

**STUDIES ON ROOT ROT OF SAFFLOWER
INCITED BY *Macrophomina phaseolina*
(Tassi) Goid**

BY
ADSUL AMARCHAND KESHAV
B.Sc.(Agri.)

T5944

DISSERTATION
Submitted to
the Marathwada Agricultural University
in partial fulfilment of the
requirement for the Degree of

MASTER OF SCIENCE
(Agriculture)
IN
PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY
MARATHWADA AGRICULTURAL UNIVERSITY,
PARBHANI 431 402 (M.S.), INDIA.

2010

A black and white photograph of a person's hands holding a book. The person's face is partially visible in the background, looking down at the book. In the foreground, there is a plant with long, thin leaves, possibly a fern or a similar foliage. The text is overlaid on the image in a white, elegant script font.

*Affectionately
Dedicated
To
My Beloved
Parents, Father,
Mother, Brothers
, Sisters, Grandmother,
&
Research Guide*

P3909-TH 5944

Candidate's Declaration

I hereby declare that this dissertation or a

part there of has not been previously

submitted by me for a degree,

diploma or distinction of

any University or

Institution.

Place : PARBHANI

Date : 27 / 04 / 2010


(ADSUL A.K.)

Prof. P.V. Khalikar
Associate Professor
Dept. of Plant Pathology
Marathwada Agricultural University,
Parbhani - 431 402 (M.S.).

CERTIFICATE-I

This is to certify that the dissertation entitled "**STUDIES ON ROOT ROT OF SAFFLOWER INCITED BY *Macrophomina phaseolina* (Tassi) Goid**" submitted by **ADSUL AMARCHAND KESHAV** to the Marathwada Agricultural University, Parbhani in partial fulfilment of the requirement for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **PLANT PATHOLOGY** is record of original and bonafide 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 the dissertation or part thereof has not been previously submitted for a degree of any university.

Place : PARBHANI
Date : 27/08/2010

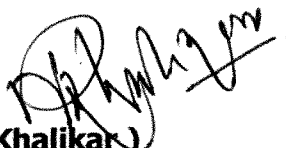

(P.V. Khalikar)
Research Guide

CERTIFICATE-II

This is to certify that the dissertation entitled "**STUDIES ON ROOT ROT OF SAFFLOWER INCITED BY *Macrophomina phaseolina* (Tassi) Goid**" submitted by **ADSUL AMARCHAND KESHAV** to the Marathwada Agricultural University, Parbhani in partial fulfilment 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 *viva-voce* examination in collaboration with the external examiner.

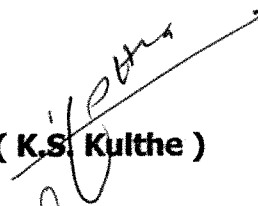

External Examiner
[D.H. DHUTRAJ]

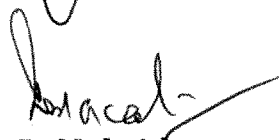
Advisory committee:


(P.V. Khalikar)
Research Guide


(G.D. Deshpande)


(K.D. Navgire)


(K.S. Kulthe)


(R.B. Mokate)


Associate Dean (P.G.)
College of Agriculture
MAU, Parbhani.

ACKNOWLEDGEMENT

“Achievement is of no mean, without the sense of gratefulness and the recognition of this is the beginning of wisdom”.

I think it is the matter of pleasure to glance back and recall the way one traverse, the days of hardwork and perseverance. It is still great at the juncture to recall all the faces and spirits in the form of teachers, friends, near and dear ones. In my opinion, this work is nothing more than incomplete, without attending to the task acknowledging, to overwhelming help I received during this endeavour of mine.

Inspiration is the best medicine can make it possible to run for crippled one and it is veritable gold of mine to get the talented and inspiring willful guidance by a man with enthusiasm and devotion Shri. P.V. Khalikar, Associate Professor, Dept. of Plant Pathology, M.A.U., Parbhani and chairman of my Advisory committee, who in his unique way, provided me with constant encouragement inspiring, scholastic guidance, love and affection offered to me during the course of my study and research. Words would be a poor vehicle to communicate him my sense of gratitude.

I sincerely express my deep sense of gratitude and great indebtedness to the advisory committee members, Dr. G.D. Deshpande, Head, Dept. of Plant Pathology, M.A.U., Parbhani, Dr. K.D. Navgire, Assistant Professor, Dept. of Plant Pathology, M.A.U., Parbhani Dr. K.S. Kulthe Assistant Professor, Dept. of Plant Pathology, M.A.U., Parbhani, Dr. R.B. Mokat, Associate Professor Dept. of Agril. Entomology for their valuable suggestions and constant encouragement.

I owe high esteemed respect to Dr. N.D. Pawar, Associate Dean and Principal, College of Agriculture, M.A.U. Parbhani, Dr. G.D. Deshpande, Head, Department of Plant Pathology for providing necessary facilities during the present investigation.

I wish to place on record my sincere thanks to Dr. G.P. Jagtap, Dr. K.T. Apet, Dr. O.D. Kohire, and other non-teaching staff of Department of

Plant Pathology for their kind co-operation during the completion of my P.G. education.

Everything in my life is nothing if I ignore the all time encouragement, ever green affection and latent love of my father Shri. Keshavrao Yashwantrao Adsul and mother Sau. Sharda Keshavrao Adsul which elevate my morals during the whole educational career. Words are insufficient to take account of encouraging and evergreen love of Grandmother Phulabai Kashinath Ghogre, my brother Vaibhav, Sanju nana, Shridhar, Haripal, Mukund, Umesh, Ranjit, Sundardas, Dhananjay, Digambar and Sister Ashwini, Jayashree, Mandakini, Vandana, Vanita.

One needs sincere friends of major junctures in life to bear strains and fatigue I have been luckier in this respect that Gajanan, Vinod, Suresh, Jagdish, Dhananjay, Nikhil, Sachin and Prakash are the stars of my friend galaxy. Who proved the phrase "friend in need is a friend in deed" did not to say about their love and affection.

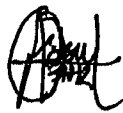
I shall failing my duties if I do not express heartfelt thanks to my all colleagues friends Sudhir, Arjun, Vijay, Sagar, Yogesh, Pramod, Sharad, Anil, Ajay and Vinay for their constant support during the post graduation period.

I take this opportunity to express my heartfelt indebtedness and deepest sense of gratitude to my respected senior Shrikrishna Zagade and Vanita Salunke for their valuable guidance and moral support.

I am especially grateful to Mr. Khaleq and Syed Sajid who carried out the difficult task of analysis and typing the revised manuscript not only swiftly and accurately but cheerfully.

Place : Parbhani

Date : 27/08/2000


(ADSUL A.K.)

CONTENTS

Chapter	Title	Pages
I	INTRODUCTION	1-4
II	REVIEW OF LITERATURE	5-20
III	MATERIALS AND METHODS	21-30
IV	RESULTS	31-61
V	DISCUSSION	62-69
VI	SUMMARY	70-71
	LITERATURE CITED	i-xi
	APPENDIX	I

LIST OF TABLE

Table. No.	Title	Page No.
1	Details of breeding material of safflower screened against <i>Macrophomina phaseolina</i>	27
1a	District wise occurrence of root rot of safflower in Marathwada region	33
1b	Occurrence of root rot disease of safflower in different tahsils of Marathwada region	33-34
1c	Root rot disease on different varieties in Marathwada region	34
2	Pathogenicity test by water culture technique	37
3a	Per cent mortality after hrs of dipping in cultivar Nira	39
3b	Per cent mortality after hrs of dipping in cultivar Mangira	39
3c	Per cent mortality after hrs of dipping in cultivar Parbhani 12	40
3d	Per cent mortality after hrs of dipping in cultivar Sharda	40
4	Pathogenicity test by sick soil technique	42
5a	<i>In vitro</i> inhibition of <i>M. phaseolina</i> by bioagents	44
5b	Mean colony diameter (mm) at 5 days	44
5c	Mean colony diameter (mm) at 7 days	45
5d	Mean colony diameter (mm) at 9 days	45
5e	Per cent reduction in colony diameter of the pathogen over control	45
6	Efficacy of fungicides against <i>M. phaseolina in vitro</i>	47
7	<i>In vitro</i> effect of fungicides on sclerotial production and inhibition of <i>M. phaseolina</i>	48
8	<i>In vitro</i> effect of plant extract on mycelia growth and inhibition of <i>M. phaseolina</i>	49

8a	<i>In vitro</i> effect of plant extracts on sclerotial production	49
9	Effect of soil amendment on germination and mortality of safflower seedling (pot culture)	52
9a	Effect of soil amendment on root rot incidence (pot culture)	53
9b	Effect of soil amendment on growth parameter of safflower (pot culture)	53
10	Effect of micronutrient alongwith RDF on root rot incidence	56
11	Reaction of safflower breeding material to <i>M. phaseolina</i>	58
12	Reaction of safflower advanced varietal material to <i>M. phaseolina</i>	60

LIST OF FIGURES

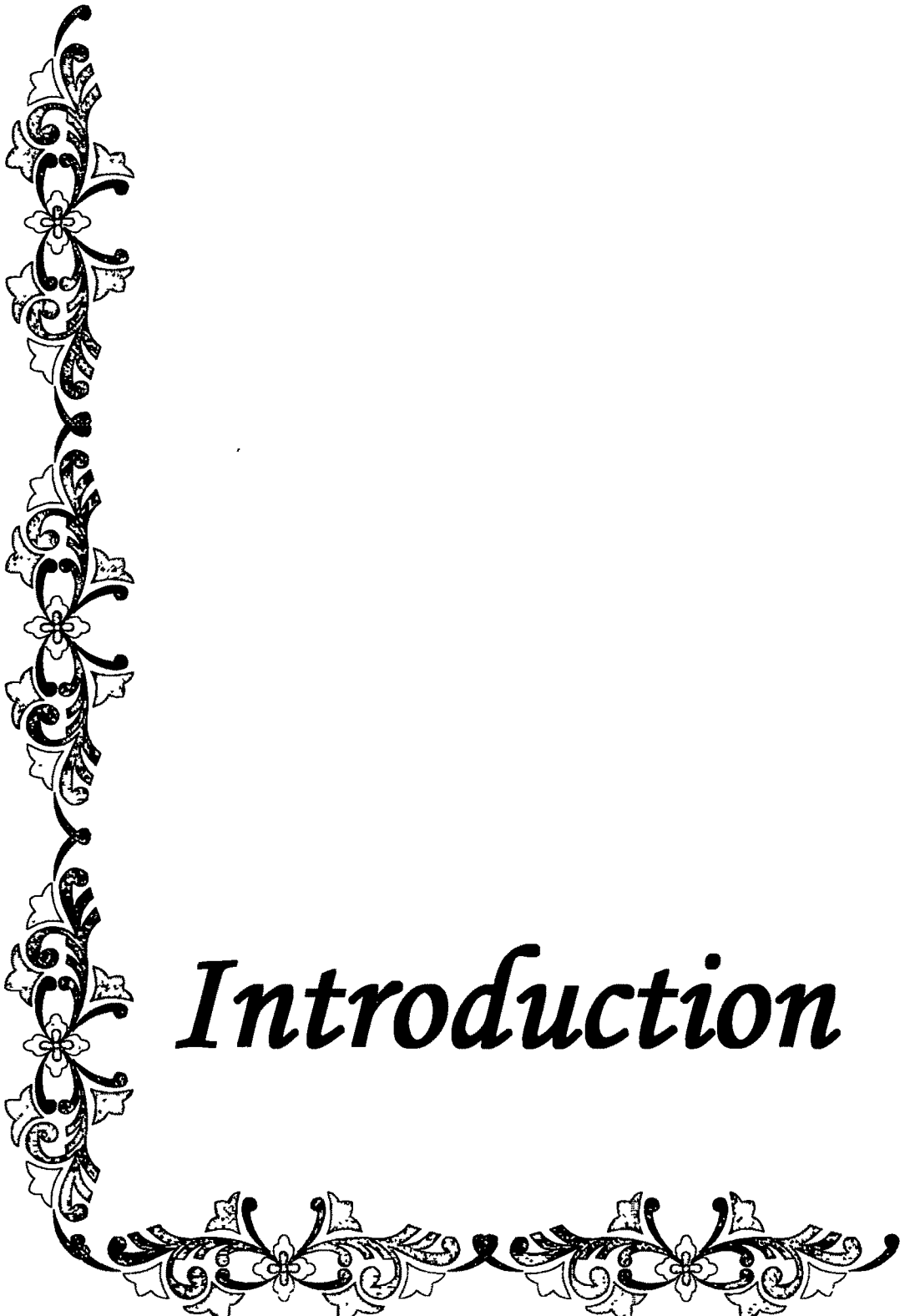
Fig. No.	Title	In between pages
1	Layout of agronomical material effect of micronutrient along with RDF on root rot incidence.	25-26
2	Layout of safflower breeding material against <i>Macrophomina phaseolina</i>	26-27
3	Layout of safflower advanced varietal trials against <i>M. phaseolina</i>	27-28
4	District wise occurrence of root rot of safflower in Marathwada region	33-34
5	Per cent mortality by water culture technique	37-38
6	Pathogenicity test by sick soil technique	42-43
6a	Pathogenicity test by sick soil technique	42-43
7	Per cent reduction in colony diameter of the pathogen over control	45-46
8	Per cent reduction in colony diameter and sclerotial production of the pathogen over control	47-48
9	<i>In vitro</i> effect of plant extracts on mycelial growth and sclerotial production	50-51
10	Reaction of safflower breeding material to <i>M. phaseolina</i>	58-59
11	Reaction of safflower advanced varietal material to <i>M. phaseolina</i>	60-61

LIST OF PLATES

Plate No.	Title	In between pages
1	General field view of safflower experimentation	27-28
2	Root rot symptoms observed under field condition	31-32
3a	Formation of Sclerotia inside the stem tissue	31-32
3b	Formation of Sclerotia inside the stem tissue	31-32
4	Pure culture of <i>Macrophomina phaseolina</i>	34-35
5	Mass multiplication of <i>Macrophomina phaseolina</i> culture	35-36
6a	Sclerotia of <i>Macrophomina phaseolina</i>	35-36
6b	<i>Pycnidiospores of Macrophomina phaseolina</i>	35-36
7a	Pathogenicity test by water culture method in Cv. Nira	37-38
7b	Pathogenicity test by water culture method in Cv. Manjira	37-38
8a	Pathogenicity test by water culture method in Cv. Parbhani-12	37-38
8b	Pathogenicity test by water culture method in Cv. Sharda	37-38
9	Pathogenicity test by sick soil technique	40-41
10	<i>In vitro</i> evaluation of fungicide	45-46

ABBREVIATIONS

%	- Per cent
/	- Per
@	- At the rate of
°C	- Degree centigrade
C.D.	- Critical difference
cfu/ml	- Colony forming unit/milliliter
cm	- centimeter
Cv.	- cultivar
DAI	- Days after inoculation
DAS	- Days after sowing
Dia	- diameter
e.g.	- <i>Exempli gratia</i> (for example)
<i>et al.</i>	- <i>et alia</i> (and his association)
etc.	- etcetera
fig.	- Figure
g	- Gram
ha	- hectare
i.e.	- That is
lb	- pounds
MAU	- Marathwada Agricultural University
mg	- Milligram
Mm	- Millimeters
N.S.	- Non significant
No.	- Number
PDA	- Potato dextrose agar
ppm	- Parts per million
RH	- Relative Humidity
rpm	- revolution per minute
S.E.	- Standard error
spp.	- Species
t	- tonne
Var.	- Variety
<i>viz.</i>	- Namely
w/v	- weight per volume
WP	- wettable powder
wt.	- weight

A decorative L-shaped border in the bottom-left corner, composed of repeating floral and scrollwork motifs.

Introduction

CHAPTER-1

INTRODUCTION

India has the unique distinction to present one of the largest vegetable oil economy in the world. Oilseed crops have been the backbone of agricultural economy of India from time immemorial. It is anticipated that the demand for edible oil and oil seeds by 2009-2010 would be 19.0 million tonnes and 56 million tonnes respectively, if per capita income grows by 5 per cent. This challenging demand has to be met by increasing the production of seven annual edible oil crops viz., rapeseed and mustard, groundnut, soybean, sunflower, safflower, sesame and niger (Sengupta and Das, 2003).

Safflower (*Carthamus tinctorius* L.) is one of the important oilseed crops of the world valued for its highly nutritious edible oil. It belongs to family *Compositae* and believed to be native of Afganistan and Abyssinia. The word *Carthamus* is arabic word *quartum* (means the colour of dye obtained from florets). It is described as “Kusumbha” in ancient Sanskrit literature. Other Indian names, like Kusum, Karrad (Hindi), Kusumpuli (Bengali), Kusumbo (Gujrathi), Kardi, Kurdi (Marathi), Sendurakam (Tamil), Kusuma (Telgu), Kusube, Kusume (Kannada), Kusumba (Punjabi) seem to have been derived from “Kusumbha”. Presently the most common name being “Kusum” or “Kardi”. Safflower plant is much branched herbaceous annual plant. An individual flowering head composed of 20 to 100 florets terminals each branch. The head are protected by several series of involucral bracts. The plant usually flowers

over a 40 days period depending upon temperature, date of planting and humidity in the environment. Out crossing to the extent of 5 to 40% was found in safflower depending upon genotype and insect activity. Honeybee (*Apis indica* Fab) is the major pollinator. Pollen and nectar secreted at the base of filaments are highly attractive to pollinators (Anonymous, 2006).

It is multipurpose crop having various uses like source of edible oil, cattle feed, medicinal and industrial products. It is used in preparation of Ayurvedic medicines, colour dyes in India. Safflower medicines are used for menstrual problems, cardiovascular diseases, pain and swelling associated with trauma. In domestic practice, these flowers are used in children and infant's complaints: measles, fevers and eruptive skin problems. Suitably dried flowers are useful to prepare infusions that are helpful in the circulatory system for inflammation and muscular fatigue. The safflower plant also makes an acceptable livestock forage in times of scarcity, it is cut at or just after bloom stage. It can also be used after converting into silage (Anonymous, 2003)

Undecorticated safflower cake is generally used as manure where as decorticated safflower cake is fed to ruminants and monogastric animals. Safflower as a bird food ingredient has also gained growing acceptance in recent years. It is used as poultry feed after fortification with lysine and methionine. Decorticated safflower meal has the potential to be utilized as human food if the bitter principles and phenolics are removed. Hulls can be used in the manufacture of cellulose, insulations, abrasions, hard boards and as fuel. Safflower leaves are rich in carotene, riboflavin and vitamin C and hence young seedlings, thinnings, prunings are used as

green vegetable. The seeds are parched and eaten either mixed with wheat or its flour or alone. Specialty traditional dishes are prepared using safflower florets. Flowers are also used in the preparation of cosmetics. The flowers are laxative and diaphoretic (Anonymous, 2006).

The safflower crop is grown in India in *rabi* or winter season i.e. from October / November to March/April on all types of soil including sandy soil but crop is best suited to deep, well drained fertile soil with high water holding capacity and neutral pH. It is primarily grown as mixed or sole crop under rainfed condition but in some area it is grown under irrigation.

The important safflower growing countries are: India, California, Mexico, USA, Argentina, Canada, China, Spain, Italy, Turkey, Iraq, Iran, Egypt, Ethiopia and Sudan. In India it is mainly grown in Maharashtra, Karnataka, and part of Andhra Pradesh, Madhya Pradesh, Orissa and Bihar. In Maharashtra it is mainly grown in Solapur, Pune, Ahmednagar, Latur, Osmanabad, Parbhani, Hingoli and Jalna districts.

Economically India occupies first position in hectareage followed by USA. Recent statistics shown that safflower occupied 771.5 thousand ha area in India with production of 418.8 thousand tonnes with average productivity of 465 kg/ha. Maharashtra ranks first in area and production accounting as 67% and 63%, respectively, in India (Anonymous, 2006). In India diseases play an important role in safflower cultivation and responsible to cause 25-60 % yield losses every year.

Some of the important diseases of safflower occurring in India (Bhale *et al*, 1998) are listed below.

A) Fungal diseases

1. Root rot [*Macrophomina Phaseolina* Tassi (Goid)]

2. Leaf spot/blight (*Alternaria carthami*)
3. Wilt (*Fusarium oxysporum* f. sp. *carthami*)
4. Powdery mildew (*Erysiphe cichoracearum* DC)
5. Anthracnose (*Colletotrichum capsici*)

B) Bacteria

1. Leaf blight/spot (*Pseudomonas syringae* Van Hall)

C) Viral diseases

1. Mosaic (Cucumber mosaic virus)
2. Necrosis (Tobacco streak virus)

D) Nematode diseases

Root knot (*Meloidogyne hapla*)

Amongst the diseases, *Macrophomina phaseolina* Tassi (Goid) is a serious and commonly occurring soil born disease. The fungus produces stem splitting symptoms on plant.

The present investigation is undertaken considering the economic importance of the *Macrophomina phaseolina* Tassi (Goid) in Maharashtra with following objectives.

Objectives

- 1) Survey and surveillance for root rot disease of safflower in Marathwada region.
- 2) Isolation and purification of pathogen.
- 3) To prove the Koch's postulate.
- 4) *In vitro* inhibition of pathogen by using bio-agents, chemicals, plant extracts and soil amendments.
- 5) Field screening of available safflower germplasm under natural epiphytotic conditions.



*Review of
Literature*



CHAPTER-2

REVIEW OF LITERATURE

2.1 Survey and distribution of pathogen

Uppal (1931) reported *Macrophomina phaseolina* (*R. bataticola*) for the first time in India on Jowar, where it was responsible for seedling blight and hollow stem.

Hillderbrand *et al.*, (1945) observed that the *Macrophomina phaseolina* causes stem rot, root rot, fruit rot, foliage blight, tuber decay, pod and seed rot. In the absence of host plant, the pathogen survives predominantly as small black sclerotia in diseased plant parts. The fungus is highly variable in both pathogenicity and morphological characteristics.

Moniz (1963) reported root rot of cotton in Maharashtra and Gujarat state caused by *M. phaseoli* (Maubl) Ashby.

Dingra and Sinclair (1978) reported that *Macrophomina phaseolina* causes charcoal rot disease on a wide variety of hosts in the arid and semi arid areas of world.

Kore and Deshmukh (1979) concluded that root rot caused by *Rhizoctonia bataticola* is a very devastating disease of safflower causing heavy reduction in yield.

Saksena (1979) observed that *Rhizoctonia bataticola* (*Macrophomina phaseolina*) is the most wide spread destructive and

important plant pathogen. Disease caused by *Macrophomina phaseolina* have received great attention all over the world, because earlier, the pathogen was causing only root rot, collar rot, stalk rot etc. Now it is known to attack different aerial plant parts to cause stalk rot, leaf spot and blights.

Garg and Chauhan (1981) reported the occurrence of *Macrophomina phaseolina* in the onion stored bulbs for the first time from India during the survey of market diseases of onion. *M. phaseolina* was isolated from decayed strawberry roots for the first time in Egypt (Madkour and Aly, 1981).

Carta and Franceschini (1982) reported *Macrophomina phaseolina* for the first time on safflower in Italy.

Shambharkar and Indi (1987) reported that root rot is one of the major soil-borne disease of safflower and appears sporadically all over country.

Sandhu (1999) observed that disease development is favoured by high temperature followed by moisture stress.

Suriachandraselvan and Seetharaman (2000a) reported that survival of *Macrophomina phaseolina* causing charcoal rot of sunflower in soil seed and infected plant debris. The pathogen survived for 13 and 10 months in sterilized and unsterilized soil respectively. The pathogen persisted upto 39 months in the in tested seeds under ambient conditions.

Govindappa *et al.*, (2005) observed that new stem splitting symptoms in *Macrophomina phaseolina* infected plants of safflower. Stem split was observed in 30 days old safflower plants as minute crack 2-3 cm above the soil surface.

Suriachandraselvan *et al.*, (2005) reported that *Macrophomina phaseolina* isolated from sunflower infected all the 22 plants tested.

