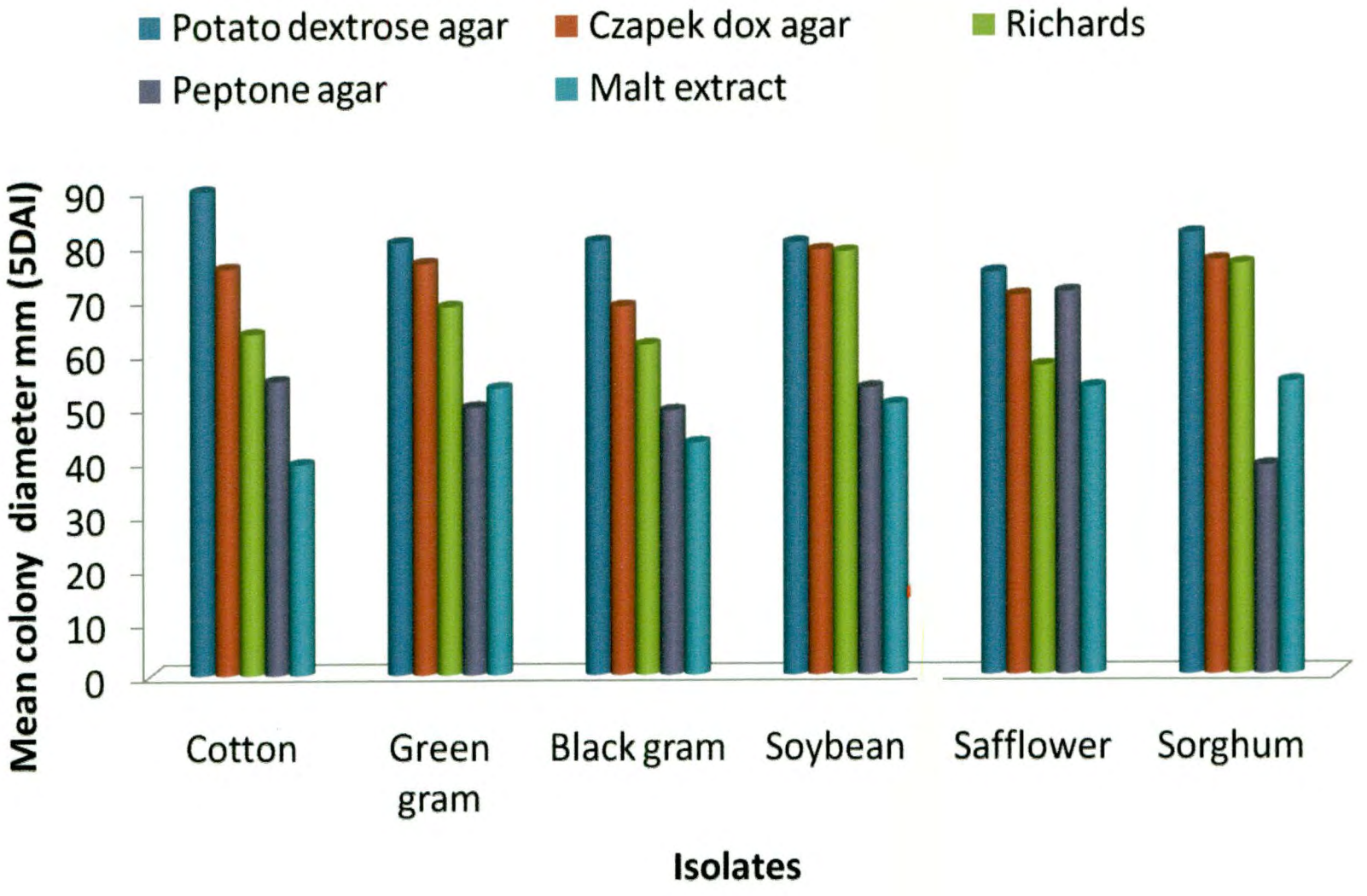


Fig. 2: Effect of different culture media on growth (mm) of *Macrophomina phaseolina*

Table 8 Effect of carbon sources on radial growth (mm) and sclerotial production of *M.phaseolina* isolates.

Isolates from	Cotton			Green gram			Black gram		
Carbon sources	3DAI	5DAI	7DAI	3DAI	5DAI	7DAI	3DAI	5DAI	7DAI
Dextrose	43.96 (+)	77.59 (++)	89.63 (+++)	37.43 (+)	73.66 (+++)	89.66 (+++)	49.33 (+)	78.10 (++)	89.80 (+++)
Manitol	37.40 (N)	70.44 (+)	90.00 (++)	30.16 (N)	67.36 (+)	90.00 (++)	43.66 (N)	72.67 (+)	89.96 (++)
Starch	33.00 (+)	68.23 (++)	89.80 (+++)	26.46 (+)	65.17 (++)	90.00 (+++)	32.33 (+)	58.30 (++)	88.33 (+++)
Cellulose	23.23 (++)	62.00 (+++)	84.16 (+++)	22.16 (++)	59.33 (+++)	72.36 (+++)	22.20 (++)	42.26 (+++)	63.25 (+++)
Sucrose	37.46 (N)	71.66 (++)	90.00 (+++)	31.33 (N)	69.79 (++)	90.00 (+++)	44.63 (N)	74.10 (++)	90.00 (+++)
Maltose	31.22 (+)	64.34 (++)	89..96 (+++)	24.33 (+)	61.66 (++)	74.40 (++)	09.60 (+)	18.0 (++)	32.66 (++)
F- test	sig	sig	sig	sig	sig	sig	sig	sig	sig
SE m ±	0.53	0.78	0.28	0.65	0.67	0.33	0.65	0.65	0.78
CD at 0.01%	1.65	2.42	0.87	2.00	2.07	1.04	2.01	2.02	2.42

Table 8 contd....

Isolates from	**Soybean**			**Safflower**			**Sorghum**		
Carbon sources	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**
Dextrose	55.60 (N)	81.52 (++)	89.83 (+++)	44.33 (N)	72.66 (++)	90.00 (+++)	55.35 (++)	90.00 (+++)	90.00 (+++)
Manitol	47.63 (N)	73.63 (+)	90.00 (++)	38.40 (N)	69.03 (++)	90.00 (++)	37.60 (N)	71.16 (+)	90.00 (++)
Starch	44.32 (+)	67.24 (++)	90.00 (+++)	35.11 (+)	65.22 (++)	80.30 (+++)	31.45 (+)	67.46 (++)	80.00 (+++)
Cellulose	33.32 (++)	69.00 (+++)	84.41 (+++)	21.36 (++)	53.70 (+++)	83.52 (+++)	23.30 (++)	52.13 (+++)	71.66 (+++)
Sucrose	49.61 (N)	78.59 (++)	89.90 (+++)	39.74 (+)	71.33 (++)	90.00 (+++)	46.80 (+)	83.00 (++)	89.93 (+++)
Maltose	41.33 (+)	73.36 (++)	89.93 (++)	04.33 (+)	13.66 (++)	33.33 (++)	06.33 (+)	12.66 (++)	24.00 (+++)
F- test	sig	sig	sig	sig	sig	sig	sig	sig	sig
SE m $\pm$	0.71	1.52	0.14	0.63	0.76	0.68	0.67	0.69	0.57
CD at 0.01%	2.19	4.69	0.45	1.94	2.35	2.10	2.08	2.12	1.75

Sclerotial formation

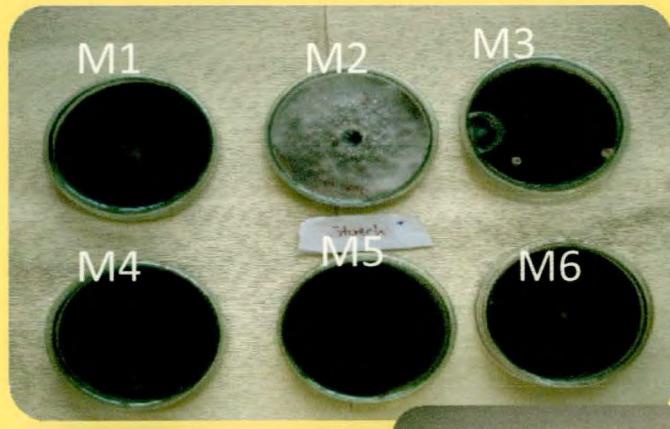
N: No formation

+: Rare (10-20 sclerotia per microscopic field)

++: Medium (21-30 sclerotia per microscopic field)

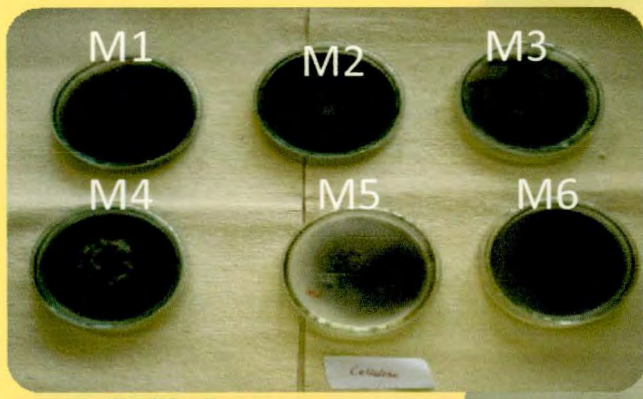
+++: Abundant (above 30 sclerotia per microscopic field)