2.2 Media requirement

Uppal *et al.* (1936) reported that two isolates: Broach and Mohol from jowar causing charcoal rot, grew very well in Mayer's liquid medium forming abundant sclerotial bodies. Mean sclerotial diameter was 130 and 110 μm respectively. Potato dextrose agar, corn meal agar and Richard's liquid media also supported good growth and sclerotial formation.

Vasudeva (1936) found that *Rhizoctonia bataticola* from cotton rot gave mycelial growth on Richard's medium and very few sclerotia were formed on this medium.

Patil (1962) studied cultural characters of *Macrophomina phaseolina* isolates from cotton, groundnut, sesame and castor on eight different natural, semisynthetic and synthetic media. He concluded that all the isolates formed fairly good growth on almost all the media except on plain agar. Maximum growth was obtained on PDA.

Wyllie and Fry (1973) studied two strains of *Macrophomina phaseolina* on Czapek's agar medium, which was found to be suitable for the sclerotial formation, whereas PDA was good for the mycelial growth.

Raut and Bhombe (1977) reported that Elliot's medium at 30°C supported the maximum growth of two isolates of *Rhizoctonia bataticola* (*Macrophomina phaseolina*) from jowar.

Gupta and Kolte (1981) studied cultural characteristics of leaf and root isolates of *Macrophomina phaseolina* (Tassi) Goid; from groundnut, on different synthetic and non synthetic media.

Singh and Chohan (1982) obtained best mycelial growth on Czapek-Dox medium with abundant sclerotia on nearly all the twelve media used.

Sahi *et al.* (1992) found that potato dextrose agar was the best medium for the growth of *Macrophomina phaseolina*. The maximum colony diameter and sclerotial formation by this fungus was recorded at 30°C and at pH 6. Richard's agar supported maximum sclerotial production.

Singh and Kaiser (1994) studied growth and cultural characteristics of *Macrophomina phaseolina* on 4 culture media. The pathogen showed abundant growth on Potato Dextrose Agar (PDA), Richard's Agar & Czapek's Dox Agar media. The colour of the mycelium varied from dark brown on PDA and Corn Meal Agar medium to light brown on Richard's Agar and Czapek's Dox Agar medium. The mycelium had a texture like wool on PDA and like cotton on Richhard's Agar, while it was a mixture of the two on Czapek's Dox Agar medium and the mycelia were fully submerged on Corn Meal Agar medium. Sclerotia were produced in all the media tested and there colour varied from black to black brown. Linear growth was maximum on PDA medium.

Rodrigues *et al.* (1997) investigated *in vitro* the effect of culture media on mycelial growth, fertility, pycnidial production and sporulation of *Macrophomina phaseolina*.

Prameela Devi and Singh (1998) stated that all isolates grew best in Richard's medium and produced maximum sclerotia at 30-35 °C.

Bainade *et al.*, (2005) tested growth of *Macrophomina phaseolina* on eleven different soil media, pH levels and temperature. The highest growth of *Macrophomina phaseolina* was observed on mung leaf media followed by soybean seed media and potato dextrose media. The optimum temperature 30-35°C and pH 7 recorded the maximum growth and sclerotial production.

2.3 Pathogenicity test

2.3.1 Pathogenicity by water culture

Many workers isolated *M. phaseolina* from the seed, root and stem portion from variety of crops and proved its pathogenicity either *in vitro* or *in vivo* (Saxena 1971, Mayer *et al.*, 1974, Dhingra and Sinclair, 1978, Chohan, 1979).

Moulin *et al.*, (1994) analyzed 39 hydroponic isolates of *Pythium spp.* from cucumber and tomato plants and noted that *Pythium aphanidermatum*, *Pythium irregulare*, *Pythium sylvaticum* and *Pythium ultimum* were highly pathogenic and in sand peat. But only *Pythium aphanidermatum* strains were pathogenic in soilless conditions.

Suriachandraselvan and Seetharaman (2000a) reported that most virulent isolate maximum mycelial growth was recorded irrespective of the culture broth. Among culture broths Richard's broth (790 mg)

followed by Czapeks dox (752 mg) showed maximum mycelial dry weight.

Suriachandraselvan and Seetharaman (2000b) reported that survival of *Macrophomina phaseolina* causing charcoal rot of sunflower in soil seed and infected plant debris. The pathogen survived for 13 and 10 months in sterilized and unsterilized soil respectively. The pathogen persist upto 39 months in the in tested seeds under ambient conditions.

Suriachandraselvan and Seetharaman (2002) reported that most virulent isolate showed significant faster growth (41.3 mm) on PDA even 24 hours after inoculation, while the least virulent exhibit slowest growth (19.7 mm).

2.3.2 Pathogenicity by sick soil technique

Klisiewicz and Houston (1963) demonstrated pathogenicity of the fungus on safflower by inoculating injured and uninjured roots of plants. Injured roots were inoculated with spore suspension of the fungus while for inoculation with uninjured roots, seeds were planted in sick soil.

Miller and Cooper (1967) suspended one week old seedlings of safflower for five minutes in fungal inoculums of 50,000 conidia per ml and transplanted in sterilized soil wilt symptoms appeared in there plants 4 to 5 weeks later at 85 – 90 °F.

Nirmal (1985) demonstrated pathogenicity of the fungus on the basis of expression of wilt symptoms on safflower plant.

Hooda and Grower (1988) collected 14 isolates of *M. phaseolina* from different plant species and reported that isolates producing

abundant sclerotia in culture were more pathogenic than isolates producing few sclerotia.

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

Devi and Singh (1998) collected *M. phaseolina* isolates of black gram and green gram crops from 11 different localities and categorized them as highly virulent (MP₂ and MP₃), moderately virulent (MP₁, MP₄ and MP₆) and weakly virulent (MP₅) on the basis of disease intensity on black gram and green gram.

Chavan (1998) sown seeds of safflower in pathogenicity sick soil and reported that symptoms of wilt were visible after 25 days of sowing of seeds.

Sandhu and Singh (1999) studied *M. phaseolina* causing charcoal rot of cow pea. They reported that the pathogen caused pre- as well as post-emergence mortality under extreme too low (40%) or 599 high (100%) soil moisture. Temperature of 35°C favoured growth of the fungus *in vitro* as well as development and severity of the disease under artificial epiphytotics. They also reported that young culture of *M. phaseolina* was more aggressive in infecting seedlings.

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

Suriachandraselvan and Seetharaman (2002) reported that most virulent isolate showed significant faster growth (41.3mm) on PDA

even 24 hours after inoculation while the least virulent exhibit slowest growth (19.7 m).

Arya *et al.*, (2004) reported that the pathogen is soil as well as seed borne causes more losses due to moisture stress.

Govindappa *et al.*, (2005) reported that disease incidence and severity are often greatest when maturing plants are stressed by drought and high temperature which leads to premature plant death, in some cases *M. phaseolina* kills upto 25% of the plants in commercial field of safflower.

2.3.3 Cropping patterns

Satishlodha *et al.* (1990) studied the effect of cropping pattern on *Macrophomina phaseolina*, the population of fungus increased under continuous monocropping with a susceptible host but considerable reduction was recorded with crop rotation.

2.4 Growth studies on *Macrophomina phaseolina*

2.4.1 Effect of pH

Moniz and Bhide (1963) reported a wide range of pH (3.0 to 11.8) for the growth of fungus isolated from cotton roots, with optimum near neutrality. Kishansingh *et al.* (1973) observed that charcoal rot fungus of sugarbeet grew at all pH levels ranging from 3 to 9. Ratnoo and Bhatnagar (1991) recorded the optimum pH 7.0 for growth.

2.5 Host range

Uppal (1936), Likhite (1936) showed that *M. phaseoli* had several hosts In India.

Reichert (1997) also reported that *Macrophomina phaseolina* causes charcoal rot in more than 400 plant species including corn, sorghum and soybean.

Suriachandraselvan *et al.*, (2005) reported that *M. phaseolina* isolated from sunflower infected all the 22 plants tested but failed to infect finger millet, hyryali, pearl millet, nutgrass, mook kuthioopndu and saranai. Sunflower and blackgram were the most aggressive while those of sesame and cotton were least virulent. There was a positive correlation between *M. phaseolina* infection and crop of plants belonging to botanical families fabaceae, pedaliaceae, asteraceae and malvaceae.

2.6 Management of *Macrophomina phaseolina* in vitro

2.6.1 In vitro evaluation of fungicides

Jhooty and Bains (1972) evaluated systemic and non systemic fungi toxicants against *Rhizoctonia solani* *in vitro*. They found that Dithane M-45 at 10 ug/ml and 44 ug/ml inhibited the growth of fungus completely and 50% respectively.

Kapoor and Chohan (1974) concluded that thirum when mixed in Richard's agar and incubated at 30°C inhibited growth of *Macrophomina phaseolina* completely.

Goel and Mehrotra (1981) tested nine fungicides *in vitro* against *Rhizoctomia bataticola* (*Macrophomina phaseolina*) of gram and found that bavistin inhibited fungal growth completely.

Giri and Peshney (1983) tested nine fungicides *in vitro* and found that carbendazim (0.1%) and mancozeb (0.25%) inhibited the spore germination and mycelial growth of *Colletotrichum graminicola* and

Rhizoctonia bataticola (*Macrophomina phaseolina*) causal agent of leaf spot of mungbean.

Hooda and Grover (1983b) stated that among 39 fungicides tested in the lab, carbendazim, benomyl, guazatine, dichlozoline, iprodione and RH-893 (2 n octyl 4 isothiazolin 3 one) were highly toxic to mycelial growth of *R. bataticola* (*Macrophominaphaseolina*) in Czapek's sol.

Martin *et al.* (1984) tested chlorothalonil at 0.1% against 16 isolates of *Rhizoctonia solani in vitro* and reported that fungus was sensitive to chlorothalonil.

Patil and Wangikar (1984) concluded that among the non systemic fungicides tested mancozeb followed by copper oxychloride were most effective in inhibiting the growth of *Rhizoctonia bataticola* (*Macrophomina phaseolina*).

Basu *et al.* (1988) tested fourteen fungicides and found that Dithane -M-45 increased inhibition with increase in concentration.

Thakre; and Gahukar (1989) studied leaf and stem blight caused by *Macrophomina phaseolina* of mungbean and showed that out of 13 fungicides, thirum (0.3%), mancozeb (0.2%) carbendazim (0.10%) inhibited 100% growth of *Macrophomina phaseolina*.

Gautam and Narain (1996) tested efficacy of seven fungicides against *Macrophomina phaseolina* causing blight of cowpea and found that bavistin, thirum and topsin-M inhibited growth of fungus completely.

Devi and Singh (1997) reported that carbendazim and Thiophenate methyl (@ 0.2 %) significantly inhibited mycelial growth of *M. phaseolina*, causing seedling mortality in black gram.

Sheela and Packiaraj (1999) reported that seed treatment with carbendazim (@ 0.1 %) significantly reduced the incidence (5.3 %) of root rot (*M. phaseolina*) of groundnut.

Lambhate *et al.* (2002) studied efficacy of different fungicides against *M. phaseolina* by poisoned food technique. They reported that Bavistin, Redomil and Topsin-M respectively at 0.1, 0.2 and 0.3 per cent significantly inhibited mycelial growth (70 to 83 per cent) of the test fungus.

2.6.2 Biological control

Raguchander *et al.* (1997) evaluated seed pelleting of *T. viride* isolates when talc was used as carrier against dry root rot of mung bean caused by *M. phaseolina*. There was significant reduction in disease incidence. Among the isolates *T. viride* 111 supported higher plant growth, better native *Rhizobium* nodulation and grain yield.

Chavan (1998) reported that *T. hamatum* alone or in combination with *T. viride* or *T. harzianum* reduced seedling mortality of safflower in pot culture study.

Biswas and Sen (2000) investigated that T8 and T10 isolates of *T. harzianum* reduced stem rot of groundnut incited by *S. rolfsii*. Per cent disease reduction through seed dressing was from 33 to 50 per cent over control and through direct soil application it was up to 72 to 83 per cent.

Shanmugam *et al.* (2001) applied *P. fluorescens* in different ways with seed treatment with *Rhizobium* under field condition to black gram. Glass house and field studied indicated that *P. fluorescens* pf-1 was

found to be the most effective treatment in reducing black gram root rot incidence and increasing the grain yield.

Sajeena *et al.* (2003) found that seed treatment with *T. viride* 4 g/kg seed along with soil application of neem cake @ 150 kg/ha significantly reduced the diseased incidence of black gram root rot (*M. phaseolina*) as compared to control and also increased yield.

Kaswate *et al* (2003) reported that *Trichoderma viride* was found to be complete suppressed growth of eight isolates of *Rhizoctonia bataticola* as compared to *Pseudomonas fluorescens* and *Bacillus subtilis*. *P. fluorescens* was found least effective inhibition amongst two organisms viz., *T. viride* and *B. subtilis* over control.

Malathi and Sabitha (2003) reported that *T. harzianum* with carbendazim at 5 and 100 ug/ml improves the performance for disease management.

Sethuramna *et al.*, (2003) reported that seed treatment + soil application of *T. viride* (Gypsum based) recorded less incidence of root rot of black gram caused by *M. phaseolina*.

Chirame and Padule (2005) reported that *T. viride* was significantly superior biological agent in inhibiting the growth of the pathogen *M. phaseolina* was isolated from cotton seed.

Dhoke and Kurundkar (2006) tested efficacy of *P. fluorescens* against *M. phaseolina* on PDA by dual culture technique found reported that the bacterium significantly reduced mycelia growth and formed zone of inhibition indicating their antagonistic nature.

Lokesh and Benagi (2007) reported that seed treatment + soil application with *T. virens* recorded less root rot incidence in pigeonpea.

Ray *et al.*, (2007) tested *in vitro* the efficacy of bioagents against the *Rhizoctonia solani* the cause of aerial blight of soybean. the reported that *T. harzianum* (82.43 mm) was most effectively inhibited the mycelia growth of *R. solani* after 96 hrs of incubation followed by *T. viridae* (80.36 mm) and *P. fluorescens* (19.13 mm).

Kar and Sahu (2008) evaluated *in vitro* and *in vivo* six fungal antagonists (*T. viride*, *T. viride-5-1*, *T. harzianum* *G. virens*, *Streptoverticillium* and *Chaetomium globosum*) against *M. phaseolina* causing seed rot, seedling blight and leaf blight disease of mungbean. They reported that all the six fungal antagonists significantly reduce radial growth of the test fungus. However, maximum inhibition was recorded with *T. harzianum* (90.60%) followed by *T. viride*, *G. virens* and *C. globosum* seed treatment with antagonists significantly reduced seed rot/seedling blight and leaf blight over untreated control and also enhanced seed germination and seedling vigour.

2.6.3 Plant extracts and soil amendments

Mathur and Sinha (1970) found reduction in gram wilt and guar root rot caused by *S. rolfsii* by the application of farm manures.

Desai and Kulkarni (2002) reported that ten per cent neem flower extract, nimbidin and neem oil were found to be highly inhibitory to hypha and sclerotial formation. Extract of ring, pulp, dernel, fruit were less inhibitory compared to flower extract neem oil and nibicidin.

Sharma *et al.*, (2002) evaluated *in vitro* antifungal activity of six plant extracts against *Macrophomina phaseolina*, *F. oxysporum* f. sp. *pisii*, *Alternaria alternate* and *R. solani* of pea. They reported that *Datura stramonium* extract was effective against all test fungi and significantly inhibited growth of the test fungi.

Kaushal *et al.*, (2003) evaluated 24 botanicals *in vitro* for their fungitoxicity against *R. solani* causing damping off, root rot and collar rot in coffee and cotton. They reported that maximum inhibition of mycelia growth was caused by *Xanthium strumarium* (67.3%) and *Arthenium hysterophorus* (53.5%), applied @ 1000 ug/ml.

Pathak *et al.*, (2003) evaluated *in vivo* and *in vitro* five plant extracts against *R. bataticola*, causing root rot of cluster bean. Plant extract viz., Neem, Ashwagandha, Marva and *Datura* significantly inhibited mycelia growth of the test fungus from 62.2 to 71.1 % and maximum inhibition was recorded with all plant extracts used @ 20% ranging from 44.4 to 77.1%. Neem extract recorded least disease incidence (4.66%) and highest grain yield (6.64 q/ha) followed by Marva.

Gupta *et al.*, (2005) reported that *in vitro* 13 plant extracts against *C. lindemathianum*, causing anthracnose of french bean. They reported that all plant extracts tested at different concentration (5, 10 and 20%) significantly inhibited (range 4.6 – 80.9%) mycelial growth of five test fungus. However, Neem (*A. indica*) followed by Marigold and Parthenium were reported most effective.

Suriachandraselvan *et al.*, (2005) reported that the Rhizosphere population of bacteria, fungi and actinomycetes were in-

creased in the amended soils compared to non-amended soils. Soil application of organic amendments also increased the germination, seedling growth and seedling vigour index of sunflower.

Sundravadana and Alice (2006) in black gram reported that the basal application of zinc sulphate @ 25 kg ha⁻¹ significantly increased defense related protein viz., penoxidase. Polyphenoloxidase and phenolics contained a sign of systemic resistance coupled with reduction in the root rot incidence.

Rao and Narayana (2005) observed that *in vitro* six plant extracts against *C. dematium* causing chickpea blight and reported that leaf extract of *Polyalthia longitolia* (10%) significantly inhibited mycelia growth (30.55%) of the test pathogen followed by Neem product, Nimabidicidial.

Karnde *et al.*, (2007) evaluated *in vitro* plant extract against *C. gloeosporioides* causing dieback and inflorescence blight of cashew. They reported that leaf extracts of tulsi @ 10% (68.8%) and Sadafuli @ 10% (51.1%) significantly inhibited mycelia growth of the test fungus. This was followed by the extract Bougainvillea (45.2%) onion (32.3%), Neem (29.6%) and Gliricidia (26.0%).

Tetarwal and Rai (2007) tested *in vitro* six plant extracts against *Alternaria alternata* causing *Alternaria* blight of senna. They reported that extracts of Garlic followed by clover, Neem and Datura significantly inhibited radial growth and conidial germination of the test pathogen over untreated control.

2.7 Field screening

Deshmukh R. W. (1981) reported that some germplasm lines of safflower viz., B-21, K-1, As-2-1 were reported resistant reaction to *Macrophomina phaseolina*.

Zote *et al.*, (1983) screened 14 mungbean cultivars and found none of the cultivar completely free from *Macrophomina* blight, however, ML-5, ML-26 ML-65 ML-62 were reported moderately susceptible to the disease to J-781 as highly susceptible to the disease.

Ingle *et al.*, (2004) tested the safflower genotype against root rot and reported that AKS-152 and AKS-68 showed resistant reaction.

Desai (2005) tested eight genotypes of sorghum against *Macrophomina phaseolina* and concluded that CSH-13R, SPH-641, SPH-634 and SPH-677 were charcoal rot tolerance.

Pahlvani *et al.*, (2007) tested nineteen safflower genotypes originated from different geographical regions were screened for their response to *Macrophomina phaseolina* and reported that four genotypes were moderately resistance, ten susceptible and five moderately susceptible genotypes. However non of the genotype was found completely disease free.

Zote and Dhutraj (2005), reported Munbean var. BPMR-145 as resistant with 3.50% intensity of M. blight as compared to the check kepergaon (47.0%), B2-4 (17.0%), AKM-8802 (22.0%) and JL-781 (52.0%).



Material and Methods



CHAPTER–3

MATERIALS AND METHODS

Present investigations on root rot of safflower caused by *Macrophomina phaseolina* were undertaken in the year 2009-10. Field experiments were carried out at All India Coordinated Research Project on Safflower, Marathwada Agricultural University, Parbhani. The materials used and methodology followed for the studies viz., survey, isolation, purification of pathogen and mass multiplication during the investigation are narrated as below.

3.1 Survey of safflower root rot disease in Marathwada regions

Four districts of Marathwada region viz., Parbhani, Latur, Nanded and Osmanabad were surveyed during January, 2010. Survey was undertaken to record the incidence and intensity of root rot in safflower (Table 1).

3.2 MATERIALS USED

3.2.1 Site of experiment:-

Land resource for conducting field experiment was utilized from A.I.C.R.P. on Safflower, College of Agriculture, M.A.U., Parbhani .

3.2.2 Glassware, chemicals & fungicides:-

For all experimental work, glasswares viz. conical flasks, measuring cylinder, test tubes, petriplates etc. and chemicals (fungicides) and bioagents were obtained from the Department of Plant Pathology, College of Agriculture, M.A.U., Parbhani.

3.2.3 Seeds

Pure seeds of safflower were obtained from All India Coordinated Research Project on Safflower, M.A.U., Parbhani.

3.2.4 Miscellaneous

Common pathological laboratory equipments such as autoclave, hot air oven, B.O.D. incubator, inoculation chamber, refrigerator, weight balance, research microscope, spray pump, seed counter, yellow and white labels, polythene bags etc. were utilized from Department of Plant Pathology M.A.U., Parbhani.

3.2.5 Sterilization and working under aseptic condition

Glasswares were sterilized by dry heat in hot air oven at $180 \pm 1^{\circ}\text{C}$ temperature for one hour. Media and water blanks were sterilized in an autoclave at 15 lbs pressure for 15 min.

Isolation of fungal organisms, their subculturing, inoculation for mass multiplication and dual culture inoculation for testing the bioagents were carried out under aseptic condition in laminar air flow.

3.3 Pathogenicity :

3.3.1 Collection of disease Sample.

The disease samples were collected from All India Coordinated Research Project on Safflower, Marathwada Agricultural University, Parbhani during the month of November 2009.

Affected plants showing typical symptoms genotypes were selected for isolation of pathogen.

3.3.2 Isolation and maintenance of culture.

The portion of the root affected by root rot disease were cut into 5 mm small pieces. These pieces were then surface sterilized in

0.1 per cent mercuric chloride solution (HgCl_2) followed by three changes in sterile water. Then these pieces were planted on sterilized potato dextrose agar (PDA) in petri dishes. The petri dishes were incubated at $28 \pm 2^\circ\text{C}$ temperature in inverted position. After 3 days, subculturing was done on potato dextrose agar slants by transferring the young mycelial bit with the help of sterile inoculating needle. The tubes were kept at $28 \pm 2^\circ\text{C}$ temperature throughout the course of studies.

Subculturing was done at regular intervals of 15 days, using potato dextrose agar slants.

3.3.3 Purification and maintenance of culture

Culture of *Macrophomina phaseolina* obtained as above was purified and maintained on PDA at room temperature by giving subsequent subculturing at periodic regular intervals. Seven days old cultures were used for further studies.

3.3.4 Mass multiplication of inoculum

Potato dextrose broth was used for mass production of inoculum. The culture of *Macrophomina phaseolina* was grown on PDA in Petri dishes. Then five mm discs of 5 days old culture were inoculated into 250 ml conical flask containing 150 g potato dextrose broth.

3.3.5 Pathogenicity test

3.3.5.1 Pathogenicity test by water culture

Test tubes were taken which sterilized in hot air oven at $180 \pm 1^\circ\text{C}$ for 1 hr. Well homogenized two week old culture of pathogen multiplied on potato dextrose broth medium was added 10 ml per test tube. Sixteen test tubes were filled with isolate number 19 (MP_1) and sixteen isolate number 13 (MP_2) remaining sixteen test tubes

were filled with distilled water which were served as control test tubes. Test tubes were kept as such for one week to allow the fungus to multiply. Single germinated seed was raised in each test tube of safflower variety Nira, Parbhani-12, Sharda and Manjira. The test tubes kept in room temperature condition and symptoms were observed after 12, 36, 60, 84, 108, 132, 156, 180 and 204 hrs. after inoculation.

3.3.5.2 Pathogenicity test (sick soil technique)

Soil was taken from field and mixed with farm yard manure (2:1) and this mixture was autoclaved at 30 lbs pressure for 30 minutes and repeated at the interval of 24 hrs. Earthen pots of 12 cm size were used for filling soil + FYM mixture. Before filling, pots were surface sterilized with 0.25 per cent copper fungicide solution. In each pot, 0.250 kg of soil was filled. Two week old culture of pathogen multiplied on potato dextrose broth medium was added @ 10 per cent of weight of soil to each pots. Sixteen pots were inoculated with isolate number 19(MP₁) other sixteen with isolate number 13 (MP₂) collected from field and pots were kept as such for two weeks to allow the fungus to multiply and Sixteen pots were filled separately with only sterile soil which were served as control. Ten plants were raised in each pot of safflower variety Nira, Mangira, Parbhani-12, Sharda. The pots were kept in glasshouse condition and watered regularly. Symptoms were observed after 30, 60, 90 days of sowing.

3.4 Re-isolation

Re-isolation of the organism was made under aseptic condition from the diseased roots of plants. The culture obtained was compared with original culture and found identical with original culture.

The re-isolated culture was maintained on potato dextrose agar slants for further studies.

3.5 Poisoned food technique

Potato Dextrose Agar medium was used as a basal medium for the fungicidal study. Potato dextrose agar medium was prepared in the 250 ml conical flask. 100 ml medium was taken in each flask. The medium then sterilized at 15 lbs vapour pressure for 15 minutes. Required quantity of test fungicides were calculated and added in the sterilized medium separately. Flasks containing poisoned medium were shaken well to have even and uniform distribution of the fungicides.

About 20 ml of poisoned PDA was poured in each of the sterilized petri plates and allowed to solidify. The plates were inoculated by pure culture of *Macrophomina phaseolina*. For this purpose, 5 mm disc of one week old culture was cut with a sterilized cork borer. The disc was lifted and transferred aseptically in the centre of Petri plates containing the medium with test fungicides. Three plates were maintained for each treatment. The control plates without fungicides were also inoculated and kept for incubation.

Treated plates were incubated at 28 + 2°C temperature. The observations on colony diameter were recorded after 5 days. The inhibition zone was calculated by using the following formula. The data was statistically analysed in C.R.D.

$$PI = \frac{C - T}{T} \times 100$$

Where,

PI = Per cent inhibition

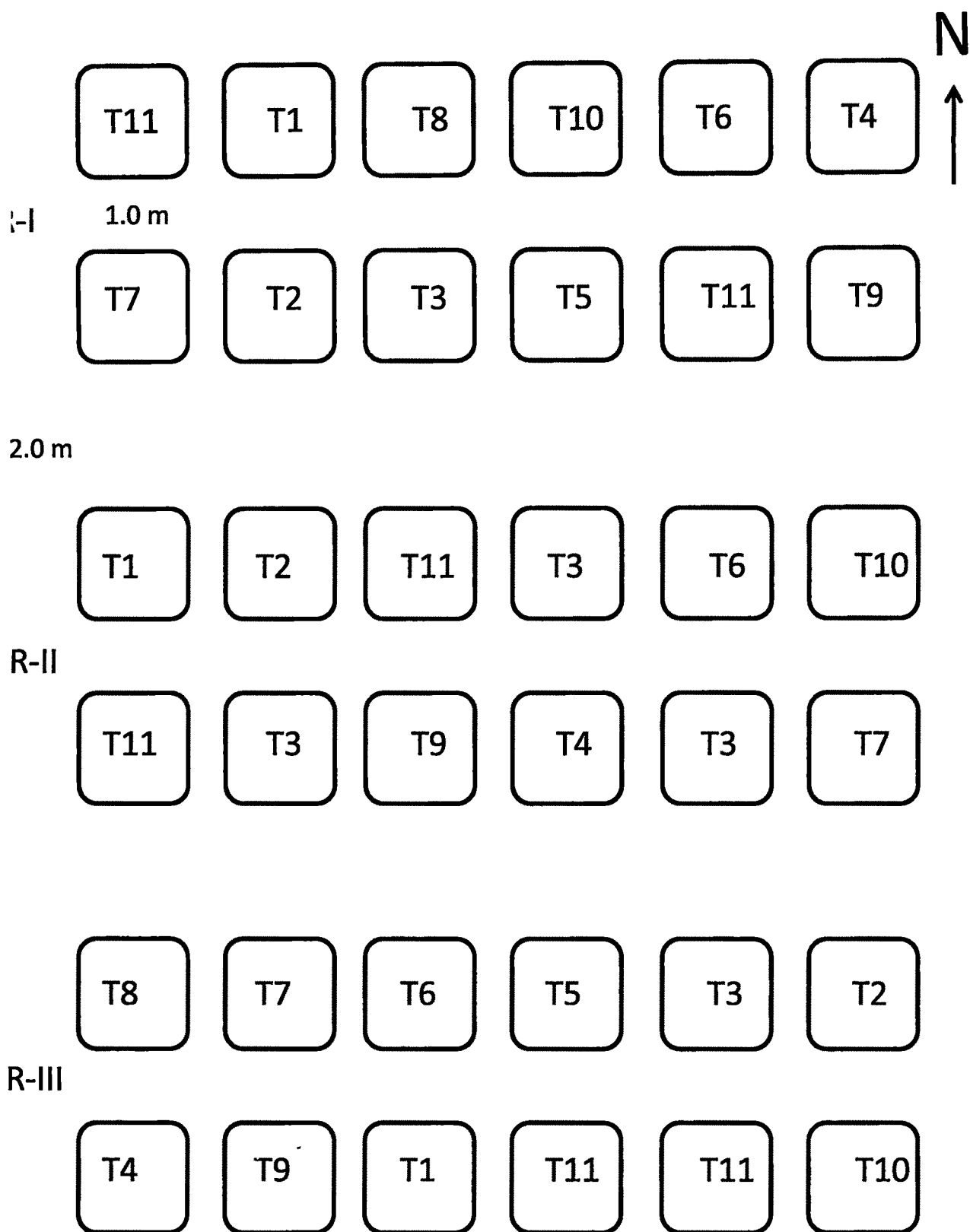


Fig. 1. Layout of agronomical experiment

C = Growth in control plates

T = Growth in plates treated with fungicides.

3.6 Methodology

A roving survey was undertaken in Marathwada region to record occurrence of *Macrophomina phaseolina* disease incidence.

3.6.1 Screening of agronomical material

One hundred twenty germplasm lines were screened separately in Randomized Block Design (RBD) during the year 2009-10. The experimental details were as follows.

1. Design : Randomised Block Design
2. Replication : Three
3. Plot size : 2.7 x 3.8 m
4. Spacing : 45 x 20 cm
5. Date of sowing : 16.10.2009

The treatments are given in Fig. 1.

3.6.2 Screening of safflower breeding material against *Macrophomina phaseolina*

Fourth generations of breeding material were screened separately in Randomized Block Design (RBD) during the year 2009-10(Fig.2). The experimental details were as follows.

1. Design : RBD
2. Replications : Three
3. Plot size : Gross : 2.25 m x 5.0 m
Net : 1.35 x 4.6 m
4. Spacing : 45 x 20 cm
5. Spacing between 2 plots – 1 m
6. Spacing between 2 replications – 1 m
7. Date of sowing : 12.10.2009

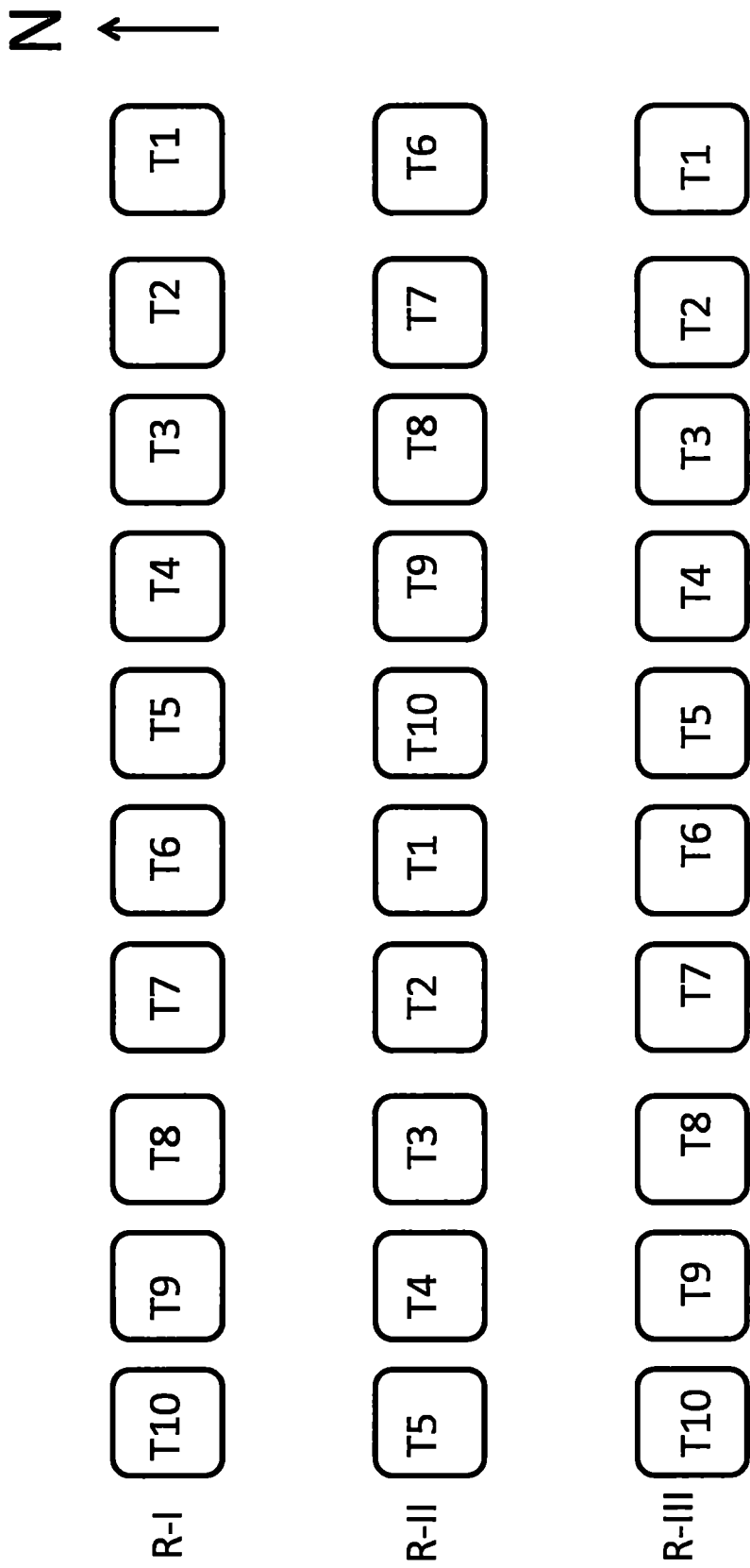


Fig. 2. Layout of crosses of breeding material

Table 1. Details of breeding material of safflower screened against *Macrophomina phaseolina*

1	MS 105 x PBNS 5-1-7
2	MS 101 x NARI-37
3	MS-II x PBNS 5-1-7
4	MS-II x GMU 1962
5	MS-II x BIP-2
6	MS-104 x PBNS 5-1-7
7	MS-105 x GMU-2523
8	MS-102 x NAIR-37
9	MS-176 x BIP-2
10	MS-105 x GMU-1962

3.6.3 Screening of safflower advanced varietal material against *Macrophomina phaseolina*

Fourteen genotypes were screened separately in Randomized Block Design (RBD) during the year 2009-10 (Fig.3). The experimental details were as follows.

Experimental details

Design	:	Randomized Block Design
Replications	:	Three
Plot size	:	2.7m x 4.6 m
Spacing	:	45 cm x 20 cm
Spacing between plots	:	1 m
Spacing between replication:		1.5 m
Date of sowing	:	11.12.2009

Treatmentt details:

B ₁	AVT-09-1
B ₂	AVT-09-2
B ₃	AVT-09-3

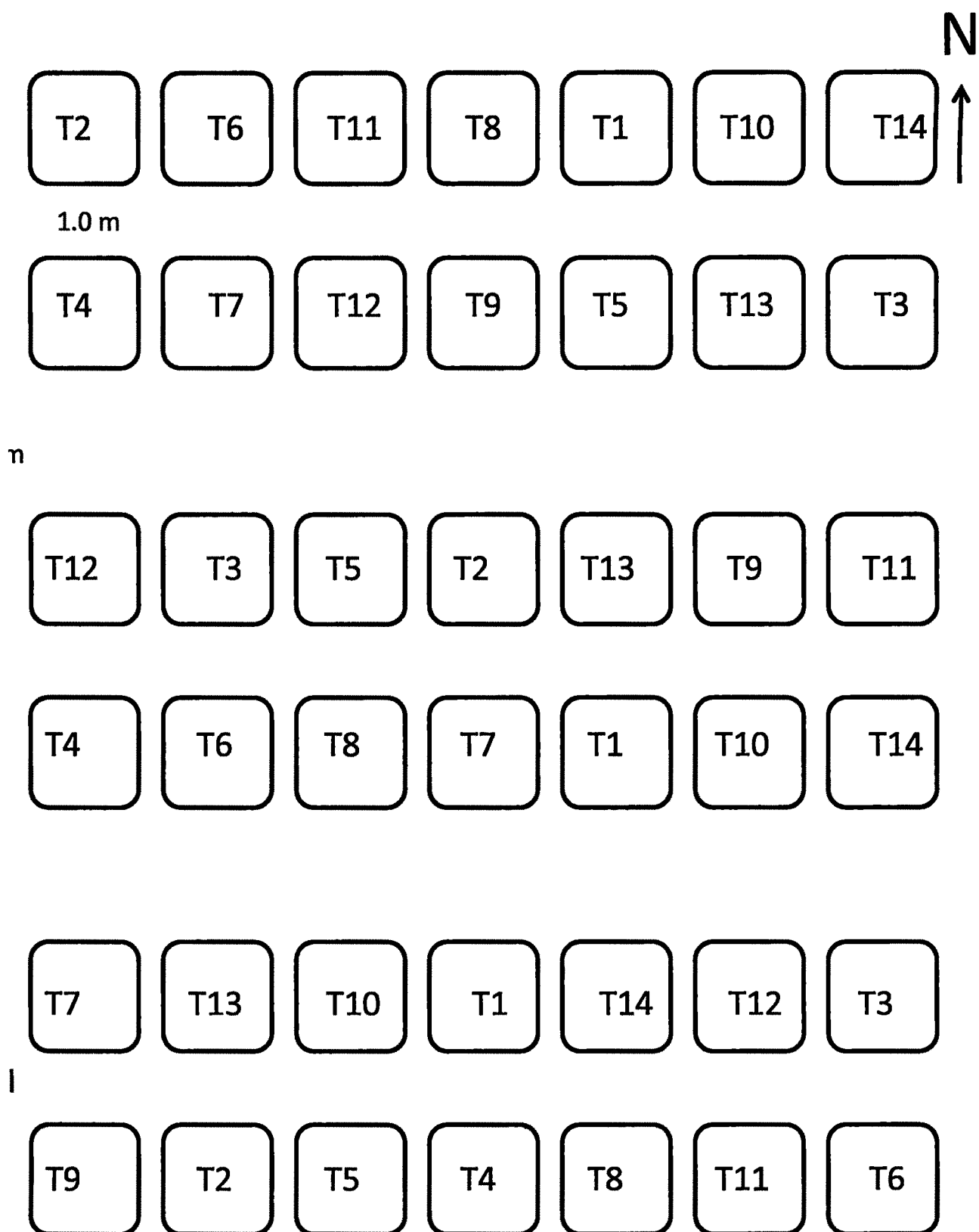


Fig. 3. Layout of AVT experiment



Plate 1. General field view of safflower experimentation

B₄ AVT-09-4
 B₅ AVT-09-5
 B₆ AVT-09-6
 B₇ AVT-09-7
 B₈ AVT-09-8
 B₉ AVT-09-9
 B₁₀ AVT-09-10
 B₁₁ AVT-09-11
 B₁₂ AVT-09-12
 B₁₃ AVT-09-13
 B₁₄ AVT-09-14

3.6.4 Per cent disease incidence

Per cent incidence was calculated from the number of infected plants against the total number of plants at the time of observation by using following formula.

$$\text{Per cent incidence (PI)} = \frac{\text{No. of plants diseased}}{\text{Total number of plants observed}} \times 100$$

3.6.5 Disease reaction

Per cent incidence	Disease scale	Reaction
No symptosm	0	Immune
Less than 1% infection	1	Highly resistant
1-10%	2	Resistant
11-25%	3	Moderately susceptible
26-50%	4	Susceptible
51-100%	5	Highly susceptible

3.7 Efficacy of *Trichoderma* spp. against *Macrophomina phaseolina*

The antagonistic potential of *Trichoderma* sp. was assessed against *Macrophomina phaseolina* by dual culture technique on PDA medium. For this 20 ml of sterilized and luke warm medium (PDA) (45°C) was poured in each petriplate and allowed to solidify. A 5 mm disc of *Macrophomina phaseolina* was cut and placed at one end of the plate with the help of sterilized cork borer and inoculating needle. Just opposite to it, a 5 mm disc of the respective bioagent was inoculated.

Observations regarding colony diameter in mm of *Macrophomina phaseolina* and bioagent were recorded at 3, 5, 7 and 9 days after inoculation by incubating at 28 ± 2 °C temperature. Experimental details were as follows.

Bioagents

Experimental design	:	CRD
Replications	:	Four
Number of treatments	:	5

Treatment details

Bioagents

T ₁	<i>Trichoderma hamatum</i> (Bonard)
T ₂	<i>T. harzianum</i> (Rifai)
T ₃	<i>T. viride</i> (Pers.)
T ₄	<i>Pseudomonas fluorescens</i>
T ₀	Untreated control

3.8 Statistical analysis

Replicated data recorded on disease incidence and disease severity was statistically analysed.

The observations of growth parameters were also used for statistical analysis. To compare two treatment means, critical difference (C.D.) at 5 % level of significance was worked out as suggested by Panse and Sukhatme (1967).



Results



T. 5944

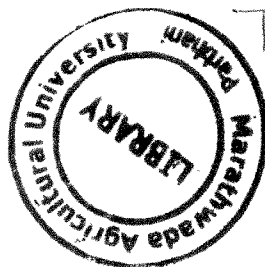
CHAPTER-4

RESULTS

Present investigation on root rot of safflower incited by *Macrophomina phaseolina* includes survey and surveillance, isolation and purification of pathogen, screening of available germplasm lines under field condition, management with the use of chemicals, bioagents and plant extracts *in vitro*. Data of the experiments were compiled, analysed statistically and results are presented.

4.1 Symptomatology

Root rot affected safflower plants showed sudden and complete wilting. The spread of the disease in the field was found in the form of concentric circles. When affected plants were pulled out and examined the entire root system was found rotted. The decay was more on thinner roots. In severely affected plants only the tap root remained attached to the plant, the remaining root system found decayed and retained in the soil. The tap root was sticky and moist. Fungal sclerotia appeared as minute black dots on the surface of the woody tissue on rotted bark. The shredding of the bark was prominent in root rot affected safflower plant and vertical section of affected tap root showed black sclerotial growth of the fungus. Stem split symptom was observed in 30 day old plants as minute cracks approximately 2-3 cm above the soil surface which later extended to both upward and downward directions. The split portion was hollow and covered with grey to black mycelial mat of the fungus on the inner surface



T 5944



Plate 2. Root rot symptoms observed under field condition



Plate 3a. Formation of Sclerotia inside the stem tissue

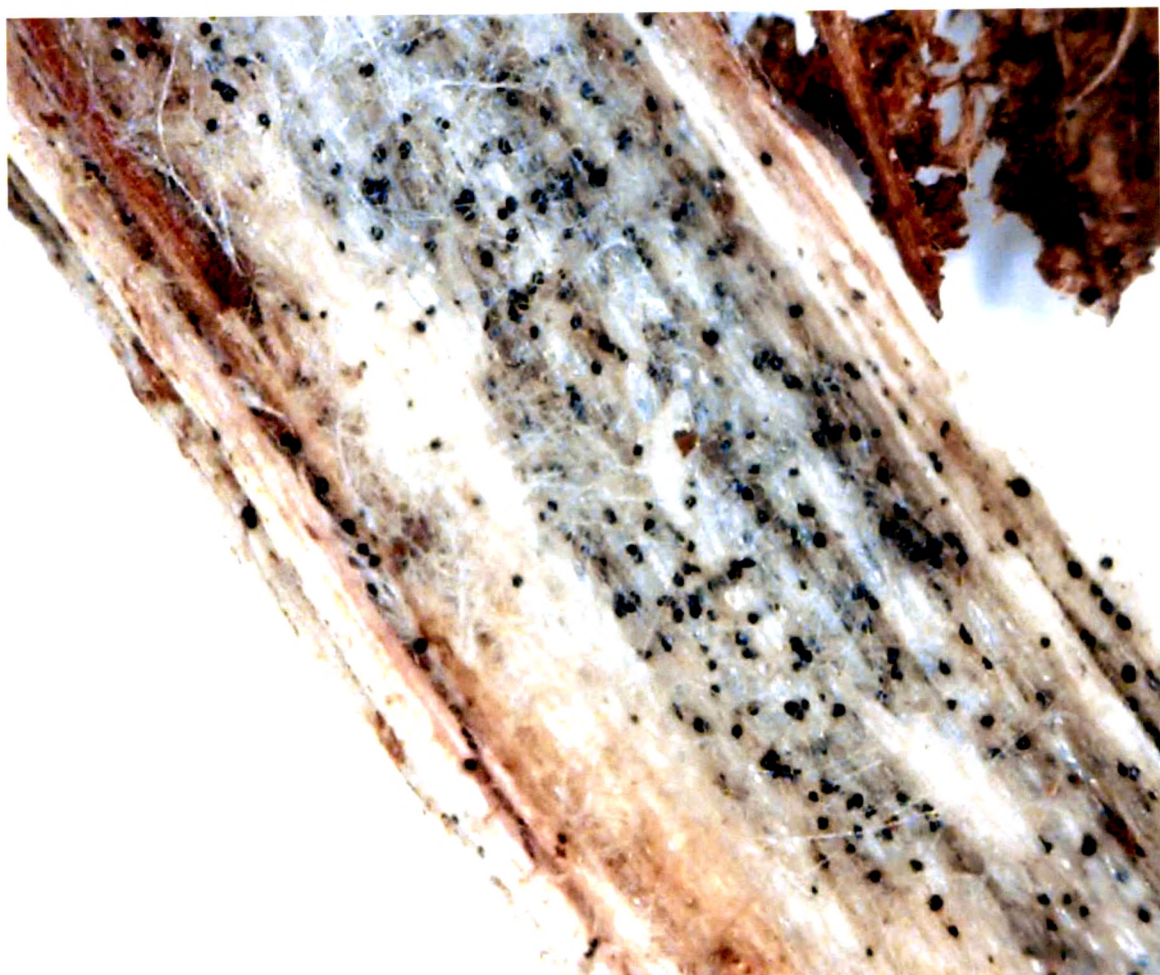


Plate 3b. Formation of Sclerotia inside the stem tissue

and infected plants results in poor growth and reduced size of inflorescence (Plate -2,3a and 3b).

4.2 Occurrence and distribution of root rot disease on farmers field

Data on survey and surveillance of root rot disease incidence on safflower is presented in Table 1a, b and c.

A roving survey was undertaken by road in Parbhani, Nanded, Hingoli, Latur, Osmanabad, Beed, Aurangabad and Jalna districts of Marathwada region. Total fifty four safflower fields were traveled, out of which 11, 8, 9, 5, 4, 4, 6 and 7 were in Parbhani, Nanded, Hingoli, Latur, Osmanabad, Beed, Aurangabad and Jalna districts, respectively. In Parbhani district 4, 3, 2 and 2 fields in Parbhani, Gangakhed, Jintur and Pathri tahsils, respectively, five in Nanded and two in Biloli tahsils of Nanded district whereas six and three fields in Hingoli and Basmat tahsils, respectively of Hingoli district. Three and two field in Latur and Chakur tahsils of Latur, two field each in Osmanabad and Tuljapur tahsils of Osmanabad, two field each in Kaij and Ambajogai tahsils of Beed district, four and two field in Aurangabad and Paithan tahsils of Aurangabad district, five and two fields in Jalna and Ambad tahsils of Jalna district were inspected for recording root rot disease incidence.