PLATE VII

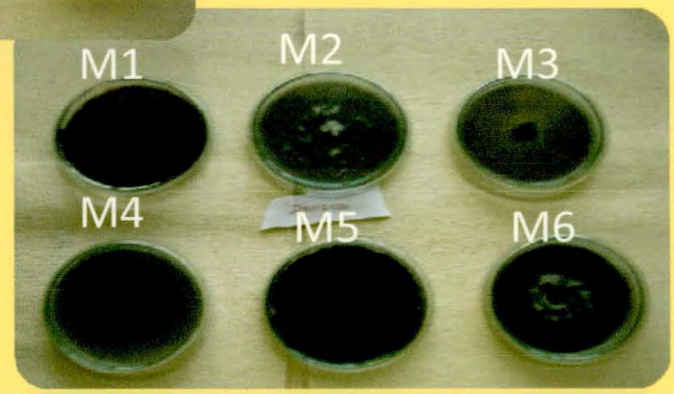


Dextrose

Manitol



Cellulose



Sucrose

Effect of different carbon sources on growth of *M.phaseolina.* (7DAI)

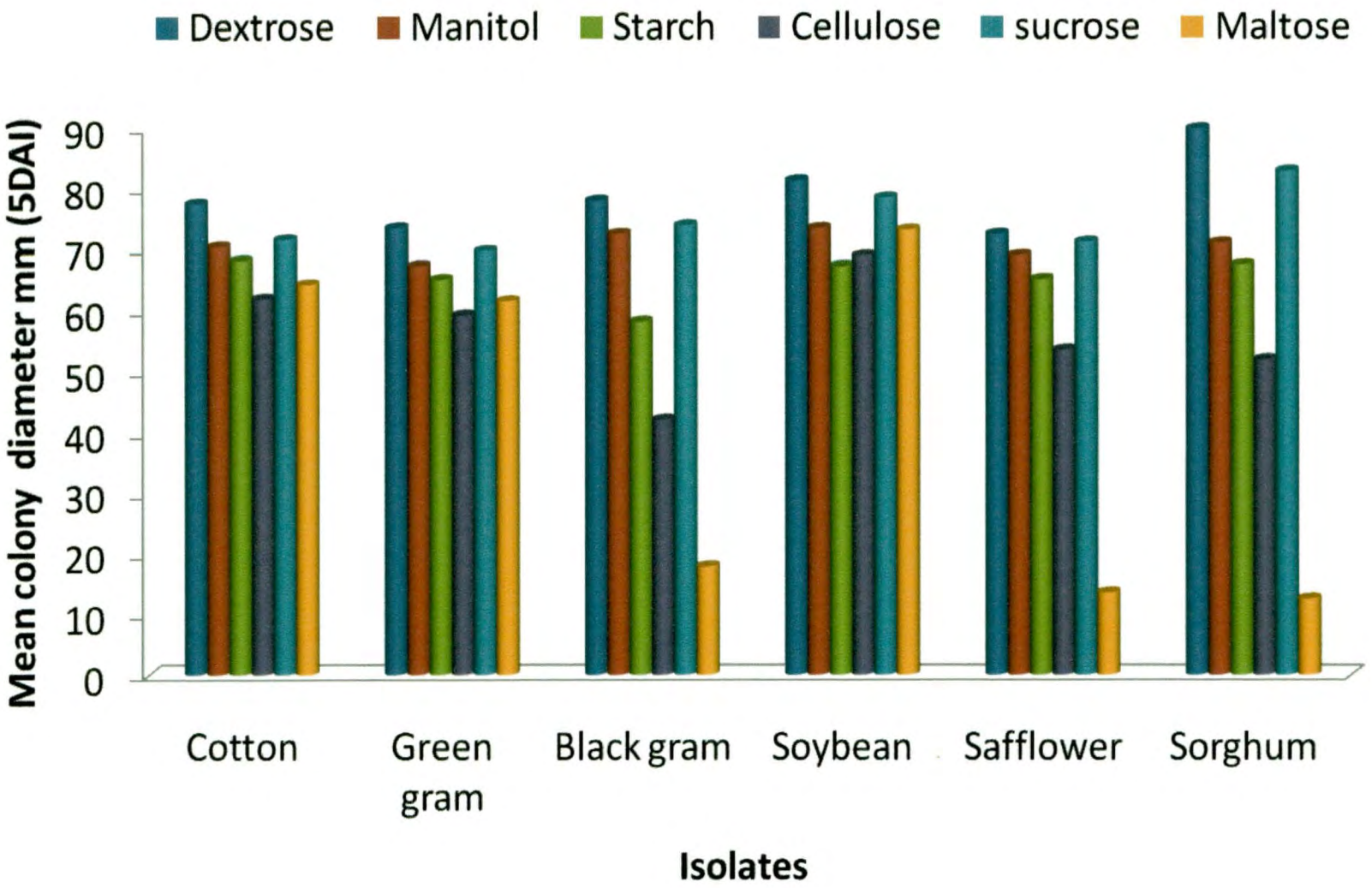


Fig. 3: Effect of carbon sources on growth (mm) of *Macrophomina phaseolina*

Results are also depicted with histogram in Fig.3 photographic presentation of growth as influenced by different carbon sources also exhibited in Plate7.

It was evident from Table 8 that growth of *M. phaseolina* isolates on all the six carbon source was significantly varied from each other.Among six carbon sources, significantly highest growth of all isolate was observed in dextrose followed by sucrose, manitol, starch, maltose and cellulose. Mycelial growth rate was poor in cellulose as compared to other carbon sources, sorghum and safflower isolates showed poor growth on maltose.

Results revealed that maximum sclerotial production was on cellulose followed by dextrose, maltose, starch and sucrose while manitol induced less sclerotia production.

4.6.3 Effect of nitrogen sources

Results (Table 9, Plate VIII, Fig.4) revealed that sodium nitrate was the best source of nitrogen as compared to ammonium nitrate. Mycelial growth was faster in sodium nitrate than ammonium nitrate. On seventh day of incubation all isolates of *M. phaseolina* showed almost maximum growth (90 mm) with of sodium nitrate.

All isolates were unable to induce the formation of sclerotia till third day of incubation. In ammonium nitrate rate of sclerotial formation was low as compared to sodium nitrate as source of nitrogen.

Table 9 Effect of nitrogen sources on radial growth (mm) and sclerotial production of *M.phaseolina* isolates.

Isolates from	Nitrogen sources					
	Sodium nitrate			Potassium nitrate		
	3 DAI	5 DAI	7 DAI	3 DAI	5 DAI	7 DAI
Cotton	37.58 (N)	71.33 (++)	90.00 (++)	21.43 (N)	46.23 (N)	74.34 (+)
Green gram	32.0 (N)	69.00 (++)	90.00 (++)	22.47 (N)	53.70 (N)	86.26 (+)
Black gram	43.38 (N)	74.58 (+)	90.00 (+)	25.60 (N)	46.33 (N)	79.25 (+)
soybean	49.00 (N)	79.16 (N)	89.93 (+)	38.14 (N)	59.86 (N)	90.00 (N)
Safflower	39.66 (N)	67.11 (++)	90.00 (+++)	29.66 (N)	49.83 (N)	82.33 (+)
Sorghum	46.31 (N)	83.00 (++)	89.96 (++)	35.11 (N)	61.56 (N)	89.96 (+)

Sclerotial formation

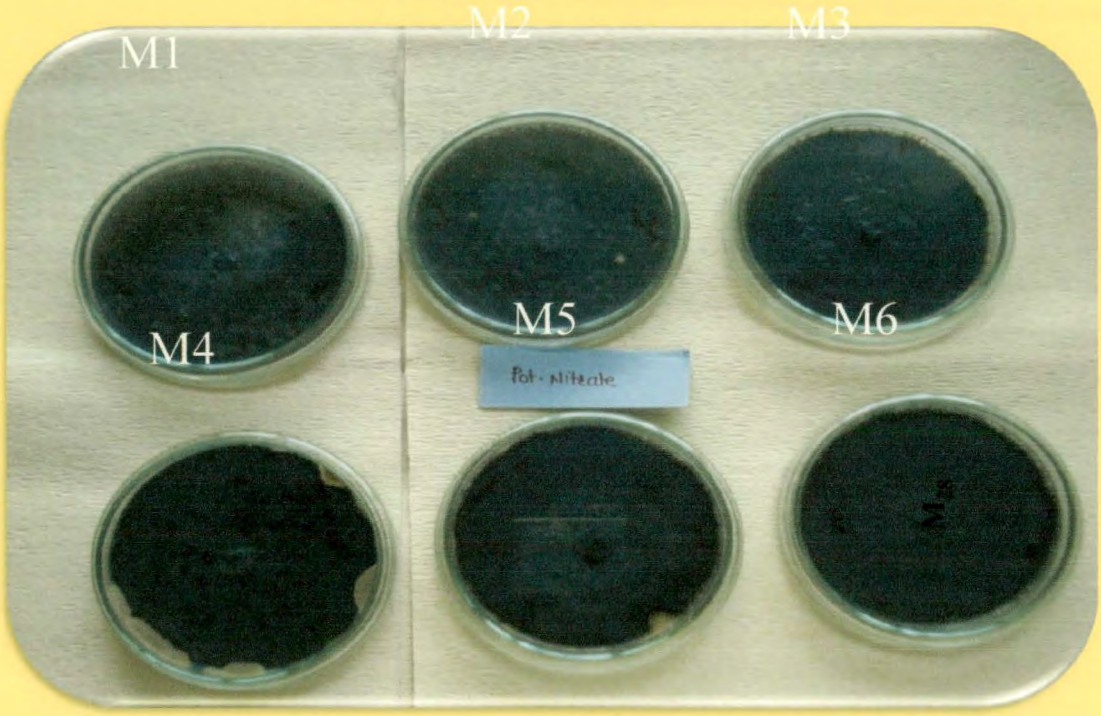
N: No formation

+: Rare (10-20 sclerotia per microscopic field)

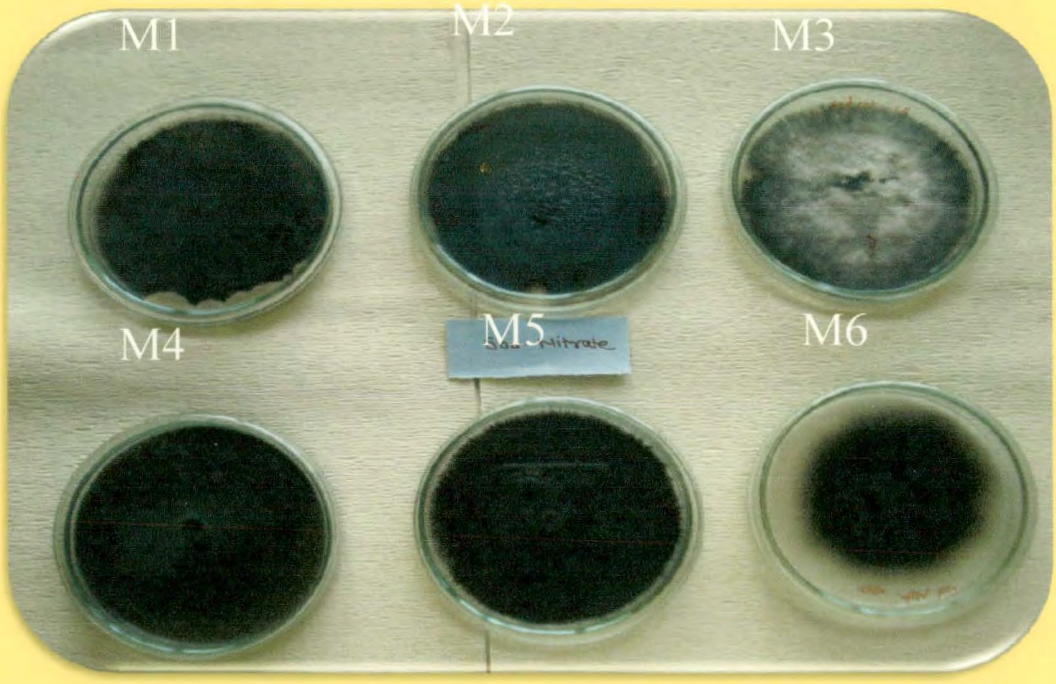
++: Medium (21-30 sclerotia per microscopic field)

+++: Abundant(above 30 sclerotia per microscopic field)

PLATE VIII



Sodium nitrate



Potassium nitrate

Effect of different nitrogen sources on growth of *M.phaseolina.* (7DAI)

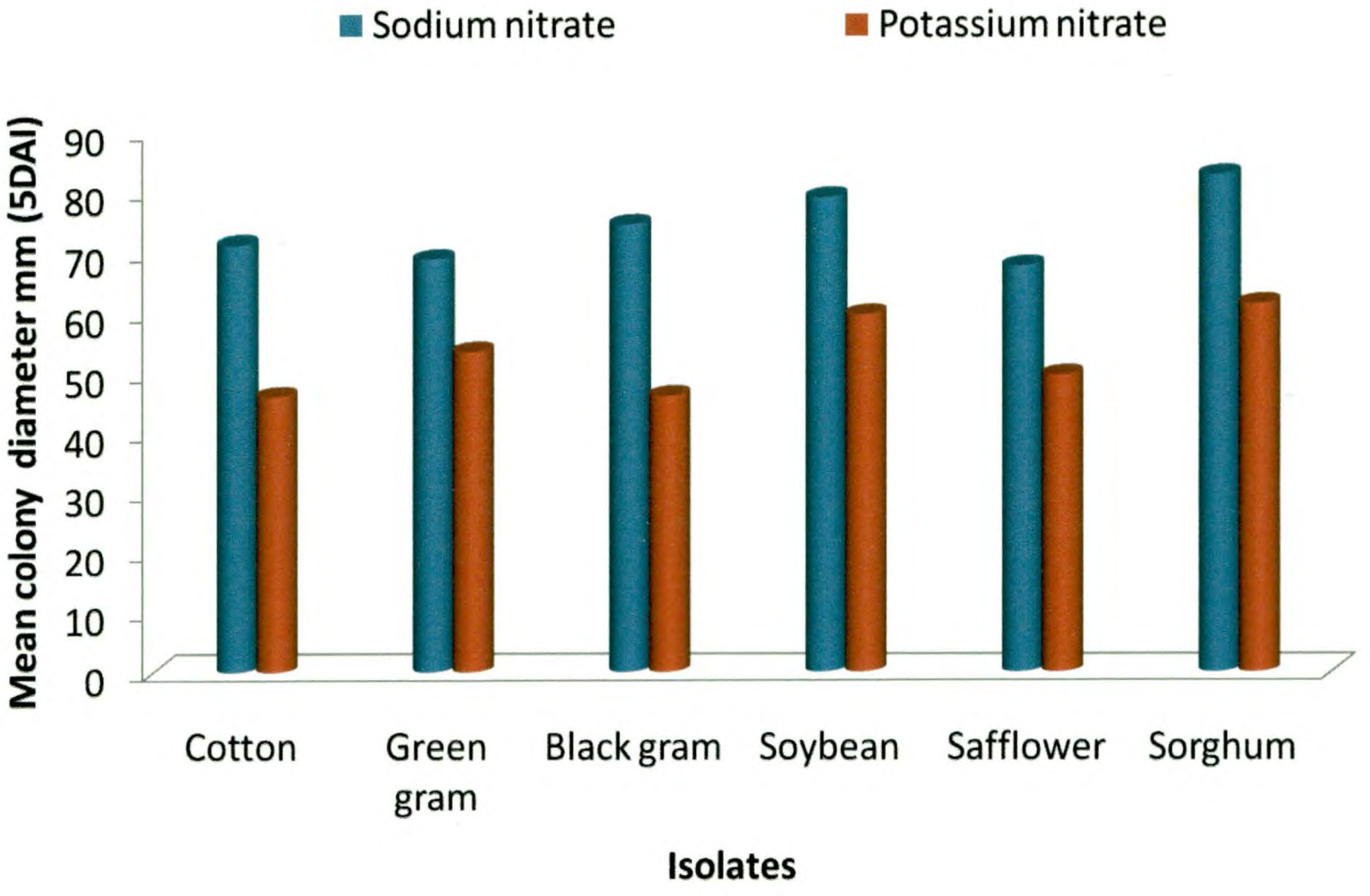


Fig. 4: Effect of nitrogen sources on growth (mm) of *Macrophomina phaseolina*

4.7 Physiological study

4.7.1 Effect of temperature

Environmental factors play an important role in the development of any disease. Among the environmental factors temperature plays an important role on the development of disease. Considering the importance of temperature the present study was conducted.

Results (Table 10, Fig.5) indicated that on third day of incubation all the isolates of *M. phaseolina* were able to grow at temperature range of 20°C to 40°C, although the maximum growth was observed at 35°C and minimum at 20°C. Mycelial growth rate increased as temperature incrased from 20°C to 35°C it ceased at 40°C.

There was a significant difference in radial growth among the isolates at all temperature on third day of incubation but on seventh day radial growth of all isolates was maximum (90 mm), except at 20°C.

Table 10 Effect of temperature on radial growth (mm) and sclerotial production of *M.phaseolina* isolates.

Isolates from	Cotton			Green gram			Black gram		
Temperature (^{0}C)	3DAI	5DAI	7DAI	3DAI	5DAI	7DAI	3DAI	5DAI	7DAI
20	27.33 (N)	56.03 (+)	85.63 (+)	27.36 (N)	45.85 (+)	67.00 (+)	22.56 (N)	52.40 (+)	73.90 (+)
25	36.66 (+)	76.66 (++)	90.00 (+++)	37.33 (+)	67.66 (++)	90.00 (+++)	42.40 (+)	64.00 (++)	84.40 (+++)
30	45.00 (++)	90.00 (+++)	90.00 (+++)	47.36 (++)	90.00 (+++)	90.00 (+++)	54.40 (++)	83.50 (+++)	90.00 (+++)
35	55.26 (+++)	90.00 (+++)	90.00 (+++)	50.00 (+++)	90.00 (+++)	90.00 (+++)	57.33 (+++)	90.00 (+++)	90.00 (+++)
40	42.16 (N)	86.00 (N)	90.00 (+)	46.66 (N)	72.26 (N)	90.00 (+)	50.00 (N)	80.20 (N)	90.00 (+)
F- test	sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE m $\pm$	1.24	0.46	0.35	0.75	0.57	0.51	0.71	0.78	0.51
CD at 0.01%	3.92	1.45	1.10	2.38	1.81	1.62	2.24	2.47	1.62

Table 10 contd....