Data presented in Table 1a and figure-4 indicated that the maximum average disease incidence was noticed in Beed district (15.17 %) followed by and Osmanabad, Jalna and Latur whereas lowest in Nanded (11.24 %).

Table 1a. District wise occurrence of root rot of safflower in Marathwada region

Sr.No.	Districts	No. of locations	Disease incidence (%)
1	Parbhani	11	12.38
2	Nanded	8	11.24
3	Hingoli	9	13.17
4	Latur	5	13.26
5	Osmanabad	4	14.73
6	Beed	4	15.17
7	Aurangabad	6	11.55
8	Jalna	7	14.62
	Average	54	13.26

Data presented in Table 1b indicated that the highest root rot disease incidence was observed in Beed, Kaij and Jalna, Ambad, whereas lowest in Nanded followed by Aurangabad tahsils.

Table 1b. Occurrence of root rot disease of safflower in different tahsils of Marathwada region

Sr.No.	Districts	Tahsils	Disease incidence (%)
1	Parbhani	Parbhani	10.60
		Jintur	12.52
		Gangakhed	13.17
		Pathri	13.25
2	Nanded	Nanded	9.27
		Biloli	13.21

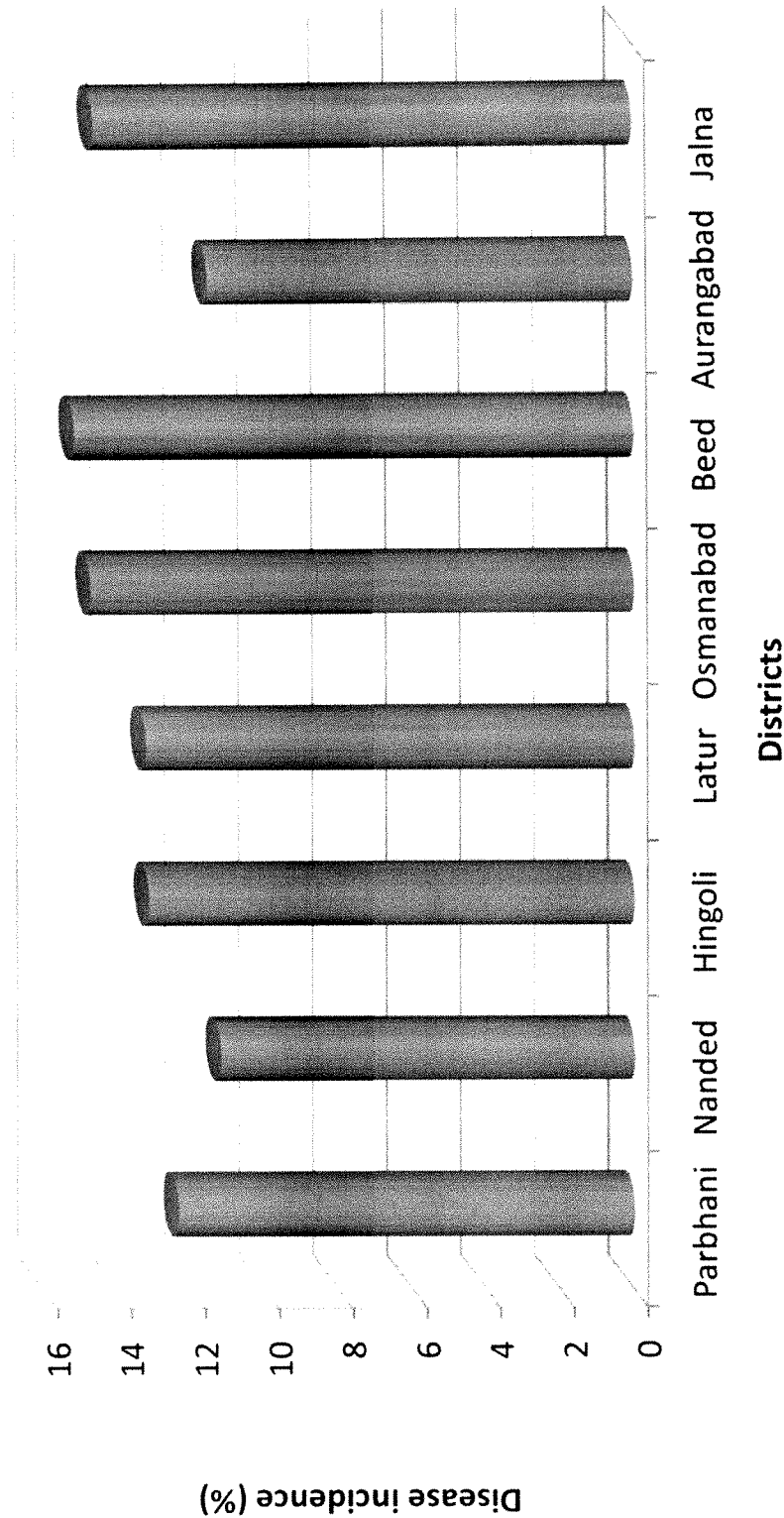


Fig. 4. District wise occurrence of root rot of safflower in Marathwada region

Sr.No.	Districts	Tahsils	Disease incidence (%)
3	Hingoli	Hingoli	14.25
		Basmat	12.10
4	Latur	Latur	11.25
		Chakur	15.28
5	Osmanabad	Tuljapur	14.42
		Osmanabad	15.05
6	Beed	Kaij	16.05
		Ambajogai	14.30
7	Aurangabad	Aurangabad	10.25
		Paithan	12.85
8	Jalna	Jalna	13.25
		Ambad	16.00

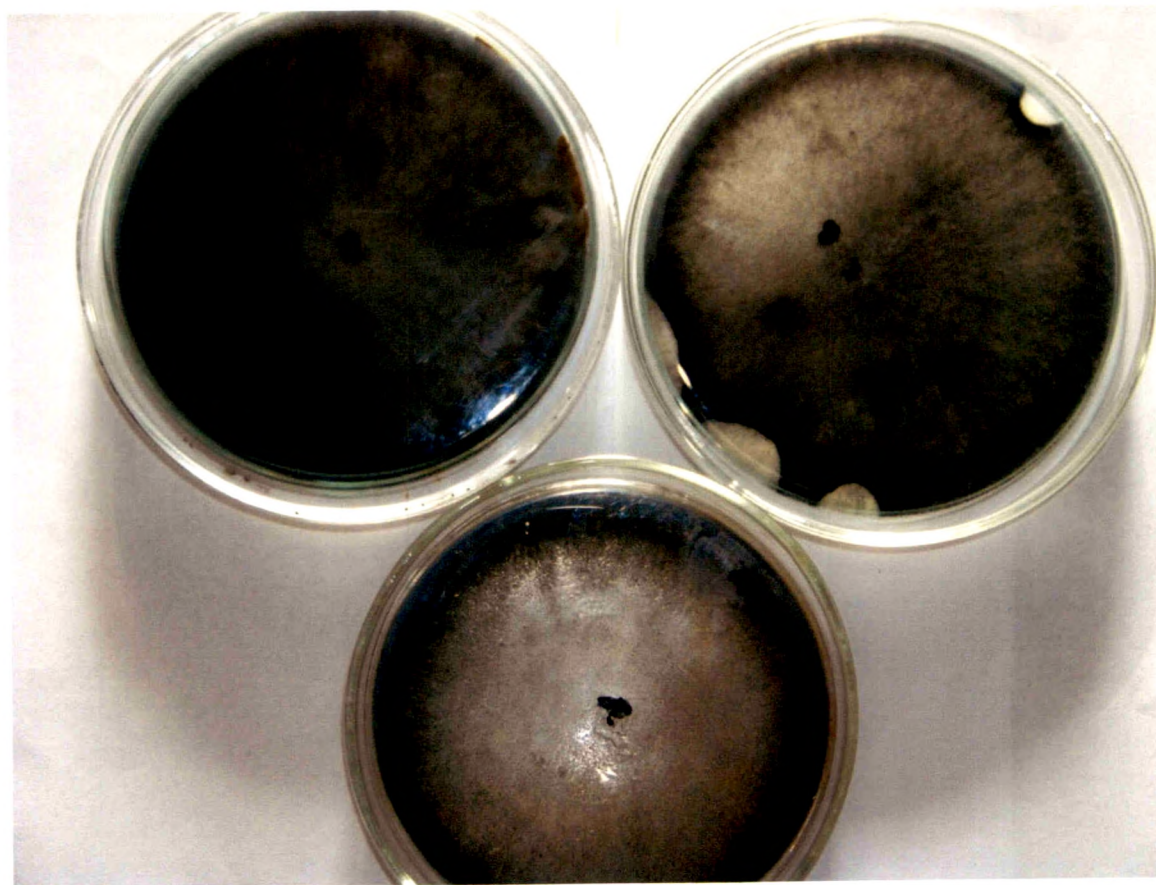
Data presented in Table 1c indicated safflower varietal response to the root rot disease on farmers field. All the observed varieties of safflower succumbed to root rot infection caused by *Macrophomina phaseolina*. Root rot disease incidence was recorded in the range of 10.20 per cent to 15.28 per cent. Maximum disease incidence was noticed on local variety followed by Sharda.

Table 1c. Root rot disease on different varieties in Marathwada region

Sr.No.	Variety	No.of location	Disease incidence (%)
1	PBNS-12	13	10.20
2	Sharda	17	13.45
3	Bhima	9	11.85
4	AVT-09-11	1	13.10
5	Local	14	15.28
	Total	54	



Test tube



Petri plates

Plate 4. Pure culture of *Macrophomina phaseolina*

4.3 Isolation and purification of pathogen

4.3.1 Collection of diseased sample

Root rot diseased samples were collected from farmers field and the All India Coordinated Research Project on Safflower, College of Agriculture, MAU, Parbhani. These diseased samples were further used for isolation of pathogen.

4.3.2 Isolation

Fresh samples of infected root were taken for isolation. The infected samples were cut into pieces. Surface sterilized with 0.1 % mercuric chloride (HgCl_2) solution were further passed through three changes of sterile water and these pieces were planted on sterilized PDA in petriplates. The petriplates were incubated at room temperature ($28 \pm 2^\circ\text{C}$).

As soon as fungus growth appeared, a uniform colonies originated from diseased bits were taken from periphery and transferred aseptically to another petriplates containing sterile PDA. After 48 hours of incubation, small tip of hypha was taken out and were transferred aseptically on PDA slants (plate-4). In this way purified cultures were maintained on PDA slants for further studies. The two isolates selected for the further studies were named as MP_1 (isolate number 19) and MP_2 (isolate number 13) based on their virulency.

4.3.3 Identification

Fungus grew well on potato dextrose agar media forming profuse growth, mycelium was dirty white in the beginning and then turned brown to black. Numerous sclerotia produced within 5 to 7 days on potato dextrose agar medium. These sclerotia were round to oval or irregular in



Plate 5. Mass multiplication of *Macrophomina phaseolina* culture



Magnified view

Plate 6a. Sclerotia of *Macrophomina phaseolina*



Magnified view

Plate 6b. Pycnidiospores of *Macrophomina phaseolina*

shape ranging from 40-220 um in diameter. They were carbonaceous dark black in colour which possibly imparts black colour to affected tissue or media.

4.4 Pathogenicity

Fungus isolated from root rot disease was grown on potato dextrose broth and used for further experimentation.(plate-5)

4.4.1 Pathogenicity by water culture technique

Water culture technique suggested by Nene *et al.*, (1981) was used to confirm pathogenicity of *M. phaseolina* isolates. Well homogenized broth was obtained by growing *M. phaseolina* for 15 days on PDB. Five days old seedling of varieties Nira, Mangira, Parbhani 12 and Sharda were removed from the blotter paper and dipped in the test tube containing 10 ml well homogenized culture. Two isolates MP₁ and MP₂ were used to study the pathogenicity test.

The data presented in Table 2 indicated statistically significant mortality differences between isolates over control. Highest mortality to the tune of 100 per cent was recorded in isolate MP₁ whereas isolate MP₂ showed 84.37 % mortality by uninoculated control (0.00%) was observe free from mortality.

Varietal response was non significant, however, varieties viz., Nira, and Sharda (64.58%) mean seedling mortality followed by Manjeera and Parbhani-12 (58.33%).

Table 2. Pathogenicity test by water culture technique

Sr. No.	Treatment	Total seedling sown	Total mortality	Mean % mortality
A	Cultivars (C)			
1	Nira	48	31	64.58 (59.99)
2	Manjira	48	28	58.33 (53.33)
3	Parbhani-12	48	28	58.33 (53.33)
4	Sharda	48	31	64.59 (59.99)
	SE $\pm$			4.04
	CD at 5%			NS
B	Isolates (I)			
1	MP ₁	64	64	100 (89.98)
2	MP ₂	64	54	84.37 (78.53)
3	Control MP ₀	64	00	00 (00.00)
	SE $\pm$			3.50
	CD at 5%			10.2
C	Interaction (C x I)			
	SE $\pm$			7.01
	CD at 5%			NS

Figures in parentheses are angular transformed values.



Plate 7a. Pathogenicity test by water culture method in Cv. Nira



Plate 7b. Pathogenicity test by water culture method in Cv. Manjira



Plate 8a. Pathogenicity test by water culture method in Cv. Parbhani-12



Plate 8b. Pathogenicity test by water culture method in Cv. Sharda

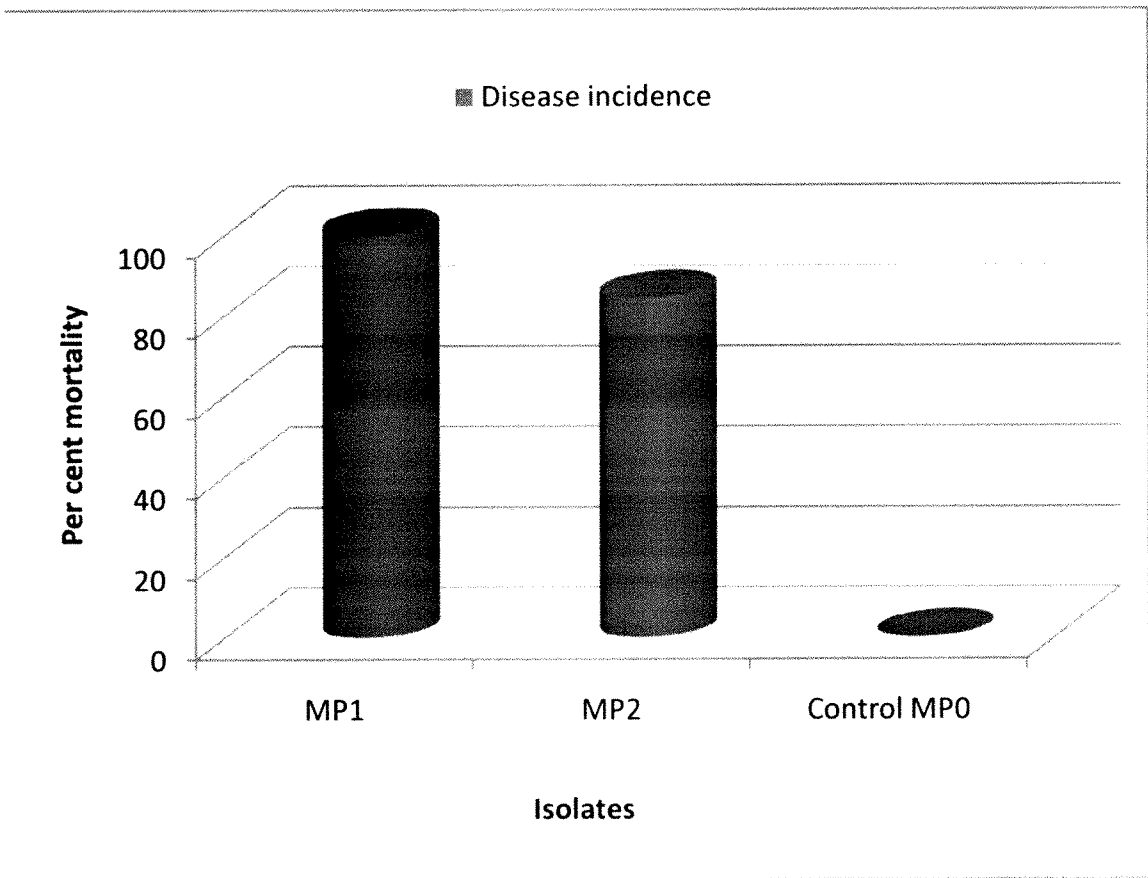
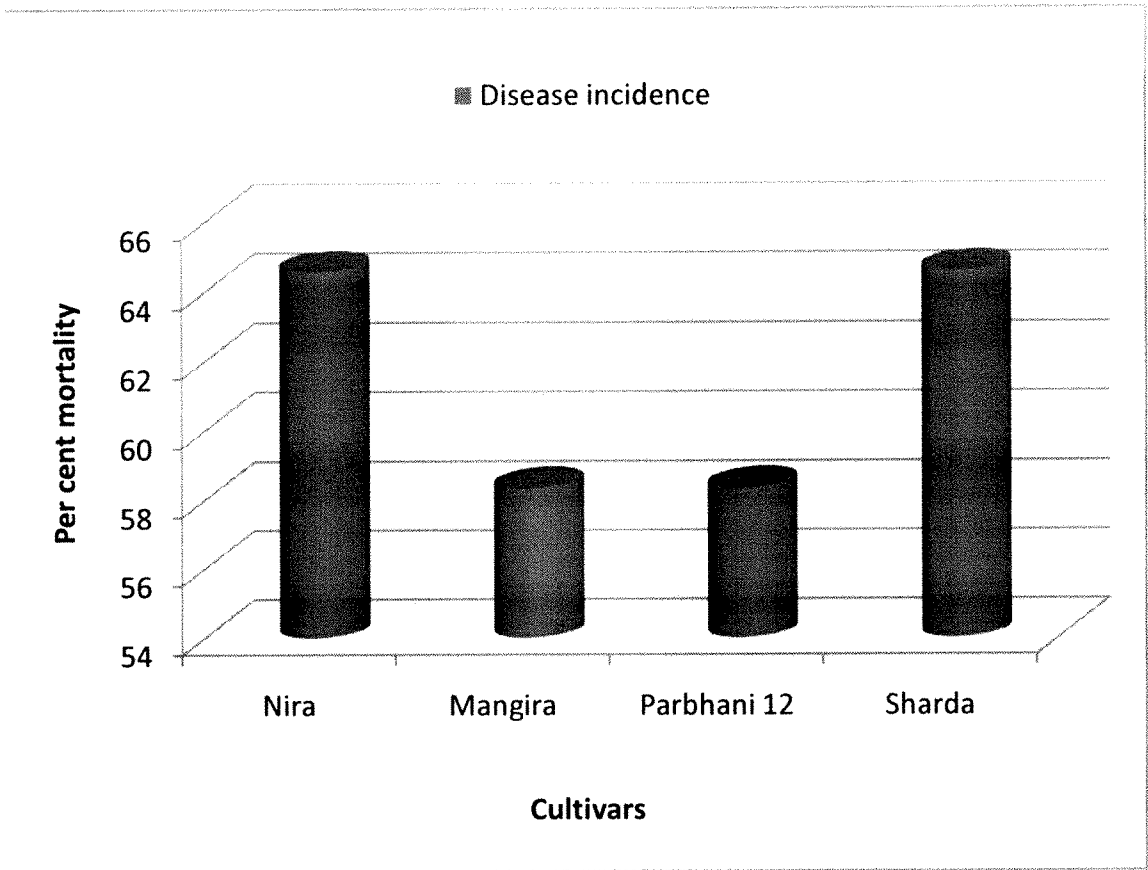


Fig 5. Per cent mortality by water culture technique

4.4.1.1 Interaction effect

Interaction between safflower cultivars and isolates for seedling mortality after dipping was absent throughout observation period (Table 2).

4.4.1.2 Per cent mortality (after hrs of inoculation/dipping)

Safflower seedling of varieties, viz., Nira, Mangira, Parbhani 12 and Sharda varieties seedling were dipped in the water culture and observed the per cent mortality of seedlings at 12, 36, 60, 84, 108, 132, 156, 180 and 204 hours of incubation separately and the data presented in Table 3 (a,b,c,d) and PLATE-7a,7b,8a,8b.

Data in respect of isolate MP-1 was recorded significant, while in case of isolate MP-2 it was non significant in all the varieties. Isolate MP-1 showed seedling mortality at 60 and 84 hours incubation to the tune of 50 and 50, 25 and 75, 25 and 75 and 0 and 100 per cent in varieties Nira, Manjeera, Parbhani-12 and Sharda, respectively. Isolate MP-2 showed seedling mortality in the range of 18.75 to 25 per cent from 84 hours to 156 hours in Nira, 25 per cent at 60, 84 and 180 hours in Manjeera, 25 per cent at 84, 132, 156 hours in parbhani-12 and total mortality from 60, 156, 180, 204 hrs was to the tune of 93.75 per cent in Sharda variety was observed.

Isolate MP₁ amongst all the varieties found more virulent (100%) at 60 and 84 hours of incubation whereas, MP₂ was non-virulent (84.37 %). Mortality of seedling in MP₂ isolate was more at 84, 108, 132, 156, 180 and 204 hours of incubation.

Table 3a. Per cent mortality after hrs of dipping in cultivar Nira

Treatment	Mortality (%)		
	MP ₁	MP ₂	MP ₀
T ₁ (12)	0 (0.00)	0 (0.00)	0 (0.00)
T ₂ (36)	0 (0.00)	0 (0.00)	0 (0.00)
T ₃ (60)	50 (44.99)	0 (0.00)	0 (0.00)
T ₄ (84)	50 (44.99)	25 (22.50)	0 (0.00)
T ₅ (108)	0 (0.00)	18.75 (12.15)	0 (0.00)
T ₆ (132)	0 (0.00)	25 (22.50)	0 (0.00)
T ₇ (156)	0 (0.00)	25 (22.50)	0 (0.00)
T ₈ (180)	0 (0.00)	0 (0.00)	0 (0.00)
T ₉ (204)	0 (0.00)	0 (0.00)	0 (0.00)
SE $\pm$	12.2	13.6	
CD at 5%	35.4	NS	

Figures in parents are angular transformed values.

Table 3b. Per cent mortality after hrs of dipping in cultivar Mangira

Treatment	Mortality (%)		
	MP ₁	MP ₂	MP ₀
T ₁ (12)	0 (0.00)	0 (0.00)	0 (0.00)
T ₂ (36)	0 (0.00)	0 (0.00)	0 (0.00)
T ₃ (60)	25 (22.50)	25 (22.50)	0 (0.00)
T ₄ (84)	75 (67.49)	25 (22.50)	0 (0.00)
T ₅ (108)	0 (0.00)	0 (0.00)	0 (0.00)
T ₆ (132)	0 (0.00)	0 (0.00)	0 (0.00)
T ₇ (156)	0 (0.00)	0 (0.00)	0 (0.00)
T ₈ (180)	0 (0.00)	25 (22.50)	0 (0.00)
T ₉ (204)	0 (0.00)	0 (0.00)	0 (0.00)
SE $\pm$	10.6	13.6	
CD at 5%	30.7	NS	

Figures in parents are angular transformed values.

Table 3c. Per cent mortality after hrs of dipping in cultivar Parbhani 12

Treatment	Mortality (%)		
	MP ₁	MP ₂	MP ₀
T ₁ (12)	0 (0.00)	0 (0.00)	0 (0.00)
T ₂ (36)	0 (0.00)	0 (0.00)	0 (0.00)
T ₃ (60)	25 (22.50)	0 (0.00)	0 (0.00)
T ₄ (84)	75 (67.49)	25 (22.50)	0 (0.00)
T ₅ (108)	0 (0.00)	0 (0.00)	0 (0.00)
T ₆ (132)	0 (0.00)	25 (22.50)	0 (0.00)
T ₇ (156)	0 (0.00)	25 (22.50)	0 (0.00)
T ₈ (180)	0 (0.00)	0 (0.00)	0 (0.00)
T ₉ (204)	0 (0.00)	0 (0.00)	0 (0.00)
SE $\pm$	10.6	13.6	
CD at 5%	30.7	NS	

Figures in parents are angular transformed values.