Isolates from	Soybean			Safflower			Sorghum		
Temperature (^{0}C)	3DAI	5DAI	7DAI	3DAI	5DAI	7DAI	3DAI	5DAI	7DAI
20	28.36 (N)	53.00 (+)	73.66 (++)	33.40 (N)	53.30 (+)	73.03 (++)	23.63 (N)	62.96 (+)	82.03 (+)
25	42.60 (+)	67.33 (++)	89.96 (+++)	43.00 (+)	63.36 (++)	76.60 (+++)	32.33 (+)	72.86 (++)	90.00 (+++)
30	63.33 (++)	83.36 (+++)	90.00 (+++)	52.63 (++)	80.83 (+++)	90.00 (+++)	46.66 (++)	78.70 (++)	90.00 (+++)
35	67.00 (+++)	90.00 (+++)	90.00 (+++)	56.60 (+++)	90.00 (+++)	90.00 (+++)	57.16 (+++)	90.00 (+++)	90.00 (+++)
40	61.66 (N)	80.60 (N)	90.00 (+)(+)	50.30 (N)	80.03 (N)	90.00 (+)	52.02 (N)	83.66 (N)	90.00 (+)
F- test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE m $\pm$	0.73	0.89	0.53	0.73	0.62	2.71	0.62	0.73	0.25
CD at 0.01%	2.31	2.80	1.69	2.32	1.98	8.52	1.95	2.32	0.81

Sclerotial formation

N: No formation

+: Rare (10-20 sclerotia per microscopic field)

++: Medium (21-30 sclerotia per microscopic field)

+++ : Abundant (above 30 sclerotia per microscopic field)

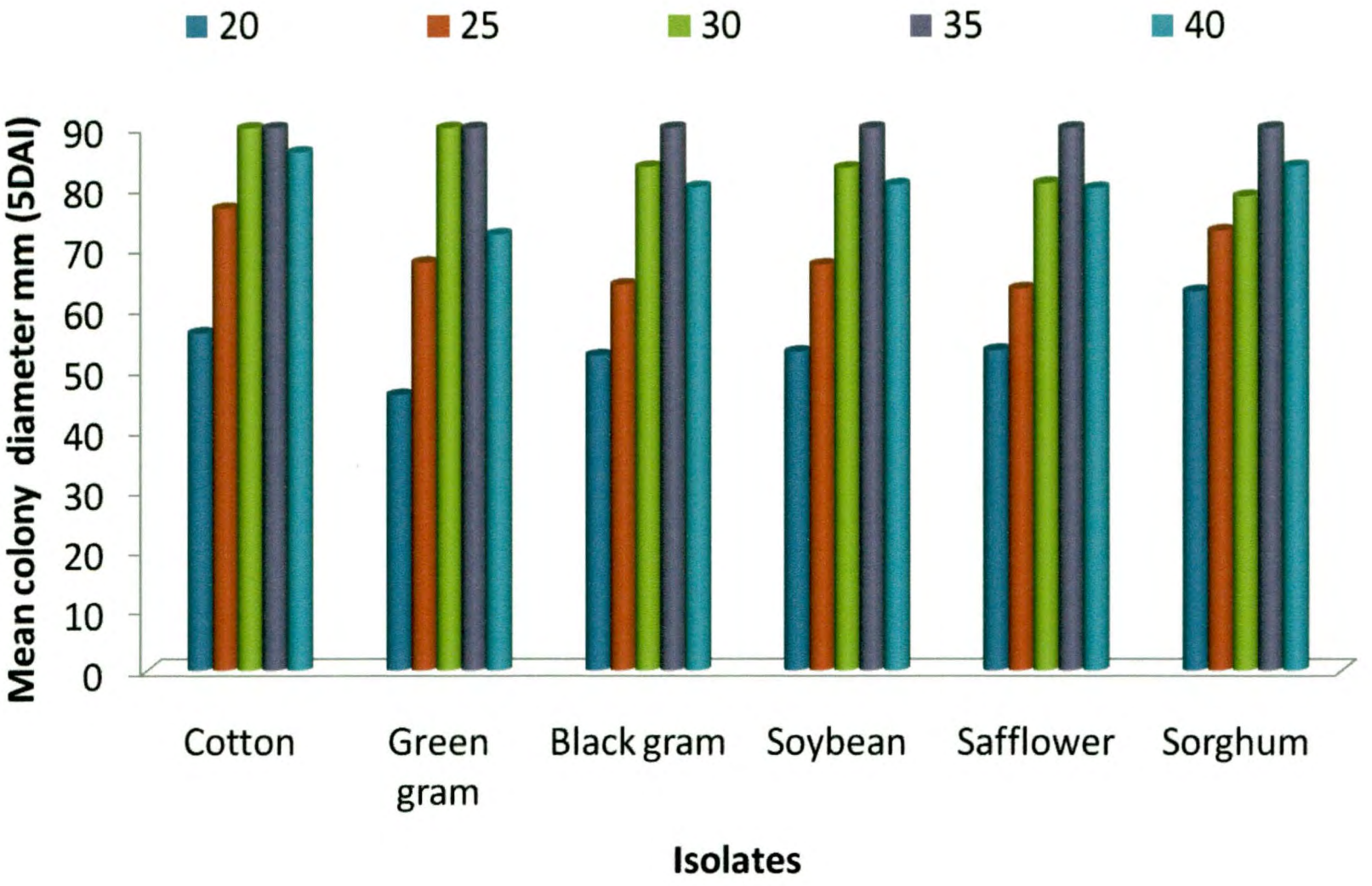


Fig. 5: Effect of temperature on growth (mm) of *Macrophomina phaseolina*

The maximum sclerotial production in most of the isolates was recorded at 35°C, followed by 30°C and no, almost nil at 20°C and 40°C.

4.7.2 Effect of pH

Different pH ranges (5, 6, 7, 8, and 9) were tested for radial growth and sclerotial production of *M. phaseolina* isolates to find out optimum pH supporting good growth and abundant sclerotial formation.

Result (Table 11 and Fig.6) revealed that after three days of inoculation all isolates showed maximum mean colony diameter at pH 7.

As the pH changed from 5 to 7 mycelial growth of all isolates increased slightly and was maximum at pH 7. As pH ranges from 7 to 9 mycelial growth decreased slightly.

At pH range 5-9 there was no change for sclerotial production of isolates. In all isolates abundant sclerotial production was observed on fifth day of incubation at pH range 5-9.

Table 11 Effect of pH on radial growth (mm) of *M.phaseolina* isolates.

Isolates from	Cotton			Green gram			Black gram		
pH	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**
5	43.66	69.17	87.50	37.86	57.00	88.33	32.73	55.33	89.33
6	50.33	70.33	90.00	38.16	59.33	88.98	33.00	56.00	89.33
7	52.83	71.99	90.00	40.00	60.00	90.00	33.86	56.83	90.00
8	49.00	70.33	90.00	39.00	59.66	87.46	38.10	54.99	89.50
9	42.00	69.36	88.50	37.33	58.00	87.10	35.33	54.30	89.00
F- test	sig	sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE m $\pm$	0.75	0.60	0.40	0.78	1.91	0.57	0.81	0.87	0.41
CD at 0.01%	2.38	1.90	1.26	2.47	6.02	1.81	2.51	2.76	1.31

Table 11 contd.....

Isolates from	Soybean			Safflower			Sorghum		
pH	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**	**3DAI**	**5DAI**	**7DAI**
5	35.00	56.17	88.33	40.17	63.66	89.30	43.17	66.73	89.00
6	36.00	57.33	89.60	41.33	64.33	89.33	47.00	67.10	89.30
7	37.66	58.00	90.00	43.26	64.16	90.00	48.16	68.32	90.00
8	37.30	57.33	87.33	46.36	67.33	89.08	41.30	63.25	90.00
9	34.33	56.03	87.66	45.66	66.38	88.17	45.40	66.83	89.66
F- test	Sig	Sig	Sig	Sig	Sig	Non sig	Sig	Sig	Non Sig
SE m ±	0.70	0.60	0.83	0.73	0.79	0.53	0.67	0.79	0.30
CD at 0.01%	2.20	1.89	2.62	2.31	2.51	1.66	2.11	2.51	0.96