Table 3d. Per cent mortality after hrs of dipping in cultivar Sharda

Treatment	Mortality (%)		
	MP ₁	MP ₂	MP ₀
T ₁ (12)	0 (0.00)	0 (0.00)	0 (0.00)
T ₂ (36)	0 (0.00)	0 (0.00)	0 (0.00)
T ₃ (60)	0 (0.00)	25 (22.50)	0 (0.00)
T ₄ (84)	100 (89.99)	0 (0.00)	0 (0.00)
T ₅ (108)	0 (0.00)	0 (0.00)	0 (0.00)
T ₆ (132)	0 (0.00)	0 (0.00)	0 (0.00)
T ₇ (156)	0 (0.00)	18.75 (12.15)	0 (0.00)
T ₈ (180)	0 (0.00)	25 (22.50)	0 (0.00)
T ₉ (204)	0 (0.00)	25 (22.50)	0 (0.00)
SE $\pm$	7.49	10.9	
CD at 5%	21.72	NS	

Figures in parents are angular transformed values.



Plate 9. Pathogenicity test by sick soil technique

4.4.2 Pathogenicity by sick soil technique

The culture isolated from diseased samples were grown on potato dextrose broth (PDB). Two weeks old culture of pathogen multiplied on potato dextrose broth was added @ 10 per cent of the weight of soil to each pot. Pots were kept for two week to allow the fungus to multiply. Ten seedlings were maintained in each pot of safflower variety Nira, Mangira, Parbhani-12 and Sharda. The pots were kept in glasshouse condition. The pre-emergence mortality and post-emergence mortality was recorded.

The results in Table 4 revealed significant mortality differences between isolates as compared to control. Maximum mortality was recorded with isolate MP₁ (100%) it was followed by MP₂ (86.87%) over control (PLATE-9).

4.4.2.1 Pre-emergence mortality

Highest pre-emergence mortality was recorded with isolate MP₁ (68.75%) followed by MP₂ (47.5%) over control (0.00%). Amongst the varieties lowest pre-emergence mortality was recorded in Mangira (35.00%), followed by Parbhani 12 (36.66%). However, highest mortality were recorded in Nira (41.66 %) and Sharda (41.66 %).

4.4.2.2 Post emergence mortality

Results on Post-emergence mortality recorded at 30, 60 and 90 DAS were non significant, however mortality differences between isolates as compared to control were significant. Post-emergence mortality was higher in MP₂ (8.75, 11.56 and 19.06, respectively at 30, 60 and 90 DAS) as compared to MP-1.

Table 4. Pathogenicity test by sick soil technique

Sr. No.	Treatment	Pre-emergence mortality (%)	Post emergence mortality (at days after sowing)			Mean	Total mortality
			30	60	90		
	Cultivars(C)						
1.	Nira	41.66 (26.03)	5.00 (2.87)	8.34 (4.79)	11.66 (6.72)	8.33	66.66
2.	Mangira	35.00 (21.84)	5.41 (3.10)	5.83 (3.58)	10.41 (5.99)	7.21	56.66
3.	Parbhani 12	36.66 (22.39)	5.00 (2.87)	5.83 (3.58)	10.83 (6.24)	7.22	58.33
4.	Sharda	41.66 (26.03)	5.41 (3.11)	7.08 (3.83)	12.91 (7.45)	8.46	67.50
	SE $\pm$	1.5	0.6	0.56	0.48		
	CD at 5%	4.1	NS	NS	NS		
	Isolates (I)						
1.	MP ₁	68.75 (43.89)	7.50 (3.94)	9.06 (4.84)	14.68 (8.81)	10.41	100
2.	MP ₂	47.5 (28.58)	8.75 (5.02)	11.56 (6.47)	19.06 (10.99)	13.12	86.87
3.	MP ₀ (control)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	00
	SE $\pm$	1.3	0.52	0.57	0.42		
	CD at 5%	3.6	1.44	1.58	1.16		
	Interaction(CxI)						
	SE $\pm$	2.6	1.04	1.14	0.84		
	CD at 5%	7.2	2.89	3.17	2.32		

Figures in parents are angular transformed values.

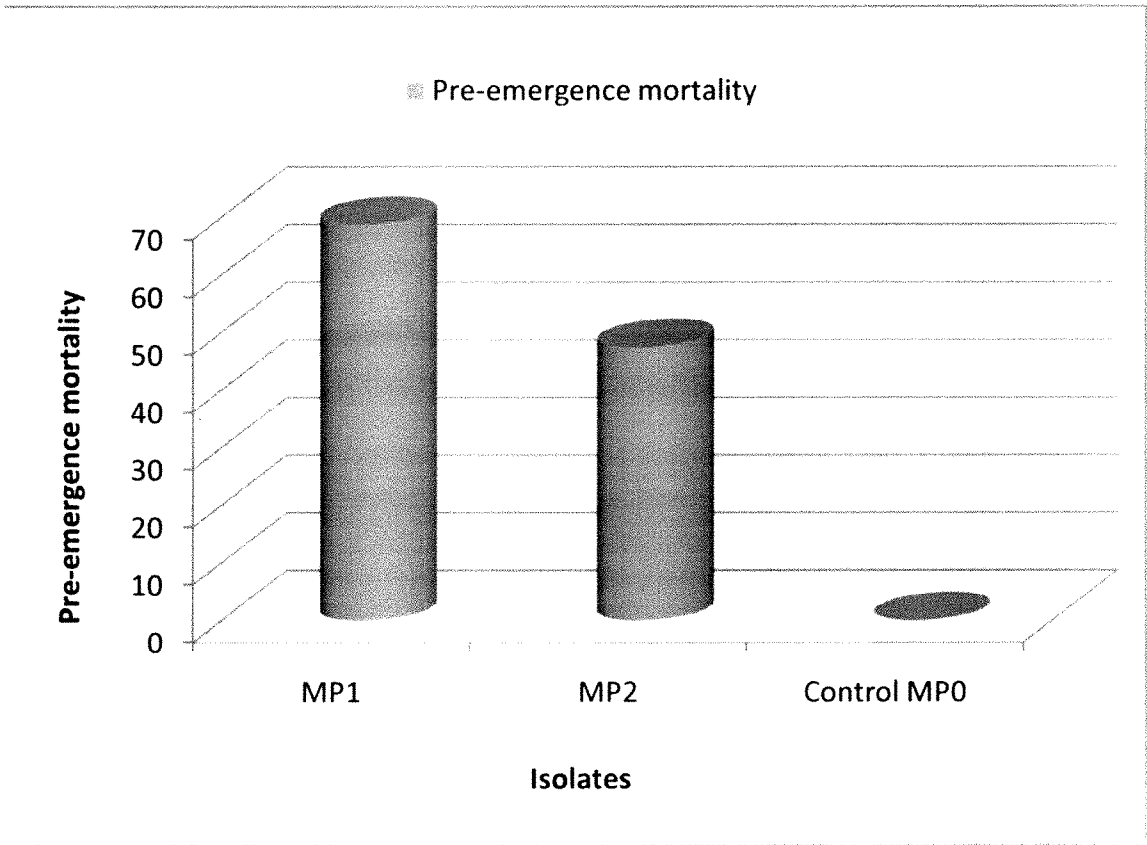
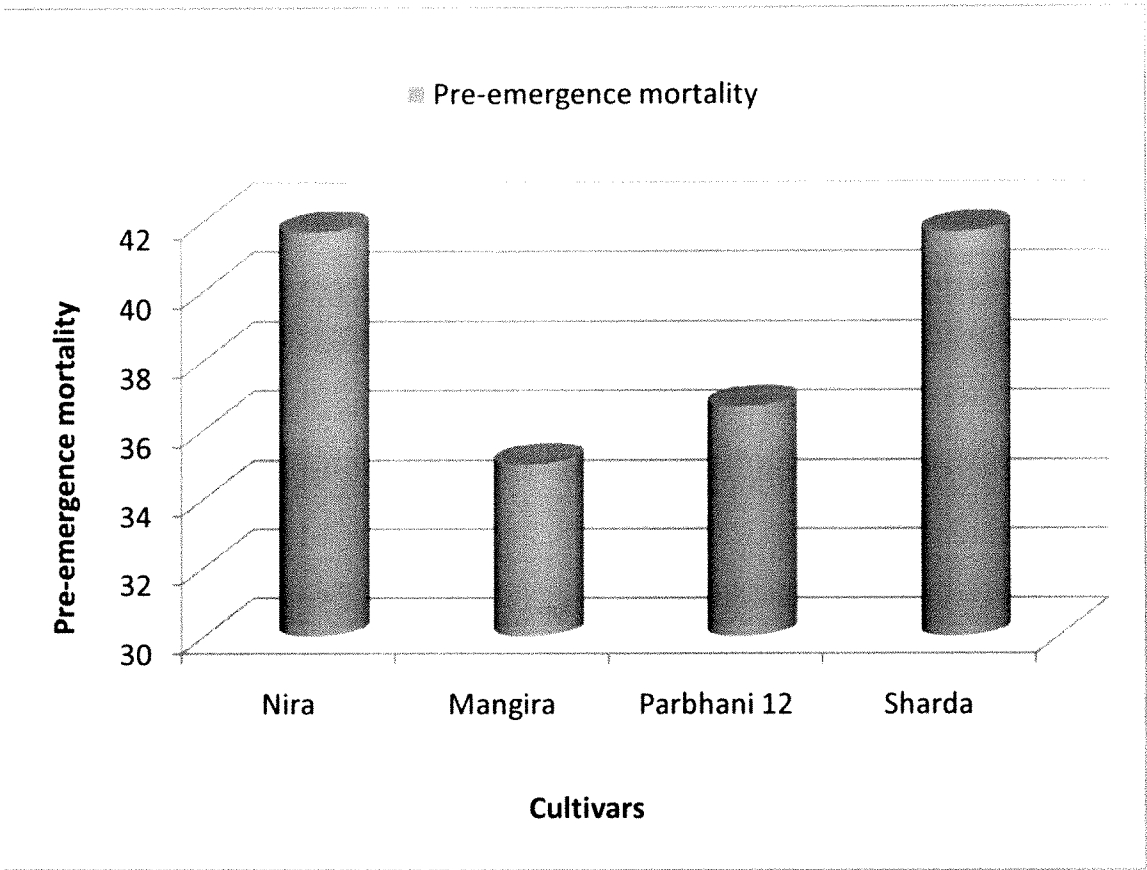


Fig 6. Pathogenicity test by sick soil technique

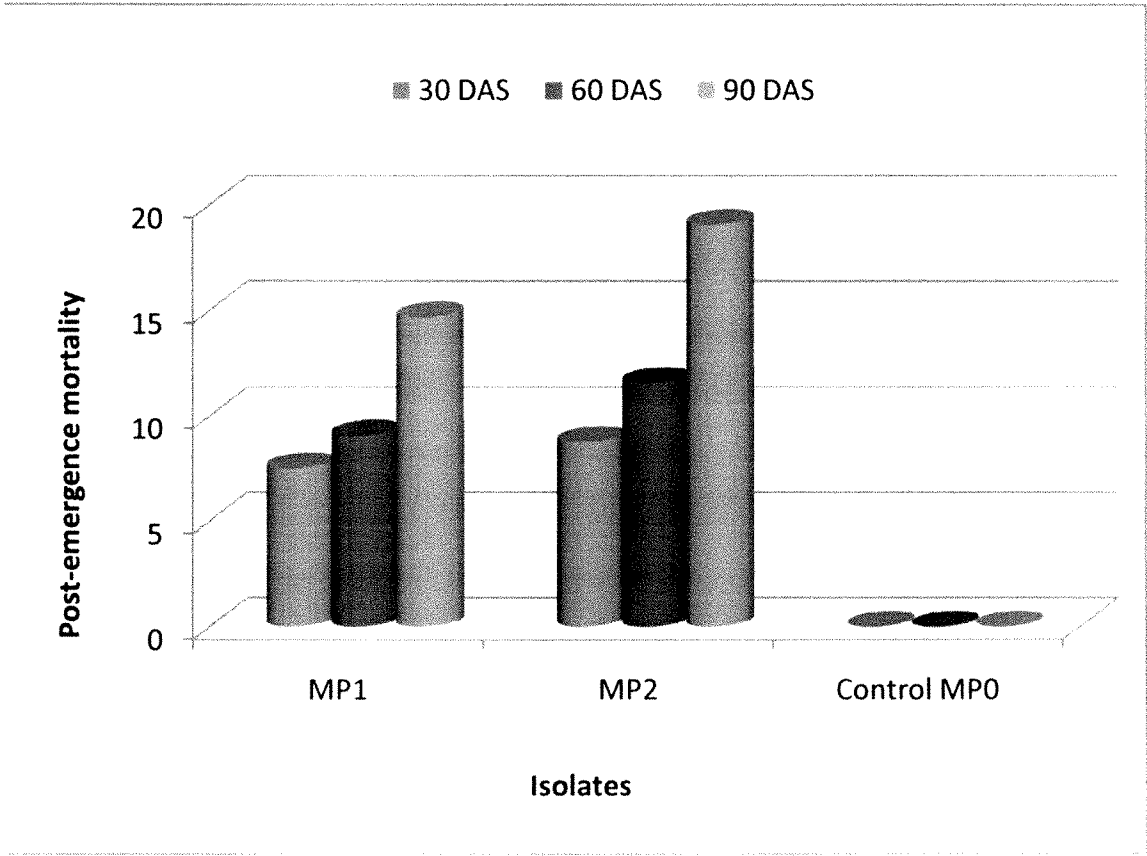
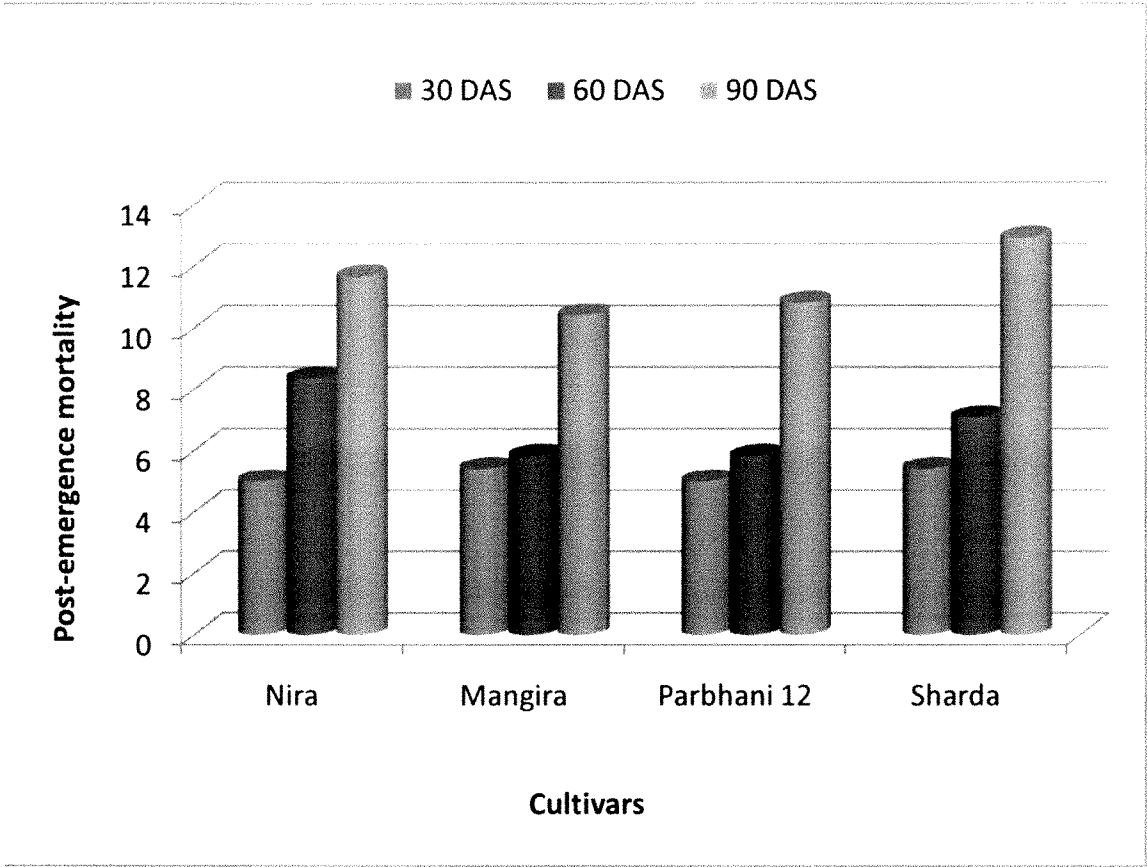


Fig 6a. Pathogenicity test by sick soil technique

Amongst the isolates tested with four varieties MP-1 isolate was found more virulent (100%). While, MP₂ was non virulent (86.87 %). Safflower varieties Sharda and Nira recorded highest mortality to the extent of 67.5 and 66.66 percent respectively. Least mortality was recorded in Mangira (56.66 %) followed by Parbhani-12 (58.33%) over control (0.00%).

4.5 Management with bioagents, chemicals, plant extracts and soil amendments

4.5.1 Management of root rot disease of safflower with bioagents *in vitro*

Three different species of *Trichoderma* viz., *T. hamatum*, *T. harzianum*, *T. viride* and one bacterial origin bioagent i.e. *P. fluorescens* were tested as biological agents for the control of *M. phaseolina*. The effect of bioagents against *M. phaseolina* were tested by dual culture technique and data are given in Table 5 (a, b c, d, e).

The mycelial growth of the pathogen and antagonist was recorded at 3, 5, 7 and 9 days of incubation and per cent reduction in colony diameter (mm) of pathogen over control was calculated. At 3 days of incubation *Trichoderma* spp. i.e. *T. harziafum* and *T. hamatum* reduced the mycelial growth of *M. phaseolina* over control. Amongst the antagonists maximum mycelial growth was observed in *Trichoderma harzianum* (25.40 mm) followed by *T. hamatum* (25.36 mm).

At 5 days of incubation maximum reduction of the pathogen was observed with *Trichoderma harzianum* (58.54 %) and it was followed by *Trichoderma hamatum* (51.18 %). Amongst antagonists maximum growth was observed with *Trichoderma harzianum* (31.88 mm) followed by *T. hamatum* (28.40 mm) and *T. viride* (26.39 mm).

Table 5a. *In vitro* inhibition of *M. phaseolina* by bioagents

Treatment	Mean colony diameter (mm) at 3 days		
	Pathogen	Antagonist	% reduction of colony diameter over control
T ₁ - <i>T.hamatum</i>	13.89	25.36	33.55
T ₂ - <i>T. harzianum</i>	8.72	25.40	58.28
T ₃ - <i>T. viride</i>	21.81	18.92	-4.3
T ₄ - <i>P. fluorescens</i>	22.19	13.75	-6.17
T ₀ - Untreated	20.90	0.00	
SE $\pm$	0.814	1.06	
CD at 5%	2.45	3.20	

Table 5b. Mean colony diameter (mm) at 5 days

Treatment	Mean colony diameter at 5 days		
	Pathogen	Antagonist	% reduction of colony diameter over control
T ₁ - <i>T.hamatum</i>	16.59	28.40	51.18
T ₂ - <i>T. harzianum</i>	14.09	31.88	58.54
T ₃ - <i>T. viride</i>	28.45	26.39	16.28
T ₄ - <i>P. fluorescens</i>	27.86	25.00	18.02
T ₀ - Untreated	33.98	0.00	0
SE $\pm$	0.855	1.10	
CD at 5%	2.57	3.58	

Table 5c. Mean colony diameter (mm) at 7 days

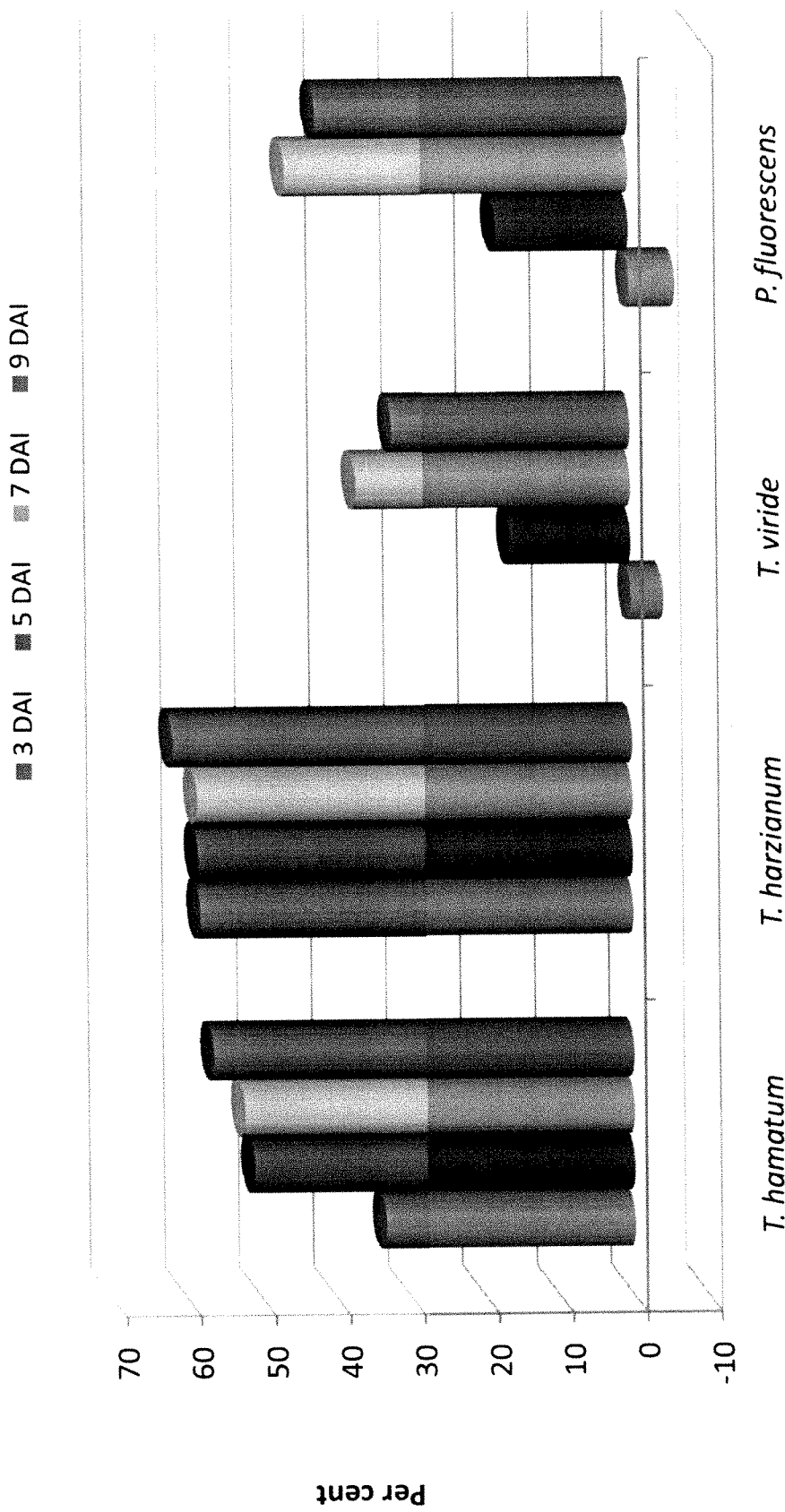
Treatment	Mean colony diameter at 7 days		
	Pathogen	Antagonist	% reduction of colony diameter over control
T ₁ - <i>T.hamatum</i>	27.35	35.30	52.51
T ₂ - <i>T. harzianum</i>	23.83	41.38	58.63
T ₃ - <i>T. viride</i>	36.24	38.37	37.08
T ₄ - <i>P. fluorescens</i>	30.93	38.31	46.30
T ₀ - Untreated	57.59	0.00	0
SE $\pm$	0.86	1.16	
CD at 5%	2.58	3.50	

Table 5d. Mean colony diameter (mm) at 9 days

Treatment	Mean colony diameter at 9 days		
	Pathogen	Antagonist	% reduction of colony diameter over control
T ₁ - <i>T.hamatum</i>	30.54	44.25	56.55
T ₂ - <i>T. harzianum</i>	26.80	49.34	61.87
T ₃ - <i>T. viride</i>	47.72	41.45	32.11
T ₄ - <i>P. fluorescens</i>	40.67	40.95	42.14
T ₀ - Untreated	70.28	0.00	0
SE $\pm$	0.749	0.734	
CD at 5%	2.25	2.21	

Table 5e. Per cent reduction in colony diameter of the pathogen over control

Treatment	Days after incubation			
	3	5	7	9
T ₁ - <i>T. hamatum</i>	33.55	51.18	52.51	56.55
T ₂ - <i>T. harzianum</i>	58.28	58.54	58.63	61.87
T ₃ - <i>T. viride</i>	-4.3	16.28	37.08	32.11
T ₄ - <i>P. fluorescens</i>	-6.17	18.02	46.30	42.14



Treatments

Fig. 7. Per cent reduction in colony diameter of the pathogen over control

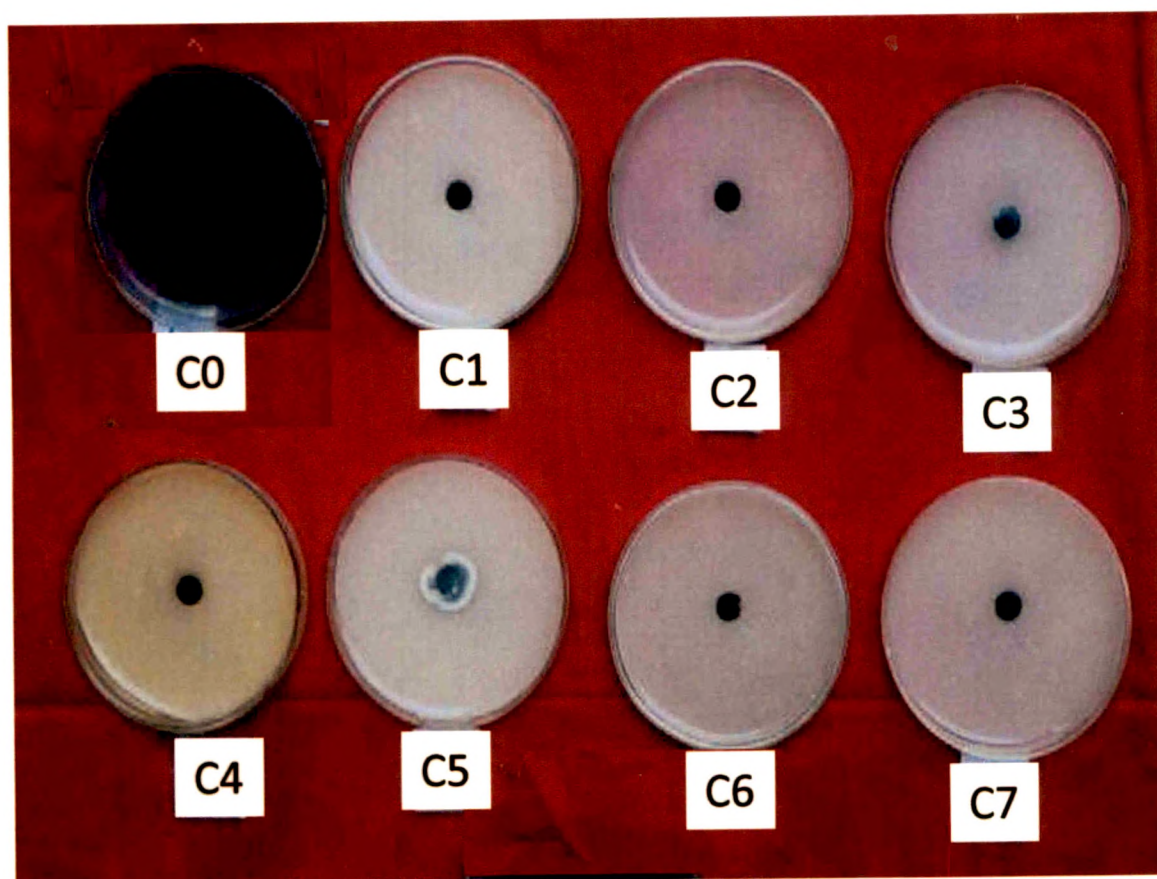


Plate 10. *In vitro* evaluation of fungicide

At 7 days of incubation all the species of *Trichoderma* reduced the mycelial growth of *M. phaseolina* over control. The maximum reduced growth of the pathogen was observed with *T. harzianum* (58.63 %) it was followed by *T. hamatum* (52.51 %) over control. Amongst antagonist maximum growth was observed in *T. harzianum* (41.38 mm) which was followed by *T. viride* (38.37 mm).

At 9 days of incubation all the species of *Trichoderma* and *Pseudomonas fluorescens* reduced the mycelial growth of *M. phaseolina* over control. The maximum reduction of pathogen was observed with *T. harzianum* (26.80 mm) followed by *T. hamatum* (30.54 mm). Amongst the antagonist maximum growth was observed in *T. harzianum* (49.34 mm) followed by *T. hamatum* (44.25 mm) and *T. viride* (41.45 mm).

Considering the per cent reduction of colony diameter (mm) of the pathogen over control after 3, 5, 7 and 9 days of incubation period the treatment *T. harzianum* was found best followed by *T. hamatum* and *P. fluorescens*.

4.5.2 *In vitro* management of *M. phaseolina* by chemicals

The results presented in Table 6 and plate-10 revealed that all the fungicides were significantly effective in reducing the radial mycelial growth of *M. phaseolina* over control. Significantly lower mycelial growth was obtained in carbendazim (500 ppm), chlorothalonil (500 ppm), hexaconazol (500 ppm) and captan (2500 ppm) over all the treatments. Mancozeb (2500 ppm) and benomyl (1000 ppm) these treatments were at par with each other. Propiconazole (500 ppm), benomyl (1000 ppm) and mancozeb (2500 ppm) were found to be least effective in reducing the growth. Maximum inhibition of *M. phaseolina* observed due to

carbendazim (100 %), chlorothalonil (100%), Hexaconazole (100%) and captan (100%).

Table 6. Efficacy of fungicides against *M. phaseolina* in vitro

Treatment	Five days after inoculation		
	Conc. (ppm)	Mean diameter (mm)	% inhibition over control
C ₁ - Carbendazim	500	0.00	100
C ₂ - Mancozeb	2500	5.05	94.39
C ₃ - Propiconazole	500	20.13	77.64
C ₄ - Chlorothalonil	500	0.00	100
C ₅ - Hexaconazol	500	0.00	100
C ₆ - Benomyl	1000	5.94	93.4
C ₇ - Captan	2500	0.00	100
C ₀ - Control	0	90.00	0.00
SE $\pm$		0.713	
CD at 5%		2.09	

4.5.2.1 Sclerotial production

The results presented in Table 7 reveals that all the fungicides tested significantly inhibited sclerotial production over untreated control (0.00). Significantly lower sclerotial production observed in carbendazim (500 ppm), chlorothalonil (500 ppm), hexaconazol (500 ppm) and captan (2500 ppm) treatments and were at par with each other. Benomyl (1000 ppm), mancozeb (2500 ppm) and propiconazole (500 ppm) these treatments were found at par with each other. However, fungicides carbendazim (500 ppm), chlorothalonil (500 ppm), hexaconazol (500 ppm) and captan (1000 ppm) recorded 100 per cent inhibition of average sclerotial production. This was followed by the fungicides mancozeb (96.59 %), benomyl (96.59 %) and propiconazole (94.43 %).

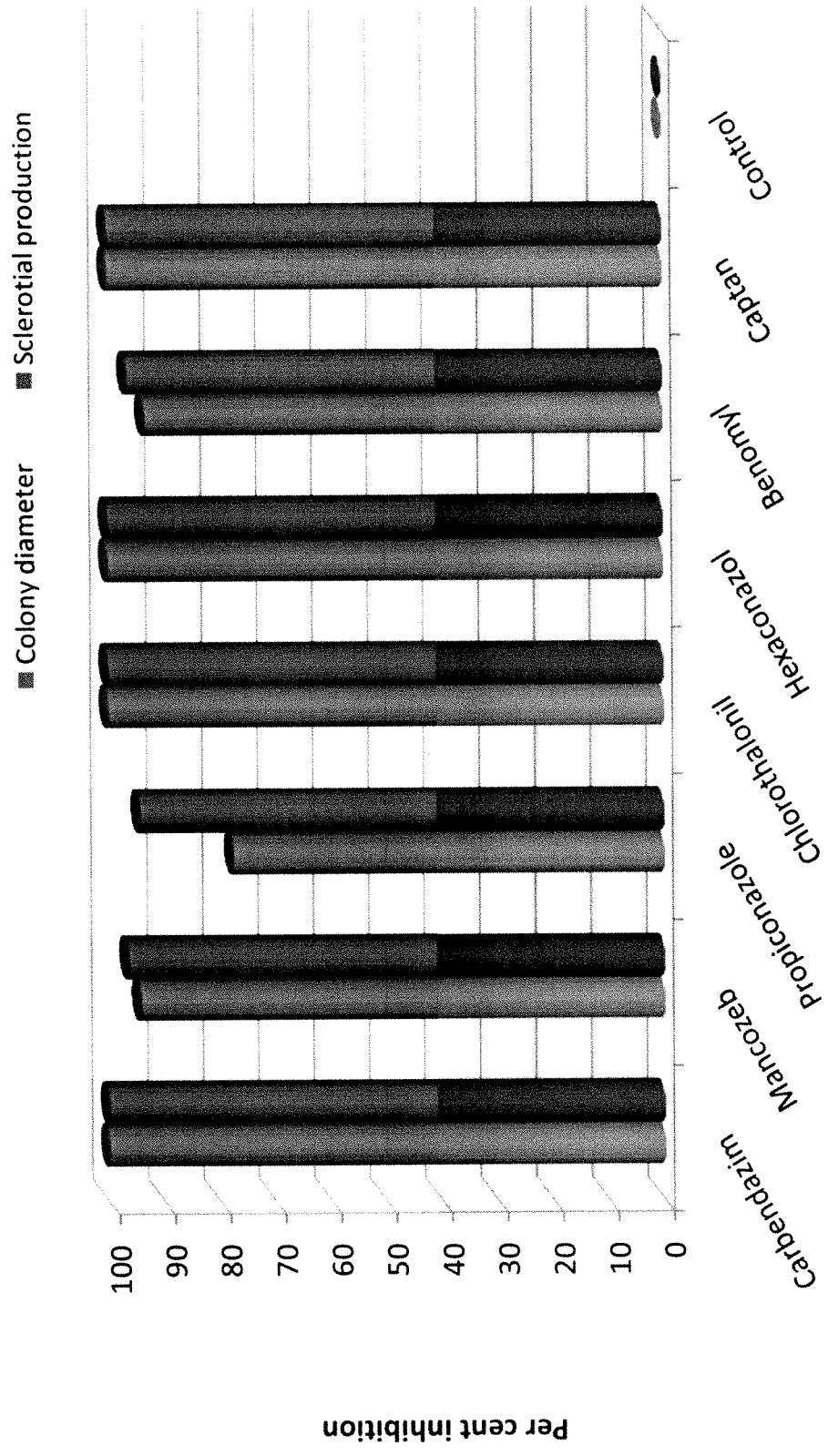


Fig. 8. Per cent reduction in colony diameter and sclerotial production of the pathogen over control

Table 7. *In vitro* effect of fungicides on sclerotial production and inhibition of *M. phaseolina*

Treatment	Five days after inoculums		
	Conc. (ppm)	Mean diameter (mm)	% inhibition over control
C ₁ - Carbendazim	500	0.00	100
C ₂ - Mancozeb	2500	4.75	96.59
C ₃ - Propiconazole	500	7.75	94.43
C ₄ - Chlorothalonil	500	0.00	100
C ₅ - Hexaconazol	500	0.00	100
C ₆ - Benomyl	1000	4.75	96.59
C ₇ - Captan	2500	0.00	100
C ₀ - Control	0	139.00	0.00
SE $\pm$		1.05	
CD at 5%		3.08	

4.5.3 Evaluation of plant extracts / botanicals

Plant leaf extract of seven botanicals were evaluated *in vitro* applying poisoned food technique and using PDA as basal medium in plate culture against *M. phaseolina* as detailed under.

Aqueous leaf extracts of seven botanicals viz., neem (*A. indica*), Aonla (*E. officinalis*), Ginger (*Zingiber officinale*), onion (*Allum cepa*), bougainvillea (*Bougainvillea* sp.), tulsi (*O. sanctum*), Ghaneri (*L. camera*) were evaluated @ 5 and 10%.

Results (Table 8) revealed that all the botanicals/plant extracts were found fungitoxic / fungistatic against the test pathogen and caused significant inhibition of mycelial growth over untreated control. Average mycelial growth of the test pathogen recorded with all botanicals was ranged from 49.73 to 82.85 mm. However, significantly least average

mycelial growth was recorded with neem (49.73 mm) followed by ginger (69.25 mm) and onion (73.36 mm).

Table 8. *In vitro* effect of plant extract on mycelia growth and inhibition of *M. phaseolina*

Treatment	Mean colony diameter (mm) at		Average colony diameter (mm)	% inhibition at		Average inhibition %
	5%	10%		5%	10%	
B ₁ - Neem leaf	58.57	40.90	49.73	34.93	54.56	44.74
B ₂ - Aonla	88.83	60.26	74.54	1.30	33.07	17.18
B ₃ - Ginger	82.87	55.81	69.25	7.93	37.99	22.96
B ₄ - Onion	86.07	60.66	73.36	4.37	32.60	18.48
B ₅ - Bougainvillea	89.73	75.98	82.85	0.30	15.58	7.94
B ₆ - Tulsi	87.45	60.65	74.02	2.84	32.62	17.73
B ₇ - Ghaneri	89.09	62.20	75.64	1.02	30.89	15.95
B ₈ - Control	90.00	90.00	90.00	0.00	0.00	0.00
SE $\pm$	1.5	1.8				
CD at 5%	4.5	5.3				

Table 8a. *In vitro* effect of plant extracts on sclerotial production

Treatment	Sclerotial production at		Average sclerotial production	% inhibition at		Average inhibition %
	5%	10%		5%	10%	
B ₁ - Neem leaf	47.50	26.75	37.12	69.41	82.77	76.09
B ₂ - Aonla	137.5	107.75	122.62	11.44	30.60	21.02
B ₃ - Ginger	132.70	97.00	114.85	14.53	37.53	26.03
B ₄ - Onion	136.00	105.50	120.75	12.50	32.05	22.27
B ₅ - Bougainvillea	146.00	126.00	136.00	5.96	18.85	12.40
B ₆ - Tulsi	139.50	120.00	129.75	10.15	22.71	16.43
B ₇ - Ghaneri	144.00	114.25	129.12	7.25	26.41	16.83
B ₈ - Control	155.25	155.25	155.25	0.00	0.00	0.00
SE $\pm$	2.70	3.30				
CD at 5%	8.09	9.60				

However, neem leaf extract recorded least mycelia growth (58.57 mm) at 5 per cent. While rest of the botanicals were found least effective and recorded mycelial growth in the range of 82.87 to 89.73 per cent of the test pathogen at 5% concentration.

At 10% concentration neem leaf extract recorded significantly least mycelial growth (40.90 mm), followed by ginger (55.81 mm), Aonla (60.26 mm), tulsi (60.65 mm), onion (60.66 mm) and Ghaneri (62.20 mm) were at par with each other. Maximum colony diameter recorded in bougainvillea (75.98 mm)

Average percentage inhibition of mycelial growth was ranged from 7.94 to 44.74. However, significantly highest average inhibition was recorded with neem extract (44.74 %). This was followed by botanicals ginger (22.96 %), onion (18.48 %), tulsi (17.73 %), aonla (17.18 %).

Of the botanicals tested neem was found highly fungistatic against the test pathogen and recorded highest mycelia inhibition of 34.93 and 54.56 per cent respectively at 5 and 10% concentrations. The second and third rank botanicals found inhibitory against the test pathogen were ginger (7.93 %) and onion (4.37 %) at 5 per cent concentration and onion (37.99%) and aonla (33.07 %) at 10% concentration. Bougainvelleia was found negligible inhibition (0.30%) at 5% but slight inhibitory (15.58 %) at 10% concentration.

4.5.3.1 Sclerotial production

Results (8a) revealed that all the botanical tested @ 5 and 10% significantly inhibited sclerotial production as that of the mycelial growth inhibition of the test pathogen. Average number of sclerotial production was ranged from 37.12 to 136.00 with maximum in untreated

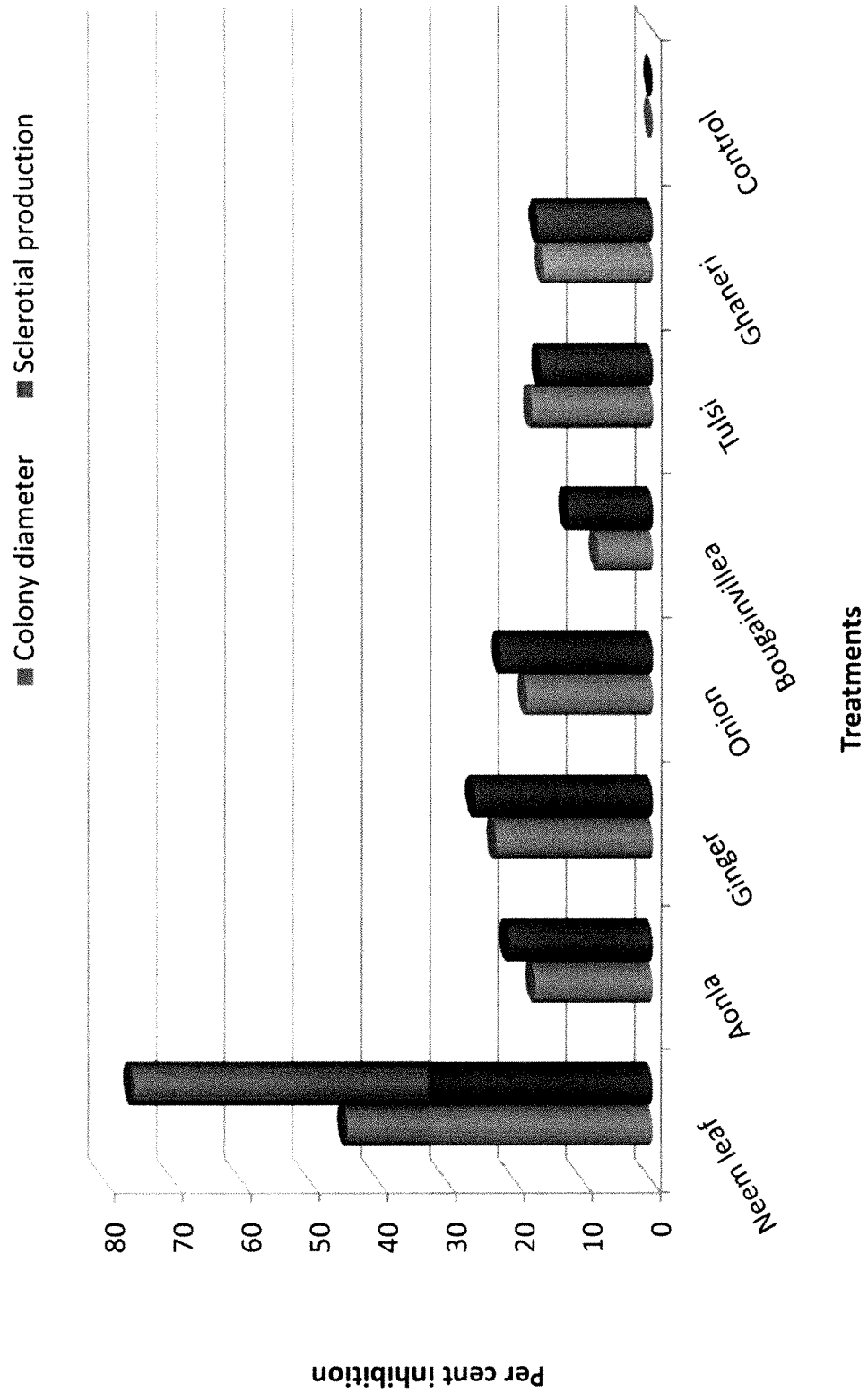


Fig. 9. *In vitro* effect of plant extracts on mycelial growth and sclerotial production

control (155.25). However, significantly least average number of sclerotia were recorded with neem leaf extracts (37.12) followed by ginger (114.85), comparatively maximum average number of sclerotia were produced in the botanicals *Bogainvillea* (136.00), tulsi (129.75), ghaneri (129.12) aonla (122.62) and onion (120.75).

All the botanicals tested significantly reduced sclerotial production at both the concentrations and it was ranged from 47.50 to 146.0 and 26.75 to 126.00 at 5 and 10 per cent concentration respectively. However, neem @ 5 and 10 % concentration recorded significantly reduced sclerotial number of 47.50 and 26.75. This was followed by ginger (132.7 and 97.00). Rest of the botanicals recorded sclerotial number in the range of 137.50 to 146.00 and 105.50 to 126.00 respectively at 5 and 10 per cent concentrations.

Neem leaf extract recorded significantly highest sclerotial inhibition of 69.41 and 82.77 per cent respectively at 5 and 10 % concentration. This was followed by ginger (14.53 and 37.53 %), onion (12.50 and 32.05 %). Rest of the botanicals recorded least inhibition of sclerotia in the range of 5.96 to 11.44 % and 18.85 to 30.60 % respectively at 5 and 10% concentrations.

Average inhibition of sclerotial production with test botanicals ranged from 12.40 to 76.09 per cent. However, highest sclerotial inhibition was recorded in neem leaf extract (76.09 %) followed by ginger (26.03 %), onion (22.27 %). Rest of the botanicals recorded average sclerotial inhibition in the range of 12.40 to 21.02 %.

Thus, all the botanicals were found effective and inhibited mycelial growth as well as sclerotial production of the test pathogen.

4.5.4 Effect of soil amendment on mortality of safflower

Soil amendment applied in soil influenced mortality of safflower significantly throughout observation period. All the soil amendments decreased seedling mortality significantly as compared to control from sowing.

In pre-emergence mortality neem seed cake (5%), karanj cake (8%) were significantly superior over rest of the treatments, however at par with each other. groundnut cake (15%) and safflower seed cake. Neem seed cake had significantly less seedling mortality than rest of the treatments (Table 9).

Table 9. Effect of soil amendment on germination and mortality of safflower seedling (pot culture)

Treatment	Mean germination (%)	Mean % mortality		Average mortality	% reduction mortality		Average reduction (%)
		Pre-emergence	Post-emergence		Pre-emergence	Post-emergence	
S ₁ - Neem cake	95 (74.43)	5 (2.86)	10 (5.73)	7.5	85.62	77.02	81.32
S ₂ - Groudnut cake	85 (59.41)	15 (8.65)	29 (16.88)	22	56.84	33.34	45.09
S ₃ - Karanj cake	92 (67.34)	8 (4.59)	24 (13.90)	16	76.98	44.83	60.90
C ₄ - Safflower cake	83.75 (57.48)	16.25 (9.36)	16.5 (9.49)	16.37	53.24	62.07	57.65
S ₀ -Control (<i>M. phaseolina</i> alone)	65.25 (40.93)	34.75 (20.38)	43.5 (25.80)	39.12	0.00	0.00	0.00
SE ±	4.21	1.95	1.12				
CD at 5%	12.68	5.87	3.87				

Figures in parentheses are angular transformed values.