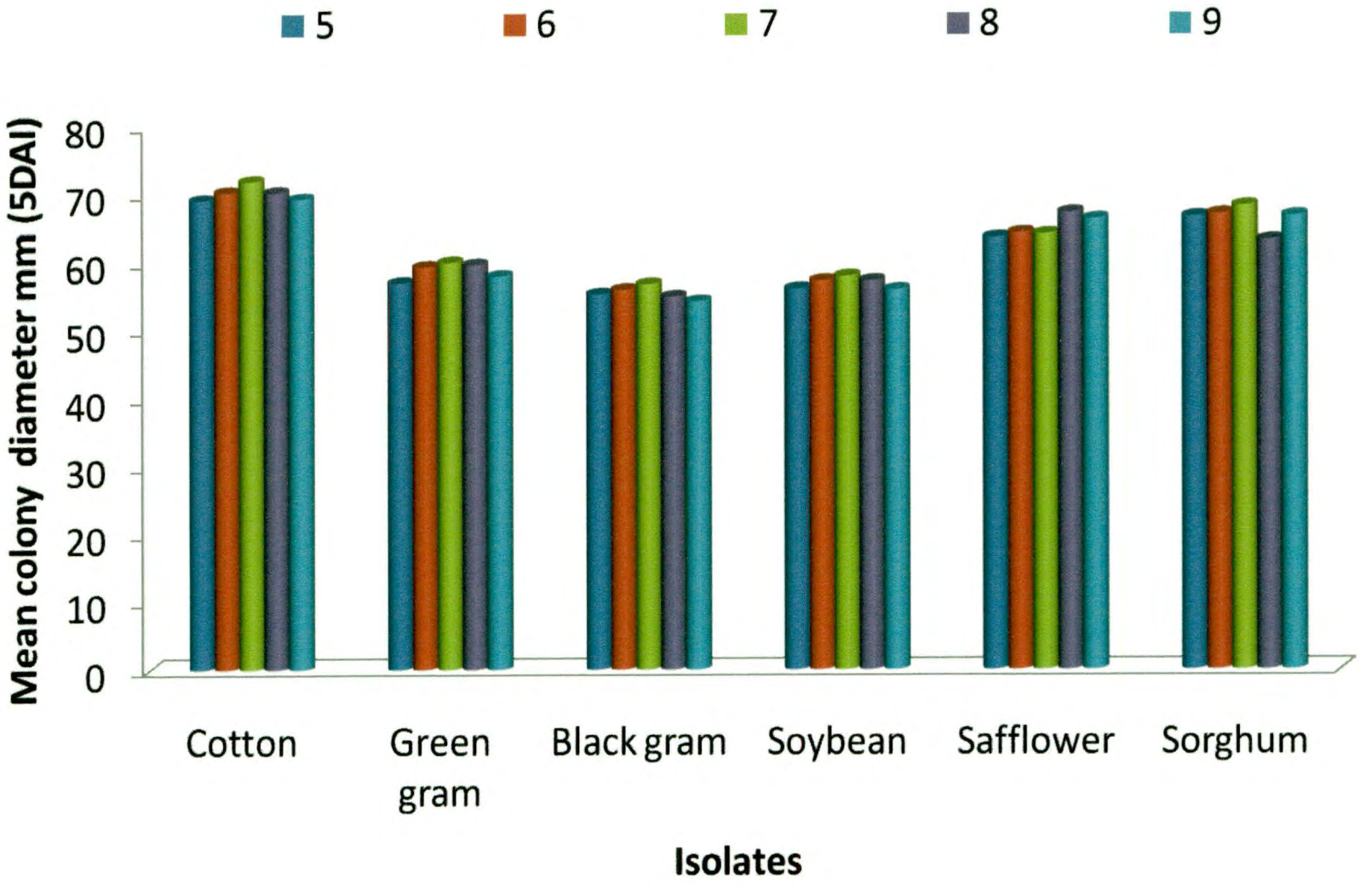


Fig. 6: Effect of pH on growth (mm) of *Macrophomina phaseolina*

4.8 Efficacy of fungicides and bioagent against *M.phaseolina* isolates.

Results (Table 12 and Fig. 7) indicated that all the treatments were significantly superior over control. The treatment T_1 (Carbendazim 0.1%) showed 59.38% to 82.89% disease control in all the hosts crops.

In cotton, treatment T_1 (Carbendazim 0.1%) reduced root rot incidence 21.11% (12.21%) followed by treatment T_7 SAAF (Carbendazim + Mancozeb) (0.2%)24.44% (14.19%) .

In green gram, treatment T_4 Iprodione (0.2 %) showed good control of root rot incidence 24.44% (14.19%) followed by treatment T_1 Carbendazim (0.1%) 28.88% (16.85%).

In black gram, treatment T_1 Carbendazim (0.1%) and (*T. viridae* (5 g/kg) treatment showed 11.99% and 27.99% disease incidence respectively followed by treatment *P. fluroscence* (T_9) (5g/kg) 21.11%.

In soybean, treatment T_8 (*T. viridae* (5 g/kg) showed 21.10% (12.84%) root rot incidence followed by treatment T_1 Carbendazim (0.1%) 22.22% ,Iprodione, *P. fluroscence all are have same value.*

In safflower, minimum incidence was observed in treatment T_1 Carbendazim (0.1%) was 13.33% (7.67%) but at par with Iprodione (T_4) (0.2%) 17.66% .

In sorghum, treatment T_1 Carbendazim (0.1%) showed reduced root rot incidence 17.77% (10.24%) followed by treatment T_8 *T. viride* 22.21 % (22.86%).

Table 12. Efficacy of fungicides and bioagent against *M.phaseolina* isolates.

Fungicide	Cotton		Green gram		Black gram	
	Root rot(%)	Disease control (%)	Root rot(%)	Disease control (%)	Root rot(%)	Disease control (%)
T_1 Carbendazim (0.1%)	21.11 (12.21)	73.60	28.88 (16.85)	59.38	19.99 (11.55)	70.97
T_2 Propiconazole (0.1%)	35.55 (20.87)	55.55	39.99 (23.61)	43.75	28.88 (16.85)	58.07
T_3 Hexaconazole (0.1%)	24.44 (14.02)	69.44	28.88 (16.85)	59.38	27.99 (16.12)	59.36
T_4 Iprodione (0.2%)	23.33 (15.48)	70.83	24.44 (14.19)	65.62	23.33 (15.48)	66.12
T_5 Thiram 75% WP (0.3%)	37.77 (22.30)	52.78	42.22 (25.08)	40.47	31.10 (18.13)	54.84
T_6 Mancozeb (0.025%)	38.88 (22.94)	51.39	44.44 (26.39)	37.49	33.33 (19.50)	51.61
T_7 SAAF (Carbendazim+ Mancozeb) (0.2%)	24.44 (18.26)	61.18	37.77 (18.13)	46.87	27.99 (16.12)	59.36
T_8 *T.viride* (5 g/kg)	31.10 (14.19)	69.44	34.44 (20.16)	51.56	19.99 (11.55)	70.97
T^9 *P. fluroscence* (5g/kg)	26.66 (15.48)	66.67	33.33 (20.91)	53.12	21.11 (12.84)	69.35
T_{10} Control	79.99 (53.44)	--	71.10 (46.24)	--	68.88 (43.72)	--
SE m±	3.31	--	3.39	--	2.19	--
CD at 5%	9.70	--	9.98	--	6.46	--

Table 12 contd.....

Fungicides	Soybean		Safflower		Sorghum	
	Root rot(%)	Disease control (%)	Root rot(%)	Disease control (%)	Root rot(%)	Disease control (%)
T_1 Carbendazim (0.1%)	22.22 (12.88)	70.58	13.33 (7.67)	82.89	17.77 (10.24)	77.78
T_2 Propiconazole (0.1%)	39.99 (23.61)	47.06	37.77 (22.38)	57.43	35.55 (20.87)	55.54
T_3 Hexaconazole (0.1%)	23.33 (13.50)	69.11	21.11 (12.21)	72.85	31.10 (18.26)	61.12
T_4 Iprodione (0.2%)	22.22 (12.88)	70.58	17.66 (10.26)	77.29	28.88 (16.85)	63.89
T_5 Thiram 75% WP (0.3%)	40.00 (23.57)	47.05	39.99 (23.61)	48.57	36.66 (21.57)	54.16
T_6 Mancozeb (0.025%)	42.21 (25.02)	44.12	40.00 (23.57)	48.56	39.99 (23.61)	50.00
T_7 SAAF (Carbendazim+ Mancozeb) (0.2%)	37.77 (22.38)	50.00	35.55 (20.87)	54.28	31.10 (18.26)	61.12
T_8 *T.viride* (5 g/kg)	21.10 (12.84)	72.07	33.33 (19.60)	57.14	22.21 (12.86)	72.23
T_9 *P. fluroscence* (5g/kg)	22.22 (12.86)	70.58	34.44 (20.16)	55.71	24.44 (14.19)	69.44
T_{10} Control	75.55 (49.34)	--	77.77 (55.43)	--	79.99 (53.44)	--
SE m $\pm$	2.99	--	2.98	--	3.43	--
CD at 5%	8.82	--	8.79	--	10.01	--

Figure given in parenthesis shows arcsin value.