Table 9a. Effect of soil amendment on root rot incidence (pot culture)

Treatment	Mean root rot incidence (DAS)			Mean PDI	% reduction over control
	30	60	90		
S ₁ - Neem cake	3.75 (2.15)	3 (1.72)	3.25 (1.86)	3.33	77.02
S ₂ - Groudnut cake	6.75 (3.87)	9.75 (5.60)	12.5 (7.18)	9.67	33.34
S ₃ - Karanj cake	6 (3.44)	6 (3.44)	12 (6.89)	8.00	44.83
C ₄ - Safflower cake	4 (2.29)	4.25 (2.43)	3.25 (4.87)	5.5	62.07
S ₀ -Control (<i>M. phaseolina</i> alone)	11.5 (6.60)	11.5 (6.60)	20.5 (11.84)	14.5	0.00
SE $\pm$	0.57	0.42	0.891		
CD at 5%	1.73	1.27	2.68		

Figures in parentheses are angular transformed values.

Table 9b. Effect of soil amendment on growth parameter of safflower (pot culture)

Treatment	Character		Per cent increase (+) or decrease (-) over control	
	Mean plant height	Mean biological yield/plot (g)	Mean plant height	Mean biological yield/plot (g)
S ₁ - Neem cake	43.51	37.90	+61.08	+99.89
S ₂ - Groudnut cake	35.00	28.71	+29.58	+51.42
S ₃ - Karanj cake	39.47	33.39	+46.13	+76.10
S ₄ - Safflower cake	33.90	27.15	+25.50	+43.19
S ₀ -Control (<i>M. phaseolina</i> alone)	27.01	18.96	0.00	0.00
SE $\pm$	2.23	1.95		
CD at 5%	6.88	6.01		

Post-emergence mortality ranged from 10 to 43.5 %. Neem seed cake was recorded significantly superior over rest of the treatments. Minimum seedling mortality recorded in neem seed cake (10%). However, maximum seedling mortality was observed in control (43.5 %).

Average reduction in per cent mortality was highest in neem seed cake (81.32 %) followed by karanj cake (60.90 %) and safflower cake (57.65 %). However, least mortality reduction was recorded in groundnut cake (45.09 %).

The results depicted in Table 9 indicated that neem seed cake was found effective than rest of the soil amendment treatments.

4.5.4.1 Post emergence mortality

At 30 DAS, neem seed cake, significantly reduced the root rot incidence (3.75%) whereas groundnut cake and karanj cake were at par with each other.

All treatments of soil amendments decreased seedling mortality significantly over control at 60 DAS. Neem seed cake and safflower cake were at par with each other. Neem seed cake (3 %) and safflower cake (4.25 %) recorded least root rot incidence over control (11.5%).

At 90 DAS, all treatments significantly reduced the disease incidence over control. Neem seed cake and safflower cake treatments were superior over rest of the treatments and recorded least disease incidence (3.25 %).

Percentage reduction in disease incidence over control was recorded in Neem seed cake (77.02 %) followed by safflower cake (62.07 %) and Karanj cake (44.83 %).

4.6 Screening of safflower genotypes

4.6.1 Super imposed observations in agronomical experiment against *M. phaseolina*

In agronomical experiment i.e. The effect of micronutrient along with recommended dose of fertilizers at 30, 60 and 90 days after sowing in PBNS-12 variety observations were recorded on root rot incidence in the field condition (Table 10).

At 30 days after sowing the incidence of root rot ranged from 1.44 to 4.27 per cent. Least incidence was recorded in RDF + ZnSO₄ @ 30 kg/ha (1.44 %) and RDF (1.95%). Highest disease incidence was noticed in RDF + elemental sulphur @ 5.1 kg/ha (4.27).

Disease incidence on 60 DAS ranged from 5.74 to 14.06 per cent. Least disease incidence was observed in RDF + ZnSO₄ @ 10 kg/ha (5.74 %) followed by RDF + FeSO₄ @ 10 kg/ha (7.05 %). However, highest disease incidence was recorded in RDF + elemental sulphur @ 5.1 kg/ha (14.06 %) followed by RDF (14.01 %).

At 90 DAS, the disease incidence ranged from 11.84 to 17.98 %. Lowest disease incidence was recorded in RDF + ZnSO₄ @ 20 kg/ha (11.84 %) followed by RDF + ZnSO₄ @ 30 kg/ha (13.89 %). Highest incidence was observed in RDF (17.98 %) followed by RDF + ZnSO₄ @ 10 kg/ha (17.33 %).

The mean disease incidence ranged from 7.96 to 11.75 per cent. Treatment RDF + ZnSO₄ @ 30 kg/ha showed least disease incidence (7.96) followed by RDF + ZnSO₄ @ 20 kg/ha (8.32 %) and RDF + ZnSO₄ @ 10 kg/ha (8.36 %). However, highest disease incidence was observed in RDF + elemental sulphur @ 5.1 kg/ha followed by RDF (11.73 %) and RDF + elemental sulphur @ 3.4 kg/ha (10.77 %).

Table 10. Effect of micronutrient alongwith RDF on root rot incidence

Treatment	Per cent disease incidence (days after sowing)			Mean disease incidence
	30	60	90	
T ₁ - RDF + ZnSO ₄ @ 10 kg/ha	2.01 (7.60)	5.74 (13.80)	17.33 (24.46)	8.36
T ₂ - RDF + ZnSO ₄ @ 20 kg/ha	3.60 (10.90)	9.52 (17.88)	11.84 (20.08)	8.32
T ₃ - RDF + ZnSO ₄ @ 30 kg/ha	1.44 (6.66)	8.57 (16.71)	13.89 (21.61)	7.96
T ₄ - RDF + FeSO ₄ @ 10 kg/ha	3.66 (11.00)	7.05 (15.35)	14.5 (22.28)	8.40
T ₅ - RDF + FeSO ₄ @ 20 kg/ha	3.40 (10.57)	8.52 (16.49)	17.07 (24.39)	9.66
T ₆ - RDF + FeSO ₄ @ 30 kg/ha	3.23 (10.26)	11.14 (19.34)	16.85 (24.23)	10.40
T ₇ - RDF + Elemental sulphur @ 1.70 kg/ha	3.14 (9.74)	11.92 (20.13)	15.57 (23.22)	10.21
T ₈ - RDF + Elemental sulphur @ 3.4 kg/ha	3.54 (10.75)	12.36 (20.58)	16.42 (23.75)	10.77
T ₉ - RDF + Elemental sulphur @ 5.1 kg/ha	4.27 (11.77)	14.06 (21.92)	16.92 (24.23)	11.75
T ₁₀ - RDF + FYM	2.68 (9.39)	11.58 (19.78)	15.82 (23.41)	10.02
T ₁₁ - RDF	3.56 (10.84)	13.66 (21.40)	17.98 (25.04)	11.73
T ₁₂ - RDF	1.95 (7.41)	14.01 (21.93)	14.43 (21.80)	10.13
SE $\pm$	1.4	1.44	1.73	
CD at 5%	NS	4.24	NS	

Figures in parents are angular transformed values.

4.6.2 Screening of breeding material of safflower

Total ten crosses of F4 generation were screened in replicated experiment against root rot of safflower incited by *M. phaseolina*. Root rot disease was recorded at 30, 60 and 90 DAS in each cross the data are presented in Table 11.

The root rot incidence at 30 DAS ranged from 5.06 to 11.69 per cent. Least disease incidence was recorded in cross MS II x PBNS 5-1-7 (5.06 %) followed by MS II x GMU 1962 (6.07%), MS 11 x BIP 2 (6.18), MS 104 x PBNS 5-1-7 (8.28 %) and MS 105 x PBNS 5-1-7 (9.03). Highest disease incidence was recorded in cross MS 102 x NARI 37 (11.69) followed by MS 1076 x BIP 2 (11.48 %) and MS 105 x GMU 1962 (9.36 %).

At 60 DAS disease incidence ranged from 8.52 to 15.52 per cent. Lowest disease incidence was observed in cross MS II x PBNS 5-1-7 (8.52 %) followed by MS II x BIP 2 (11.18 %), MS II x GMU 1962 (11.34 %) and MS 105 x PBNS 5-1-7 (12.41 %). Highest incidence was recorded in MS 102 x NARI 37 (15.52 %), MS 1076 x BIP 2 (15.17 %), MS 104 x PBNS 5-1-7 (15.11 %) and MS 105 x NARI 37 (14.09 %).

Disease incidence at 90 DAS was ranged from 14.84 to 21.03 per cent. Highest disease incidence was recorded in the MS 102 x NARI 37 (21.03 %), MS 104 x PBNS 5-1-7 (19.38 %), MS 105 x GMU 1962 (19.15 %), MS 105 x GMU 2523 (18.94 %) and MS 105 x PBNS 5-1-7 (18.77 %). However, lowest disease incidence was observed in MS II x PBNS 5-1-7 (14.84 %) followed by MS II x GMU 1962 (15.43 %) and MS II x BIP 2 (16.62 %).

Table 11. Reaction of safflower breeding material to *M. phaseolina*

Entry / crosses	Per cent disease incidence (days after sowing)			Mean disease incidence	Disease reaction
	30	60	90		
MS 105 x PBNS 5-1-7	9.03 (17.46)	12.41 (20.58)	18.77 (25.67)	13.40	MS
MS 105 x NARI 37	9.13 (17.53)	14.09 (22.01)	18.24 (25.24)	13.82	MS
MS II x PBNS 5-1-7	5.06 (12.96)	8.52 (16.77)	14.84 (22.64)	9.47	R
MS II x GMU 1962	6.07 (14.24)	11.34 (19.59)	15.43 (23.11)	10.94	MS
MS II x BIP 2	6.18 (14.29)	11.18 (19.50)	16.62 (20.05)	11.32	MS
MS 104 x PBNS 5-1-7	8.28 (16.69)	15.11 (22.87)	19.38 (26.11)	14.25	MS
MS 105 x GMU 2523	9.14 (17.46)	13.4 (21.41)	18.94 (25.79)	13.82	MS
MS 102 x Nari 37	11.69 (19.98)	15.52 (23.19)	21.03 (27.27)	16.08	MS
MS 1076 x BIP 2	11.48 (19.76)	15.17 (22.77)	18.10 (25.13)	14.91	MS
MS 105 x GMU 1962	9.36 (17.72)	13.52 (21.53)	19.15 (25.81)	14.01	MS
SE $\pm$	1.05	1.24	1.10		
CD at 5%	3.13	3.68	NS		

Figures in parents are angular transformed values.

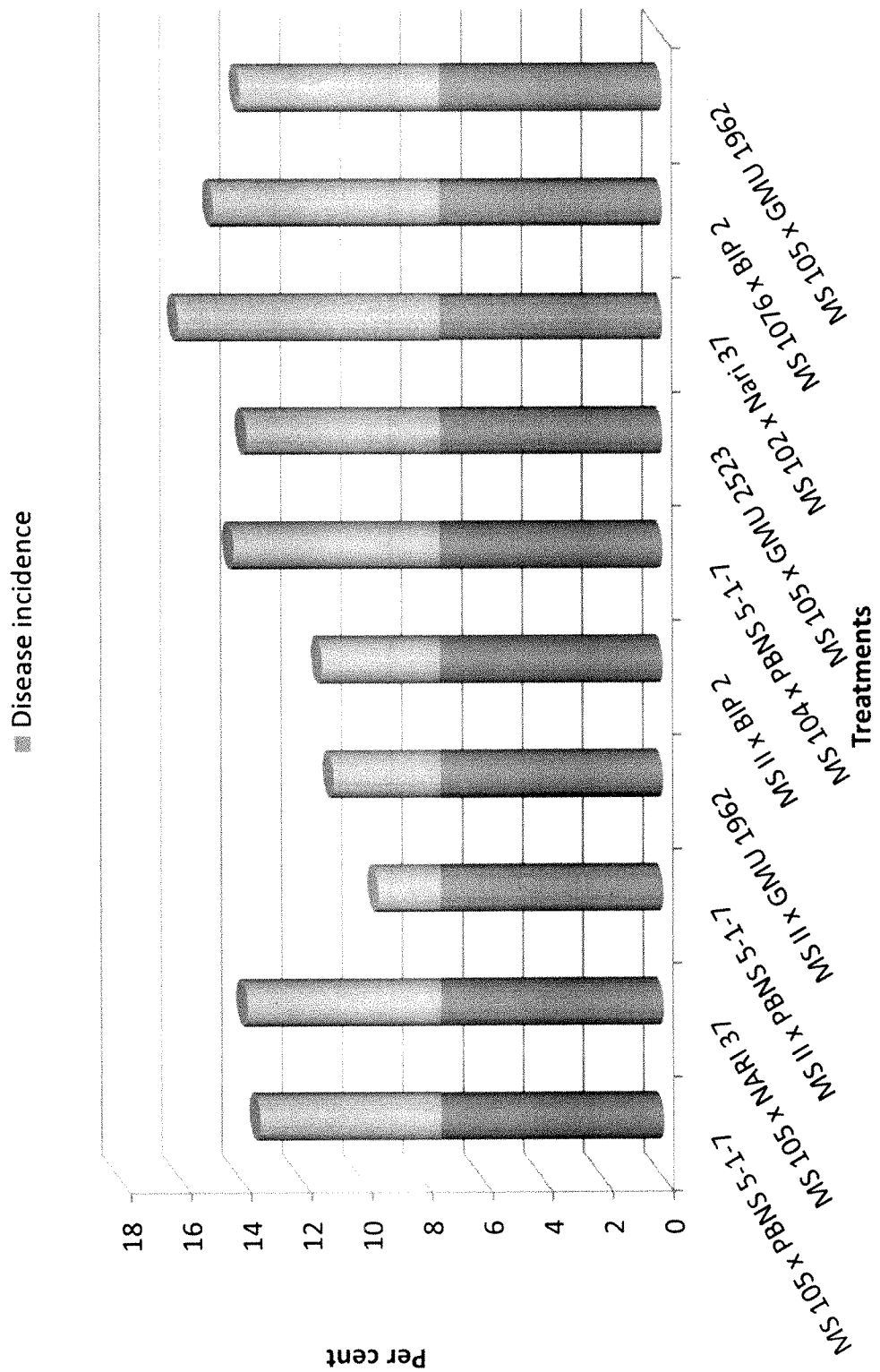


Fig. 10 Reaction of safflower breeding material to *M. phaseolina*

The mean disease incidence ranged from 9.47 to 16.08 per cent. Least incidence was recorded in MS II x PBNS 5-1-7 (9.47 %) followed by MS II x GMU 1962 (10.94 %), MS II x BIP 2 (11.32 %) and MS 105 x GMU 2523 (13.82 %). However, mean highest root rot incidence recorded in MS 102 x NARI 37 (16.08 %) followed by MS 1076 x BIP 2 (14.91 %) and MS 104 x PBNS 5-1-7 (14.25). Thus cross MS II x PBNS 5-1-7 showed resistant and other crosses showed moderately susceptible disease reaction.

4.6.3 Screening of safflower advanced varietal material against *M. phaseolina*

Total of thirteen advanced varieties were screened with local check against root rot of safflower. The advanced varietal trial further categorized into different disease reaction types (Table-12).

Observations on disease incidence recorded at 30 DAS ranged from 2.62 to 8.19 %. Least disease incidence recorded in AVT 09-01 (2.62 %) followed by AVT 09-06 (2.78 %), AVT 09-8 (3.23 %), AVT 09-03 (3.83 %) and AVT 09-02 (4.53 %). Highest disease incidence was observed in local check (8.19 %) followed by AVT 09-4 (7.85 %) and AVT 09-05 (7.65 %).

Disease incidence at 60 DAS ranged from 10.44 to 15.38 per cent. Highest disease incidence was recorded in local check (16.39 %) followed by AVT 09-10 (15.38 %), AVT 09-11 (14.58 %) and AVT 09-3 (13.00 %). Least disease incidence was observed in AVT 09-01 (10.44 %), AVT 09-04 (11.23 %), AVT 09-09 (11.23 %) and AVT 09-05 (12.23 %).

At 90 DAS, the root rot incidence was ranged from 13.34 to 20.60 per cent. Lowest disease incidence was recorded in AVT 09-01 (13.34 %), followed by AVT 09-3 (13.78 %), AVT 09-10 (16.09 %) and

Table 12. Reaction of safflower advanced varietal material to *M. phaseolina*

Entry / crosses	Per cent disease incidence (days after sowing)			Mean disease incidence	Disease reaction
	30	60	90		
AVT 09-01	2.62 (19.30)	10.44 (18.83)	13.34 (25.97)	8.80	R
AVT 09-2	4.53 (11.80)	12.52 (20.70)	19.46 (26.13)	12.10	MS
AVT 09-3	3.83 (10.88)	13.00 (21.33)	13.78 (21.41)	10.20	MS
AVT 09-4	7.85 (16.26)	11.23 (19.40)	19.18 (21.52)	12.75	MS
AVT 09-5	7.65 (16.02)	12.23 (20.46)	17.53 (24.75)	12.47	MS
AVT 09-6	2.78 (9.59)	12.57 (20.66)	18.16 (25.20)	11.17	MS
AVT 09-7	4.56 (12.02)	12.72 (20.83)	17.4 (24.38)	11.56	MS
AVT 09-8	3.23 (9.90)	11.61 (19.73)	16.93 (24.22)	10.59	MS
AVT 09-09	5.95 (14.07)	11.23 (19.40)	17.70 (24.81)	11.62	MS
AVT 09-10	6.17 (14.32)	15.38 (23.04)	16.09 (23.54)	12.54	MS
AVT 09-11	4.52 (12.20)	14.58 (22.24)	20.22 (26.65)	13.10	MS
AVT 09-12	6.95 (15.12)	13.00 (21.33)	17.24 (24.50)	12.39	MS
AVT 09-13	6.59 (14.57)	12.67 (19.92)	19.01 (25.76)	12.75	MS
Local check	8.19 (16.61)	16.39 (23.81)	20.60 (26.88)	15.06	MS
SE $\pm$	1.34	1.25	1.60		
CD at 5%	3.91	NS	NS		

Figures in parents are angular transformed values.

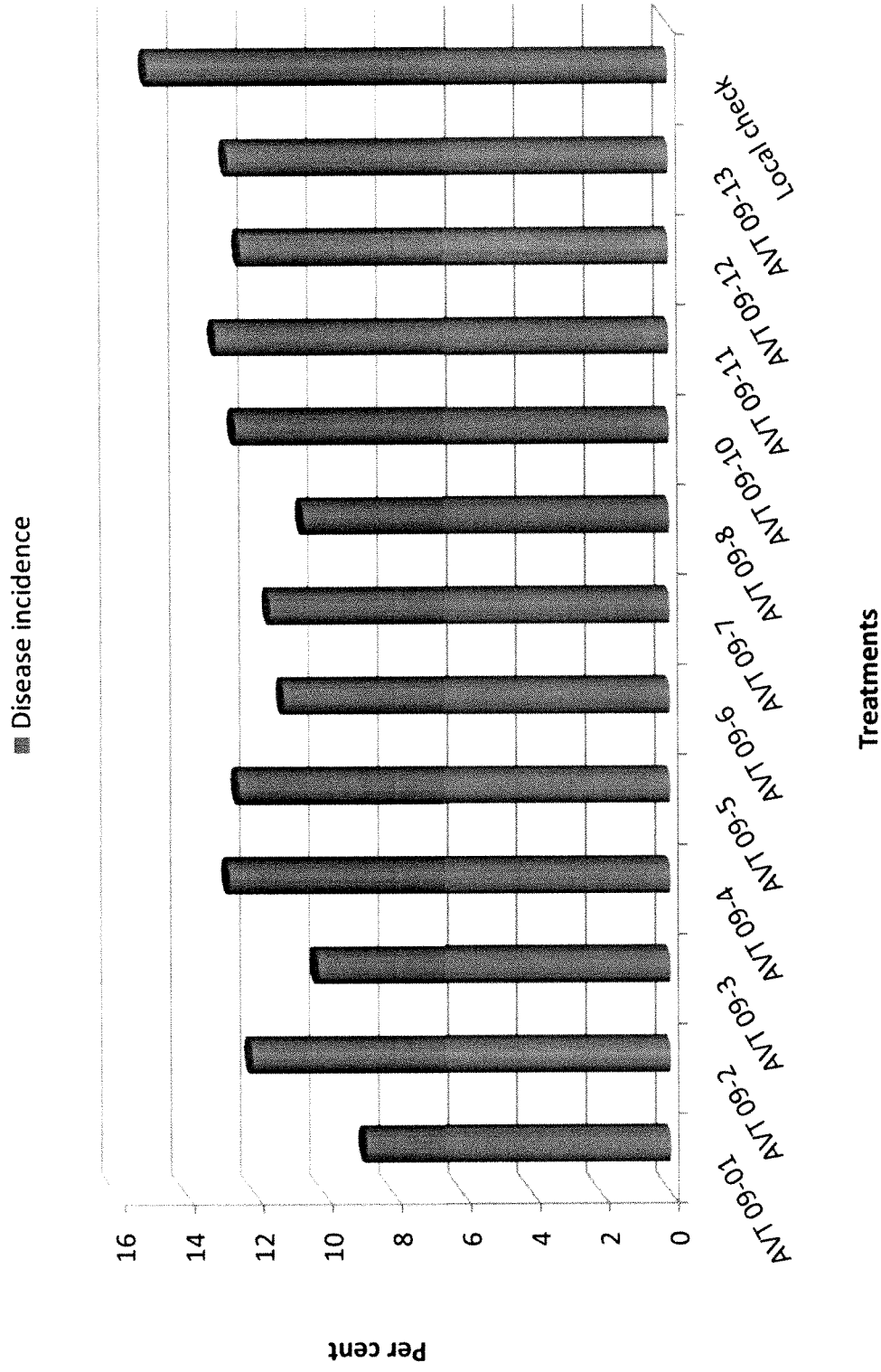


Fig. 11. Reaction of safflower advanced varietal material to *M. phaseolina*

AVT 09-8 (16.93 %). However, highest root rot incidence was recorded in local check (20.60 %) followed by AVT 09-11 (20.22 %), AVT 09-4 (19.18 %) and AVT 09-13 (19.01 %).

Mean per cent disease incidence was ranged from 8.80 to 15.06 per cent, variety AVT 09-01 (8.80 %) showed lowest disease incidence followed by AVT 09-03 (10.20), AVT 09-8 (10.59 %), AVT 09-6 (11.17 %), AVT 09-7 (11.56) and AVT 09-09 (11.62 %). Overall mean disease incidence was recorded highest (15.06 %) in local check followed by AVT 09-11 (13.10 %), AVT 09-04 (12.75 %) and AVT 09-13 (12.75 %).



Discussion

CHAPTER-5

DISCUSSION

Safflower (*Carthamus tinctorius*) belongs to family *Compositae* is one of the important oilseed crop of the world. Low level of cholesterol and adaptability to rabi conditions have created high potential demand for safflower oil in India. Diseases are the limiting factor in achieving target production of safflower. Amongst diseases root rot incited by *Macrophomina phaseolina* is the major constraint and responsible for qualitative as well as quantitative losses in safflower (Kore and Deshmukh, 1979; Shambarkar and Indi, 1987). The present investigation was therefore undertaken and results are discussed in this chapter.

5.1 Survey

Macrophomina phaseolina is an important pathogen distributed world wide and can attack different plant parts to cause leaf spot, blight, seed rot, root rot, collar rot, stalk rot, blossom and fruit rot. It is known to attack wide range of host plants including, pulses, oilseeds, sorghum, maize, jute, vegetable and fruit plants. As the organism has wide host range, they infect large number of weeds and rotation crops which function as a source of inoculums and may help in the survival of pathogen. *Macrophomina phaseolina* as one of the important plant pathogen causes root rot of safflower were reported previously by Saksena, (1979); Dingra and Sinclair, (1978), Carta and Franceschini(1982). A survey was undertaken in January 2010 in eight district of Marathwada region. Higher

disease incidence was recorded in district Beed. The incidence ranged from 11.24 per cent to 15.17 per cent.