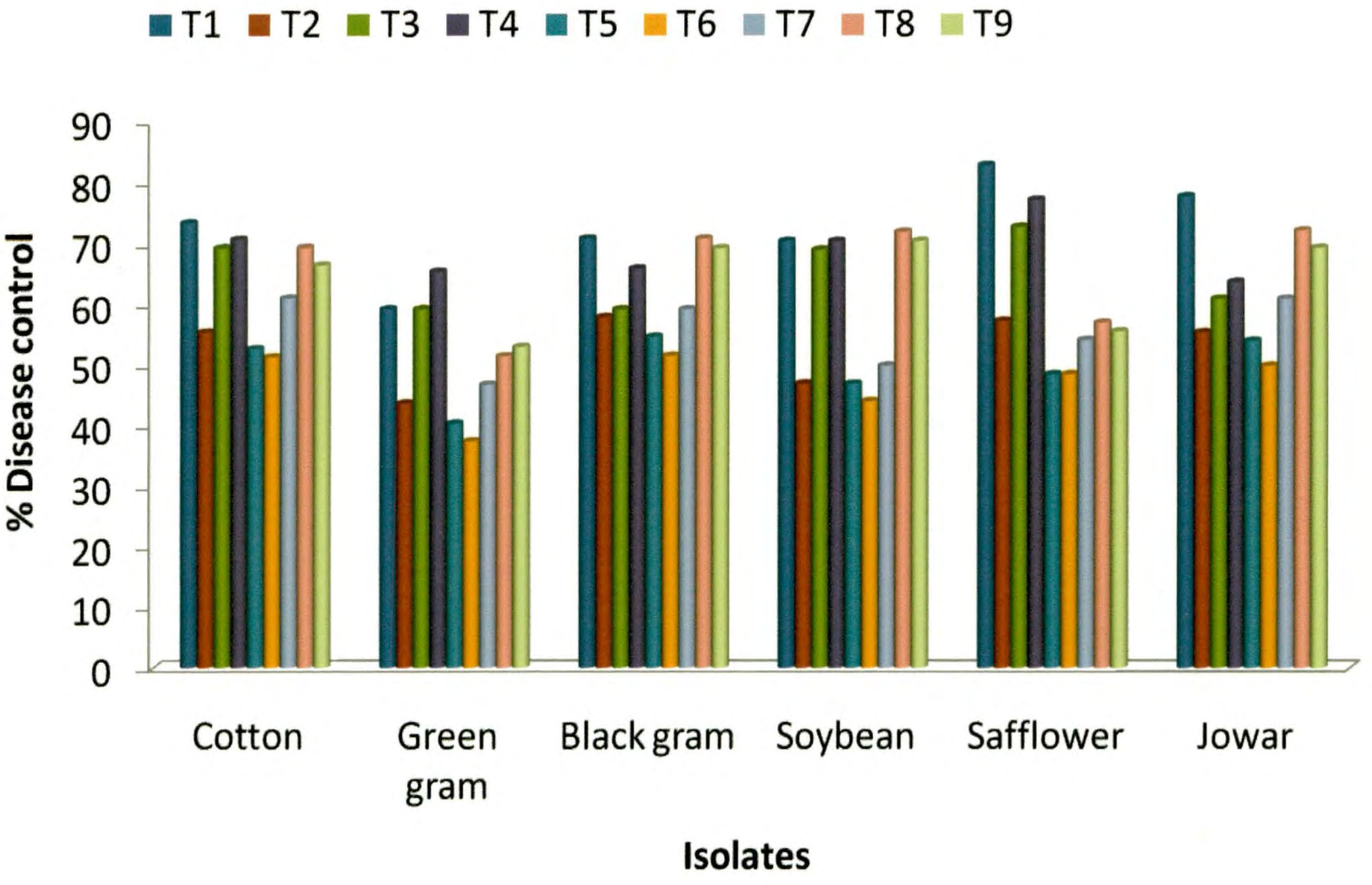
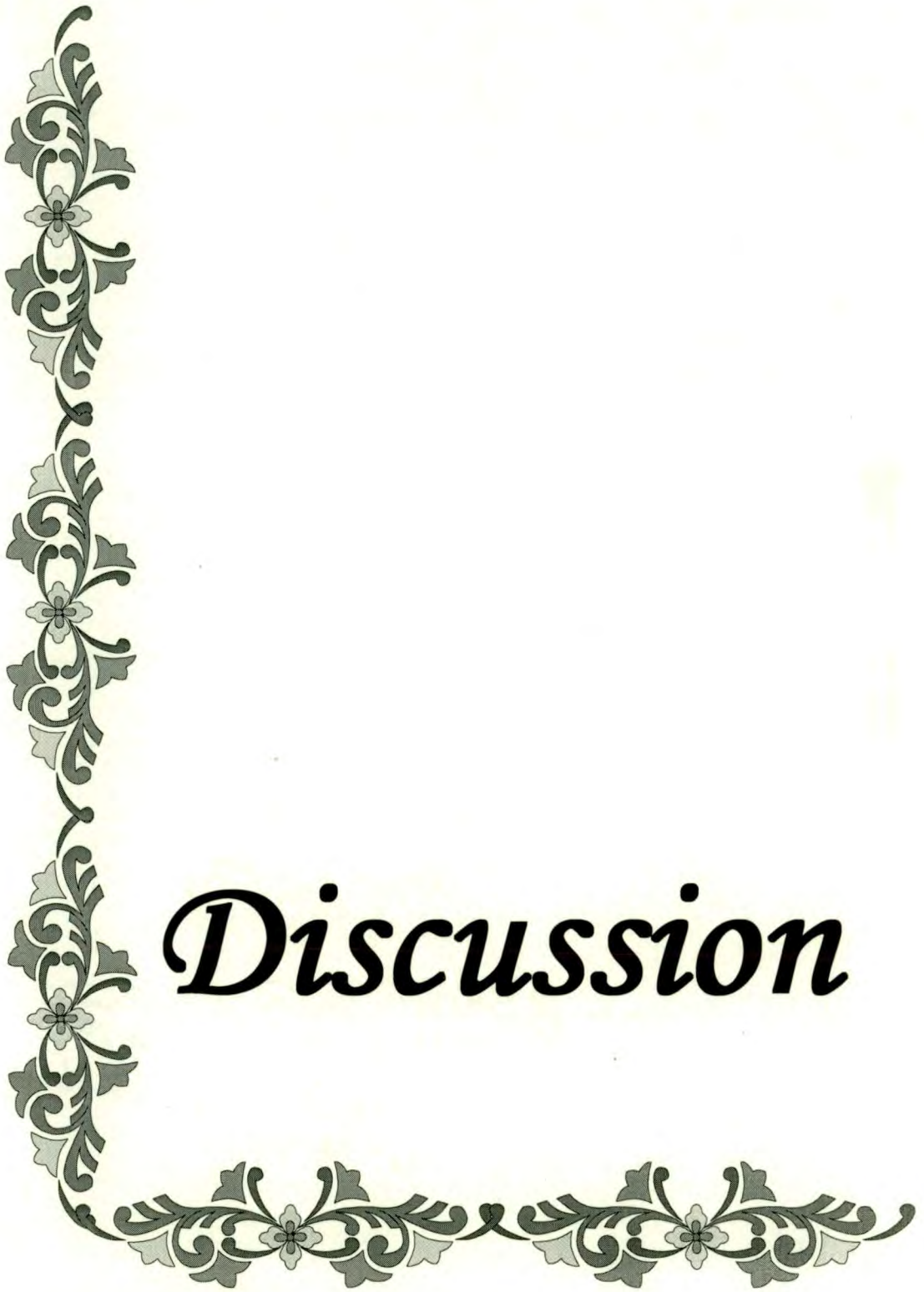


Fig. 7: Effect of fungicides and bioagent on *Macrophomina phaseolina*



Discussion

CHAPTER-V

DISCUSSION

Macrophomina phaseolina is soil borne fungus, which is most destructive, common and omnipresent pathogen with wide host range. The disease syndrome like collar rot, root rot, fruit rot, tuber decay, seed rot, pod rot, stem blight, foliage blight etc. in cereal, pulses, vegetable, oilseed and fruit crops were reported. (Singh and Bowmik 1979).

M. phaseolina affected root / stem samples of six host crops *viz.*, cotton, green gram, black gram, soybean, safflower and Sorghum were collected from various places and subjected to isolation on PDA. All the six isolates of *M. phaseolina* exhibited variations in respect of cultural and morphological characteristics on PDA. Colonies of all isolates of *M. phaseolina* appeared circular on PDA, colony colors varied form whitish to dark black due to age of sclerotial formation. These results are in consonance with Ghosh and Sen (1973), Byadgi and Hegde (1985) and Raut and Ingle (1989), who reported that isolates of *M.phaseolina* from different hosts differ in their morphological and cultural characteristics.

The isolates produced numerous sclerotia on their respective hosts as well as on PDA. These sclerotia were more or less round in shape but few of them were oval to irregular. The mean sclerotial size of cotton isolate was found largest (105.26) µm while smallest, (47.57) µm of soybean. Several workers Jain *et al.* (1972), Ghosh and Sen (1973),Byadgi and Hedge (1985), Olaya and Abawi (1996) and Shekhar

et al. (2000) reported variation in shape and size of sclerotia of *M. phaseolina*.

Pathogenicity of the test fungus was proved in earthen pots by soil inoculation method. Initiation of symptoms of disease was observed 15 days after inoculation. The cotton and safflower isolates were found more virulent showing 90 per cent mortality while green gram less virulent showing 60 per cent mortality black gram, sorghum and soybean showing 70 to 80 per cent morality . Present studies are in agreement with the findings of Bekeshi *et al.* (1970), Hooda and Grower (1988), Devi and Singh (1998), Mehetre (2000), Patil *et al.*(2005) and Tiwari and Khare (2008) was reported variations in pathogenic behavior of *M.phaseolina.*

Cross inoculation studies revealed that safflower and black gram were susceptible to all isolates of *M. phaseolina*. Soybean was non pathogenic to cotton isolate. Cotton isolate was pathogenic only to black gram and safflower. Several earlier workers Kannaiyan and Prasad (1978a),Nayak (1979), Mishra and Sinha (1982), Kaswate (2002) and Salunkhe (2007) studied cross infectivity of *M. phaseolina* on several host and reported that it was non-specific pathogen having wide host range but the reasons for higher aggressiveness on non-hosts were not clear.

In relation to compatability among the six isolates of *M.phaseolina* tested on PDA. Out of them only two combinations were non compatible while one combination was almost near to compatible reaction whereas rests of all were completely compatible. This showed that though the isolate varied in size and shape of sclerotia, most of

them were compatible to each other. The results are in conformity with Raut and Ingle (1990) Manici *et al.,* (1995) who studies compatibility among *M.phaseolina* isolates.

Results revealed that various solid media favoured the sclerotial production and growth of *M. phaseolina.* Among the media tested, PDA was found the best medium for growth and sclerotial production of *M.phaseolina* isolates over other media. All isolates showed fast mycelial growth on PDA followed by Czapeck's dox, Richard's, and peptone agar. Whereas, it was poor on malt extract although on this medium sclerotial formation was medium and peptone agar induced almost no sclerotial formation. These results are in similarity similar with Sahi *et al.* (1992), Singh and Kaiser (1994), Surianchandraselven and Seetharaman (2003), Sharma *et al.*(2004), Bainade *et al.,*(2005) and Salunkhe *et al.*(2009) who reported that PDA as the best culture medium for growth and sclerotial formation of *M.phaseolina.*

Among all carbon sources tested significant highest growth of *M.phaseolina* isolate was supported by dextrose, followed by sucrose, manitol, starch, maltose and cellulose. Sclerotial formation in cellulose was maximum followed by dextrose, sucrose, maltose, starch. In manitol sclerotia formation rate was reduced. Similar results were reported by Diaz Franco (1984) who reported that the best carbohydrate sources for growth were dextrose, fructose, sucrose and galactose. Sclerotia were formed most rapidly on sucrose followed by dextrose. Upmanyu (2009) reported that glucose was best source for maximum growth.

In respect of nitrogen source it was found that sodium nitrate was better source for growth and sclerotial formation of *M.phaseolina* isolates as compared to ammonium nitrate. Result of present investigation were in conformity with Shanmungam and Govindswamy (1973), Khune *et al.,* (1993), Lakhpale *et al.,*(1995) and Upmanyu (2009) who reported that ammonium nitrate as well as potassium nitrate was best source of nitrogen for mass growth.

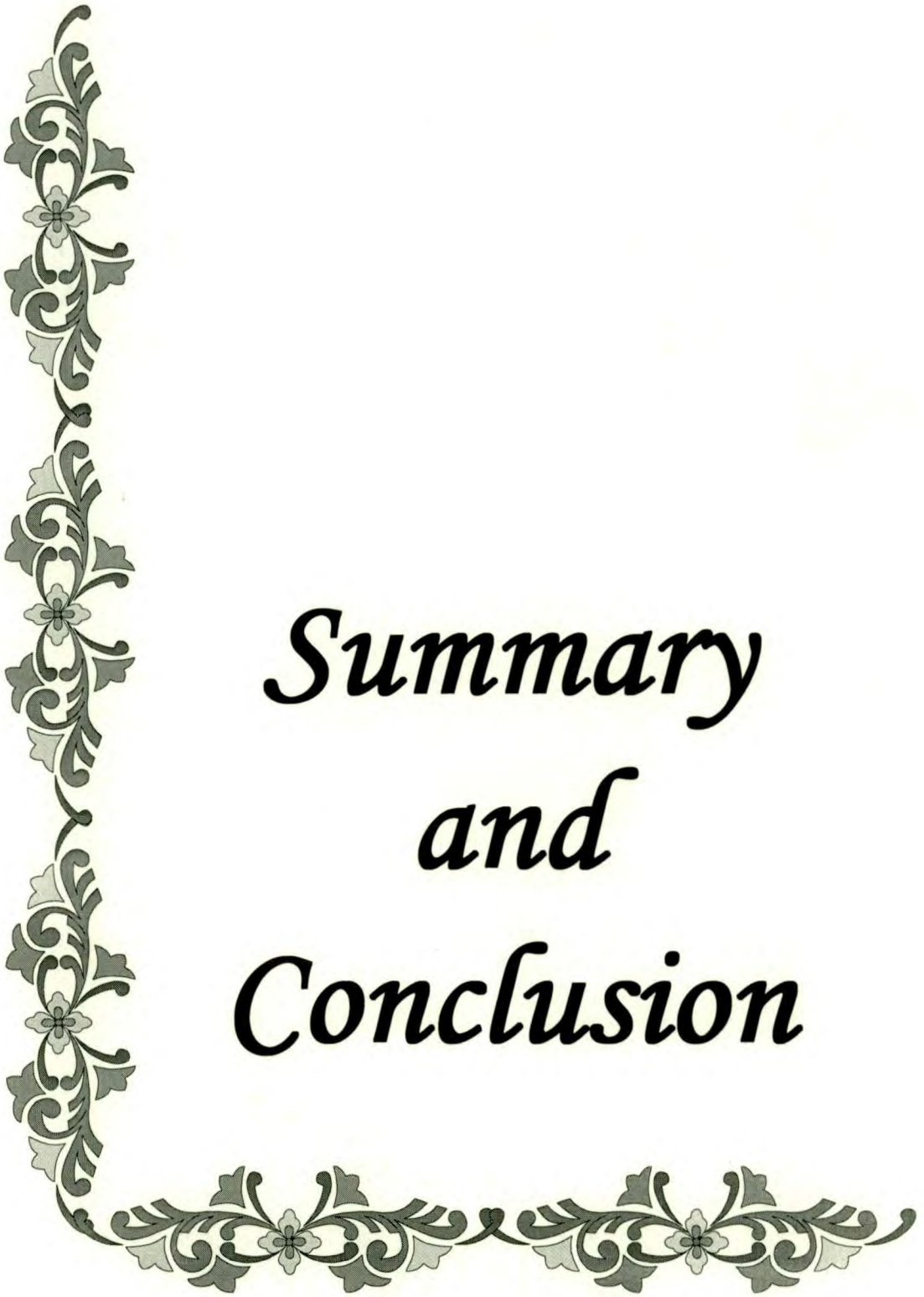
In temperature study 35^0C temperature was found favorable for the growth and sclerotial production of *M.phaseolina* isolates and minimum at 20^0C. No sclerotial production was observed at 40^0C. Similar observation were made by Byadgi and Hegde (1988), Salunkhe (2009) who reported that 35^0C was optimum temperature for the growth and sclerotial production of *M. phaseolina* with decreased mycelial growth at 40^0C.

In pH study in relation to growth and sclerotial production of *M.phaseolina* isolates was found that there were no significant differences in radial growth and sclerotial production in all isolates at pH range of 5 to 9. But at pH 7.0 growth of all isolate were comparatively superior such result were agreements with Jha and Sharma (2005), Bainade *et al.,* (2005) and Salunkhe (2009) reported that at pH 7.0 growth and sclerotial production was maximum.

Use of fungicides and bioagents is one of the measures to manage the diseases. Attempts to manage the disease by use of fungicides and bioagent was made earlier by several workers.

The highest disease control of *M.phaseolina* was observed with Carbendazim followed by Iprodione, Hexaconazole, SAAF

(Carbendazim + Mancozeb), Propaconazole, Thiram and Mancozeb. While bioagents *T.viride* followed by *P.fluroscence* were also found effective against *M. phaseolina.* These findings proved the observation reported by Devi and Singh (1997), Yadav *et al.* (2000), Jahagirdar *et al.* (2001) and Mohanbau and Seethraman (2002) reported that seed treatment with *T.viride* reduced the incidence of root rot caused by *M.phaseolina.*



Summary and Conclusion

CHAPTER-VI

SUMMARY AND CONCLUSION

*Macrophomina phaseolina (*Tassi) Goid cause various diseases in cotton, green gram, black gram, soybean, safflower and sorghum. This disease causes potential yield losses to these crops. The present investigations were undertaken with a view to understand morphological, cultural, physiological, nutritional requirement and management of disease by using fungicides and bioagents.

Diseased samples of cotton, green gram, black gram, soybean, safflower and sorghum yielded a culture of *M. phaseolina* on potato dextrose agar and the pathogen was proved to pathogenic. Cotton and safflower isolates more virulent; whereas, green gram less virulent and rest were intermediate in their virulence.

All isolates showed variations, in morphological and cultural characteristics. Cotton and safflower isolates were more vigorous in growth and sclerotial formation; whereas, green gram was slow in growth and moderate in sclerotial formation. The sclerotia at maturity varied in shape from globose to irregular and black in colour, appearing hairy owing to the emerging black hyphae. The sclerotia of cotton isolate were large (105.27 µm), whereas of soybean were smallest (47.57 µm), other isolate measured between 60 to 90 µm.

In cross inoculation studies black gram and safflower isolates were more aggressive infecting all other hosts; whereas, black gram and safflower were most susceptible host crops to all the isolates.

Compatibility studies showed that only two combinations *viz.,* cotton- black gram and cotton-sorghum were non-compatible with each other, while others were almost compatible or compatible.

Potato dextrose agar and Czapek's dox agar supported excellent growth and sclerotial production of *M. phaseolina*. Sclerotial formation on malt extract medium was moderate with poor mycelial growth while peptone agar induced rare or no sclerotial formation.

Among various carbon sources tested, dextrose was found better for growth and sclerotial production of *M. phaseolina*; followed by sucrose, manitol, starch and cellulose. Mycelial growth rate was poor in cellulose. Cellulose induced maximum sclerotial production in cellulose while manitol induced less sclerotial production. Sodium nitrate proved better nitrogen source for growth and sclerotial formation than ammonium nitrate.

In temperature studies growth of all isolates was recorded highest at 35^0C, while minimum at 20^0C. Sclerotial formation was also better at 30^0C but at 20^0C and 40^0C it was rare or nil. There was no significant difference in radial growth and sclerotial formation in isolates when pH range 5 to 9. Although pH 7.0 was some what superior to other pH values.

Seven fungicides and two bioagents were tested. Among fungicides Carbendazim gave best disease control followed by Iprodione and Hexaconazole and bioagents *T.viride* followed by best *P. fluroscence* were also found antagonistic against *M. phaseolina*.

Conclusion:

From present investigations following conclusions are emerged.

1) Variation in cultural, morphological, physiological and nutritional characters was seen among all six isolates of *M. phaseolina*.

2) Abundant sclerotia were found in cotton and safflower isolates.

3) All isolates showed variation in pathogenic behavior and cross infectivity.

4) Most aggressive isolates of black gram and safflower were found during study.

5) All combinations were almost compatible or compatible with each other except two combination *viz.*, cotton-green gram and cotton-sorghum.

6) Highest colony diameter and sclerotial production were proved on potato dextrose agar media.

7) Dextrose as carbon source and sodium nitrate as nitrogen source were the best for growth of *M. phaseolina.*

8) The optimum pH 7.0 and 35^0C temperature recorded maximum growth, and sclerotial production.

9) Carbendazim and Iprodione among fungicides achieved 59.38-82.89 % and 63.89-77.29 % respectively disease control.

10) *T.viride* controls the root rot incidence up to 72.23%.

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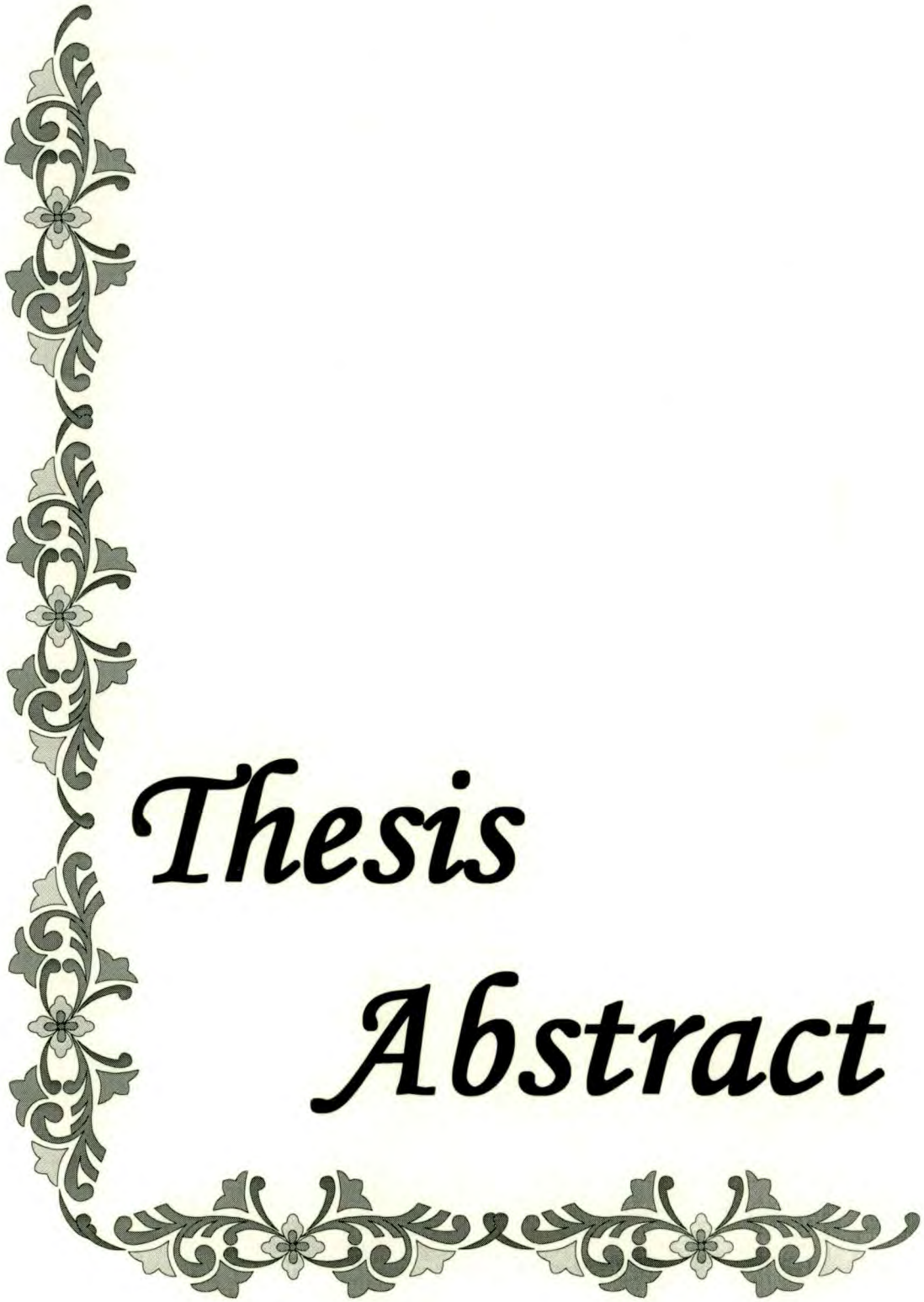


Thesis Abstract

STUDIES ON *Macrophomina phaseolina* (Tassi) Goid.

Students Name : **Patil V.D.** Reg. No. : 2009A/104M
Research Guide : **Dr. K.D. Navgire**
Designation : Assistant Seed Research Officer,
Seed Technology Research Unit, M.K.V., Parbhani

ABSTRACT

The fungus *M.phaseolina* (Tassi) Goid cuased complex disease syndromes like charcoal rot of stem, root rot, seedling blight, foliage blight tuber decay, dry rot, fruit rot, pod and seed rot in several economically important crops.

Six isolates of *M. phaseolina* were obtained from various cultivated crops *viz,* cotton, greengram, blackgram, soybean, safflower and sorghum. The isolates varied in their growth rate, sclerotial size and shape.

Cotton and safflower isolates were found more vigorous in growth and sclerotial formation. Cotton isolate produced largest sclerotial (105.26 µm) while soybean smallest (47.57 µm).

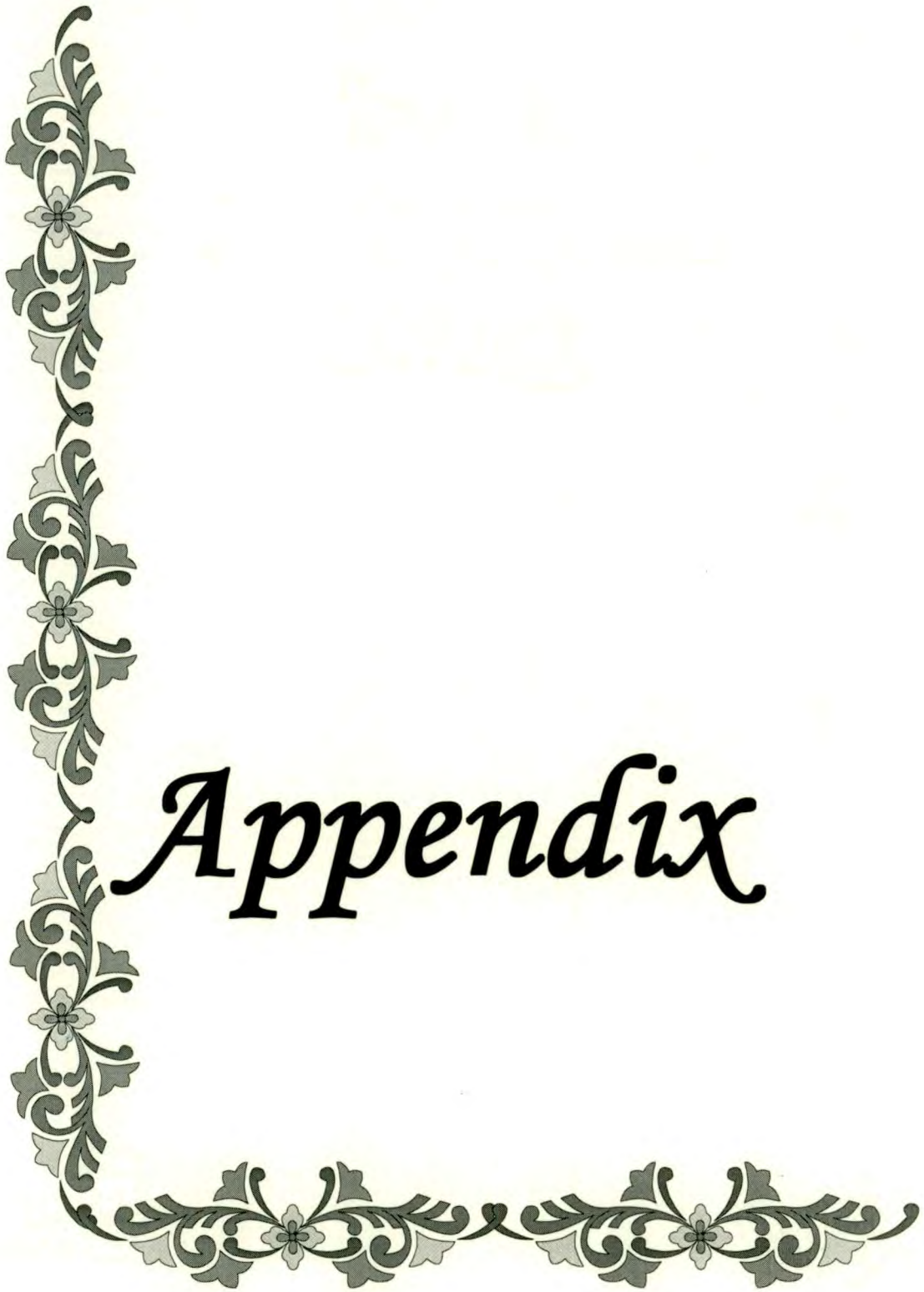
The variation in pathogenic behavior of isolates was also observed. Cotton and safflower isolates were found more virulent. Except cotton, all isolates showed cross infectivity on at least five alternate hosts.

As regard to compatibility only two combinations *viz.,* cotton-black gram and cotton-sorghum were non compatible and rest of all combinations showed almost compatible or compatible reaction.

PDA was proved to be the best medium for growth and sclerotial production of isolates. Dextrose was the best carbon source for mycelial growth followed by sucrose, manitol, starch, maltose and cellulose. Maximum sclerotial productions were recorded in cellulose followed by dextrose, maltose, starch, sucrose and manitol. Sodium nitrate was found better source of nitrogen compared to ammonium nitrate.

The optimum 35°C temperature and neutral pH was best for growth and sclerotial production of *M. Phaseolina* .

Out of seven fungicides and bioagents tested Carbendazim (0.1%) give best control followed by Iprodione (0.2%) and Hexaconazole (0.2%) and in bioagents *Trichoderma viride* was best than the *Pseudomonas fluorescens* to control *M. Phaseonila.*



Appendix

APPENDIX

Sr.No.	Name of media	Quantity/litre
A)	**Potato Dextrose Agar (PDA) media**	
i)	Peeled potato	200g
ii)	Dextrose	20 g
iii)	Agar	20g
iv)	Distiller water	1000ml
B)	**Malt Extract media**	
i)	Malt extract	25g
ii)	Agar	20g
iii)	Distilled water	1000ml
C)	**Czapek's box media**	
i)	Sodium nitrate ($NaNO_3$)	2g
ii)	Dipotassium hydrogen phosphate (K_2HP_4)	1g
iii)	Magnesium sulphate (Mg So_4 $7H_2O$)	0.5g
iv)	Potassium chloride ($FeSO_4$)	0.5g
v)	Ferrous sulphate ($FeSO_4$)	0.01g
vi)	Sucrose ($C_{12}H_{22}O_{11}$)	30g
vii)	Agar	20g
viii)	Distilled water	1000ml
D)	**Richard's media**	
i)	Potassium nitrate (KNO_3)	10g

ii)	Potassium dihydrogen phosphate (KH_2PO_4)	5g
iii)	Magnesium chloride ($FeCL_3$)	0.5g
iv)	Sucrose ($C_{12}H_{22}O_{11}$)	50g
v)	Distilled water	1000g