5.2 Symptomatology

The disease manifested with sudden and complete wilting. The spread of the disease in the field was found in the form of concentric circles. Root rot affected plant pulled out easily, entire root system rotted. Only tap root remained attached to plant, the remaining root system found decay and retained in the soil. Fungal sclerotia appeared as minute black dots on the surface of the woody tissue on rotted bark. Grey to black mycelial mat of the fungus on the inner surface and infected plants results in poor growth and death of plant. These symptoms typically resembled with those previously reported by Uppal (1931), Hillerbrand *et al.* (1945), saxena (1979) and Govindappa *et al.* (2005).

5.3 Pathogenicity

The Koch's postulates was proved. Safflower roots depicting typical disease symptoms were used for isolation of causal pathogen. Pathogen was purified by hyphal tip method and maintained on PDA for further studies. Pathogenicity of fungus was established by dipping the healthy seedling in test tubes filled with culture. Symptoms were expressed after 12 hours of incubation. Early symptoms were manifested on the roots and stem, entire root system was found rotted. The yellowing started from the lower portion of plant and gradually extended upwards (Nene *et al.*, 1981).

Pathogenic nature of *Macrophomina phaseolina* was studied by sowing the seeds in earthen pots filled with sick soil. Symptoms were observed after 15 days of sowing. In inoculated experiment, early symptoms were manifested on the root and entire root system was found

rotted, the decay was more in lateral and thinner roots. At the time of flowering the yellowing started from the lower portion of plant and gradually extended upwards. The collar region of the plant showed grayish black discolouration which was often shriveled and plant was broken at this point.

The symptoms produced on artificially inoculated plants were similar to those produced naturally. The isolated and reisolated fungus was found to be identical on the basis of their morphological characteristics. Based on colony character, morphology of the fungus and pathogenicity test the pathogen was identified as *Macrophomina phaseolina*. The identification was further confirmed with the description of *Macrophomina* given by Hilderbrand *et al.* (1945), Sahi *et al.* (1992), Moulin *et al.* (1994), Prameela Devi and Singh (1998) and Bainade *et al.* (2005).

5.4 Management with bioagents, chemicals, plant extracts and soil amendments

5.4.1 Effect of bioagents on *Macrophomina phaseolina* in vitro

Three species of *Trichoderma* viz., *T. hamatum*, *T. harzianum* and *T. viride* and one bacterial bioagent i.e. *Pseudomonas fluorescens* were tested for their antagonistic effect on root rot causing pathogen *Macrophomina phaseolina* in safflower.

The mycelial growth (diameter in mm) of the pathogen and antagonist recorded at 3, 5, 7 and 9 days of incubation period were statistically significant over control. *Trichoderma viride* recorded very poor performance in suppressing the growth of the pathogen at all the incubation periods tried. However, *Trichoderma harzianum* recorded significantly superior in per cent reduction of mycelial colony diameter (mm) of the pathogen at all the incubation periods tested.

Per cent reduction in colony diameter (mm) of the pathogen after 3, 5, 7 and 9 days of incubation in *Trichoderma harzianum* was noticed to the tune of 58.28, 58.54, 58.63, 61.87 per cent, respectively. The next best antagonist noticed was *T. hamatum*.

Results obtained in respect of efficacy of bioagents in effectively inhibiting the *Macrophomina phaseolina* are conformity with those reported earlier by Rauchander *et al.* (1997), Biswas Sen (2000) Kaswate (2003), Dhoke and Kurundkar (2006), Ray *et al.* (2007) and Kar and Sahu (2008).

5.4.2 *In vitro* effect of chemicals on *Macrophomina phaseolina*

Chemical control is one of the measures to manage the disease and avoid the losses. Attempts to manage this disease by the use of chemicals had been made by several workers. The evaluation study was therefore conducted *in vitro*. Seven fungicides were tested against the pathogen i.e. *Macrophomina phaseolina*.

The highest inhibition (100%) of *M. phaseolina* was observed due to carbendazim (500 ppm), chlorothalonil (500 ppm), hexaconazol (500 ppm) and captan (2500 ppm) followed by mancozeb (2500 ppm), (94.39 %) and benomyl (1000 ppm) (93.4 %) and rest of the treatments significantly inhibited colony growth over control.

The significantly highest inhibition (100%) of sclerotial production was recorded due to carbendazim (500 ppm), chlorothalonil (800 ppm), hexaconazol (500 ppm) and captan (2500 ppm) followed by mancozeb (2500 ppm) (96.59 %) and benomyl (1000) (96.59%).

Results obtained in respect of the efficacy of fungicides effectively inhibiting colony growth and sclerotial production of *M.*

phaseolina are in conformity with those reported earlier in safflower, groundnut, black gram, cowpea (Jhooty and Bains, 1972; Kapoor and Chohan, 1974; Goel and Mehrotra, 1981; Giri and Peshney, 1983a; Hooda and Grover, 1983b; Martine *et al.*, 1984; Patil and Wangikar, 1984; Thakre and Gahukar, 1989; Gautam and Narain, 1996; Devi and Sidngh, 1997; Sheela and Packiaraj, 1999; Lambhate *et al.*, 2002).

5.4.3 Evaluation of botanicals by plate culture

Aqueous plant leaf extracts of 7 botanicals viz., neem (*A. indica*), aonla (*E. officinalis*), ginger (*Zingiber officinale*), onion (*Allum sepa*), Bougainvillea (*Bougainvillea* spp.), Tulsi (*O. sanctum*) and Ghaneri (*L. camera*) were evaluated against *M. phaseolina* applying poisoned food technique in plate culture.

Results (Table 8) revealed that all the botanicals evaluated *in vitro* (@ 5% and 10% each) significantly inhibited mycelial growth of the test pathogen over untreated control. However, neem leaf recorded significantly least mycelial growth of 58.57 mm and 40.90 mm and highest mycelia inhibition of 34.93 and 54.56 per cent, respectively at 5 and 10 per cent concentrations. While rest of the botanicals were found least effective and recorded more than 92.7 % mycelia growth of the test pathogen. However, Bougainvillea recorded very poor inhibition (0.30 and 15.58 % at 5 and 10% concentration).

Similar trend was observed in respect of Sclerotial production and their inhibition with test botanicals. Results (Table 8a) indicated that all the botanicals tested (5% and 10%) significantly reduced sclerotial production and increased the inhibition of sclerotial production. Average sclerotial production was ranged from 37.12 to 136.00 compared to untreated control (155.25). The corresponding average inhibition of

sclerotial production as ranged from 12.40 to 76.09 per cent over untreated control (0.00 %). However, botanicals neem followed by ginger, onion, aonla, ghaneri, tulsi and bougainvillea recorded average sclerotial production respectively of 37.12, 114.85, 120.75, 122.62, 129.12, 129.75 and 136.00 over untreated control (155.25). The highest inhibition of sclerotia recorded with neem (76.09 per cent) followed by ginger (26.03 %) and onion (22.27 %). While bougainvillea recorded least average inhibition (12.40%).

Results obtained in present study in respect of antifungal properties and inhibitory effects of botanicals tested are in conformity with those reported earlier by several workers. Plant leaf extracts of *A. indica*, *P. longifolia*, *Eucalyptus spp.*, *P. hysterophorus*, *D. stramonium*, *V. rosea*, *L. camera*, *L. inermis*, *O. sanctum* against *R. solani*, *S. rolfsii*, *R. bataticola* and *A. alternata* were reported earlier by several workers (Desai and Kulkarni, 2002; Sharma *et al.*, 2002; Kaushal *et al.*, 2003; Pathak *et al.*, 2003; Gupta *et al.* 2005; Rao and Narayana, 2005; Karande *et al.*, 2007 and Tatarwal and Rai, 2007).

5.4.4 Effect of soil amendment on mortality and growth parameters

Four types of seed cakes were tested influence of organic soil amendment on germination of safflower and growth parameters viz., height of plant and biological yield.

Application of organic soil amendment significantly improved the seed germination, plant growth and seedling vigour. Neem cake significantly reduced pre-emergence and post-emergence mortality of safflower. Amongst the treatment neem cake found very effective.

Groundnut cake recorded very poor performance in controlling root rot pathogen.

Neem cake recorded highest average plant height (43.51 cm) and average biological yield (37.90 g/pot). However, least average plant height (33.90 cm) and average biological yield (27.15 g/pot) recorded in safflower cake. These results are in conformity with those reported earlier by Desai and Kulkarni (2002), Suriachandraselvan *et al.* (2005), Sundravadana and Alice (2006).

5.5 Screening of safflower, breeding material and advanced varietal trial

A total eleven treatments of micronutrient along with recommended dose of fertilizer in PBNS 12, ten crosses of breeding material (F4 generation) and thirteen advanced varieties were screened in separate set of experiment under natural epiphytotics.

Disease incidence in each set of experiment was recorded at 30, 60 and 90 DAS. Overall least mean disease incidence was recorded in micronutrient treatment RDF + ZnSO₄ @30 kg/ha (7.96 %) followed by RDF + ZnSO₄ @ 20 kg/ha.

Disease incidence on breeding crosses of F4 generation was recorded at 30, 60 and 90 DAS ranged from 5.06 to 11.69, 8.52 to 15.52 and 14.84 to 21.03 per cent, respectively. Based on mean performance all lines were observed as moderately susceptible except MS II x PBNS 5-1-7 (9.47 %) showed resistant reaction followed by MS II x GMU 1962 (10.94 %).

Amongst thirteen advanced varieties tested for disease incidence at 30, 60 and 90 DAS revealed that variety. AVT 9-01 (8.80 %) showed resistant reaction. However, rest of the advance varieties trial were

observed as moderately susceptible disease reaction. Similar type of work was also undertaken by several workers previously against root rot of safflower and Jowar. Deshmukh (1981), Ingle *et al.* (2004), Desai (2005), Pahlvani *et al.* (2007).



Summary



CHAPTER-6

SUMMARY

Safflower (*Carthamus tinctorius* L.) belongs to family Compositae is one of the important oil seed crop of Maharashtra state of India grown for its edible oil. Root rot disease incited by *Macrophomina phaseolina* Tassi (Goid.) play an important role in crop health.

Symptoms of disease include wilting of plant and yellow discoloration of woody tissue. Fungal sclerotia appeared as minute black dots on the surface of the woody tissue on rotted bark. Grey to black mycelial mat appeared in the fungus of the inner surface of infected root.

An extensive roving survey was conducted in major safflower growing areas of Marathwada region of Maharashtra state. Fifty four locations in eight district of Marathwada region were traveled and average disease incidence to the tune of 13.26 % has been observed.

Pathogenicity of the pathogen was tested by proving Koch's postulate on safflower seedlings of cv. Nira, Mangira, Parbhani-12 and Sharda by water culture technique and sick soil technique (pot culture). Symptoms were initiated after 12 hours incubation with water culture and 25 DAS by sick soil technique. Parbhani-12 and Mangira varieties recorded least mortality in both the methods.

Amongst antagonist tested *Trichoderma harzianum* was significantly superior in percent reduction of mycelial colony diameter (mm) of pathogen at all incubation periods tested.

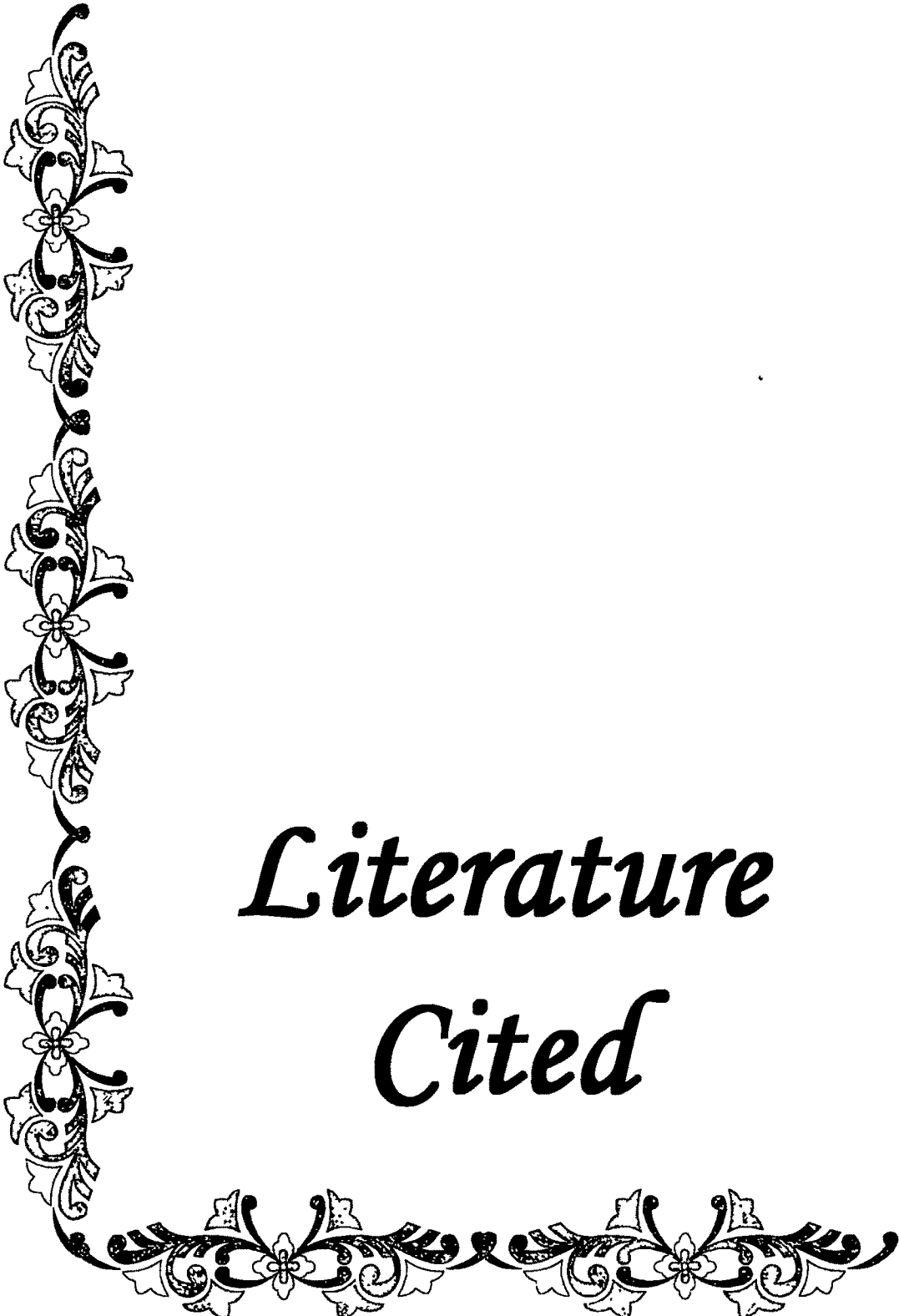
Maximum inhibition of per cent mycelial colony diameter (mm) and average sclerotial production of the pathogen were observed due

to carbandazim, chlorothalonil, hexaconazole and captan at different concentrations.

Of the plant extracts (botanicals) tested, Neem was found highly fungistatic against the test pathogen. Neem extract recorded highest average mycelial inhibition (44.74 %) and sclerotial inhibition 76.09 per cent, followed by ginger (average mycelial inhibition 22.96% and average sclerotial production 26.03%).

Neem seed cake as soil amendment gave appreciable average reduction over control (81.32%) followed by Karanj cake (60.90%). Maximum plant height and biological yield over control were noticed with Neem cake.

Screening of safflower material under natural condition of infection revealed that none of the entry was found completely free from disease, however, variety AVT-09-01 showed resistant disease reaction and rest of the advanced varieties were moderately susceptible.

A decorative floral border in black ink, featuring a vertical column of stylized flowers and leaves on the left, and a horizontal row of similar motifs at the bottom, forming an L-shape around the text.

Literature Cited

LITERATURE CITED

Anonymous, (2003). Safflower. Annual Progress Report of DOR, pp 8-10.

Anonymous, (2005). Safflower. Annual Progress Report of DOR, pp 12-15.

Anonymous, (2006). Safflower. Annual Progress Report of DOR, 3:101-107.

Arya, V.K.; Vishunuvat, and Negi N. (2004). Detection, location, and transmission of seed borne inoculation of *Macrophomina Phaseolina* in charcoal rot in soybean. J. Mycol. Pl. Pathol. 34(2): 233-237.

Bainade, P.S.; Tripathi B.P., Khare N. and Yadav V.K. (2005). Growth of *Macrophomina phaseolina* on different media pH and temperature levels. J. Pl. Dis. Sci., 1(1): 70-73.

Basu, K.C.; Choudhary A. and Sharma Y.R. (1988). *In vitro* evaluation of some fungicides against two soil pathogens. Pesticides. 22(10): 23-25.

Bhale, M.S.; Bhale, Usha and Khare, M.N. (1998). Disease of important oilseed crops and their management. In Pathological problems of economic crop plants and their management. Edts. S.M. Paul Khurana, Scientific Publishers, Jodhpur, pp 251-279.

Biswas, K.K. and Sen, C. (2000). Management of stem rot of groundnut caused by *Sclerotium rolfsii* through *Trichoderma harzianum*. Indian Phytopath., 53(3):290-295.

Carta, C. and Franceschini, A. (1982). Early blight of safflower (*Carthamus tinctorius* L.) caused by *Macrophomina*

- phaseolina*(Tass) Goid. desseccamentoprecoce declartomo (*Carthamus tinctorium* L.) Causato da *Macrophomina phaseolina* (Tassi) Goid. Studi. Sassaesi, III, **29**: 431-434.
- Chavan, R.A. (1998). Efficacy of *Trichoderma* in controlling charcoal rot of safflower. M.Sc. (Agri.) thesis, submitted to MAU, Parbhani, pp:50.
- Chirame, B.B. and Pandule D.N. (2005). Effect of *Trichoderma* sp. on the growth of *Macrophomina phaseolina*. Agric. Sci. Digest., **25**(3): 213-214.
- Desai, S.A. (2005). Field evaluation of advanced sorghum hybrids against charcoal rot disease caused by *Macrophomina phaseolina*. Indian J. Pl. Pathol., **23**(1&2). 55-57.
- Desai, S.A. and Srikant Kulkarni (2002). Effects of neem extracts and products on the growth and sclerotial formation of *Macrophomina phaseolina*. Indian J. Pl. Pathol. **20**(1&2): 58-60.
- Deshmukh, R.W. (1981). Charcoal rot of safflower (*Carthamus tinctorius* L.) caused by *Macrophomina phaseolina* (Tassi) Goid. M.Sc. (Agri.) thesis submitted to MAU, Parbhani.
- Devi, T.P. and Singh R.H. (1997). Screening of fungicides against seedling mortality of blackgram caused by *Macrophomina phaseolina*. Indian J. plant Prot., **25**(2): 123-127.
- Dhingre, D.D. and Sinccair, J.B. (1978). Biology and pathology of *M. phasiolina* Minss Geraij Brazil. University Federal de vicos, Brazil.



- Dhoke, P.K. and Kurundkar, B.P. (2006). Efficacy of fluorescent bacterial isolates against *M. phaseolina* *In vitro* J. Pl. Dis. Sci., 1(2): 203-204.
- Garg, R.P. and Chauhan, R.K.S. (1981). Occurrence of *Macrophomina phaseolina* on onion bulbs. Indian Phytopath., 34(4): 501-502.
- Gautam, V.S. and Udit Narain (1996). Efficacy of some fungitoxicants against *Macrophomina phaseolina* causing blight of cowpea. Annals Plant Prot. Sci., 4(1): 85-94.
- Giri, G.K. and Peshney N.L. (1983). Efficacy of some fungicides *in vitro* against fungi causing leaf spot in mungbean. J. soil and crops. 3(2): 112-114.
- Goel, S.K. and Mehrotra R.S. (1981). Chemical control of root rot of gram caused by *Rhizoctonia bataticola*. Acta. Botanica indica., 9(2): 228-232.
- Govindappa, M.; Lokesh, S. and Raj, V.R. (2005). A new stem splitting symptoms in safflower caused by *Macrophomina phaseolina*. J. of Phytopath. 153(2): 560-561.
- Gupta, S., Kalha, C.S., Vaid, A., Rizvi, S.E.H. (2005). Integrated management of anthracnose of french bean caused by *C. lindemuthianum*. J. Mycol. Pl. Pathol., 35(3): 432-436.
- Gupta, S.C. and Kolte S.J. (1981). Cultural characteristics of leaf and root isolates of *Macrophomina phaseolina* (Tassi). Goid. form groundnut. Indian Phytopath., 21(4): 345-346.
- Hillderbrand, A.A., Miller J.G. and Koach, L.W. (1945). Some studies on *Macrophomina phaseolina* (Mauhl.). Ashby. Sci. Agric. 25:690-706.

- Hooda, I. and Grover R.K. (1983). Comparative antifungal activity of fungitoxicants against *Rhizoctonia bataticola* causing seedling rot and foliage blight of mungbea. Indian J. Pl. Path., 1(1): 75-82.
- Hooda, I. and Grover R.K. (1990). Environmental factors affecting control of *Macrophomina phaseolina* by fungicides on mungbean. Pl. Disease Res., 5(1): 35-37.
- Ingle, V.N.; Deshmukh V.V., Jiotode D.J., Chore N.S. and Dhawad C.S. (2004). Screening of safflower varieties against root rot. (*Rhizoctonia bataticola*). Screen Crops 5(1):113-114.
- Jhooty, J.S. and Bains S.S. (1972). Evaluation of different systemic and non systemic fungicides against greengram leaf spot incidence. Karnataka J. Agri. Sci., 5(3): 241-243.
- Kapoor, S.P. and Chohan J.S. (1974). Evaluation of fungicides for the control of fruit rot of papaya caused by *Macrophomina phaseolina* Indian Phytopath, 27: 251.
- Kar, A.K. and Sahu, K.C. (2008). Effect of some biological agents on *M. phaseolina* causing root rot, seedling blight and leaf blight of mungbean. In national symposium of plant disease scenario in organic agriculture for eco-friendly sustainability and 60th annual meeting IPS, regional wheat rust research station, Mahabaleshwar, :59.
- Karande, M.G.; Raut, S.P. and Gawande, A.D. (2007). Efficacy of fungicides, bio-organics and plant extracts against *Colletotrichum gloeosporioides* and *Fusarium oxysporum*. Annl. Pl. Prot. Sci., 15(1): 235-281.

- Kaswate, N.S.; Shinde S.S. and Rathod R.R. (2003). Effect of bioagents against different isolates of *Rhizoctonia bataticola* butler *In vitro*. *Ann. Plant Physiol.* **17**(2): 167-168.
- Kaushal, G.; Rao; P.B. and Chauhan, S.V.S. (2003). Efficacy of some botanicals against *R. Solani*, *J. Mycol. Pl. Pathol.*, **33**(2): 230-235.
- Kishansingh, V.P.; Agnihotri, S.N. and Srivastava, S.K. (1973). Studies on the carbon and nitrogen requirement of *Rhizoctonia bataticola* and its response to variation in pH and temperature. *Phytopathology*, **27**: 85-88.
- Klisiewicz, J.M. and Houston, B.R. (1963). A new form of *Fusarium oxysporum*. *Phytoph.*, **53**(2): 241.
- Kore, S.S. and Deshmukh, R.W. (1979). Charcoal rot of safflower caused by *Macrophomina phaseolina*. *Res. Bul MAU*, **6**: 43-45.
- Lambhate, S.J.; Chaudhari, G.K., Mehtre, S.S. and Zanjare, S.R. (2002). *In vitro* evaluation of chemicals against root rot of cotton caused by *M. phaseolina*. *J. Maharashtra Agric. Univ.*, **27**(1):99-100.
- Likhite, V.N. (1936). Host range of the Gujrat root rot cotton. *Proc. Assan. Econ. Soi., Coimbatore*, **31**: 1820.
- Lokesh, N.M. and Benagi V.I. (2007). Biological management of pigeonpea dry root rot caused by *Macrophomina phaseolina*. *Karnataka J. agric. Sci.*, **20**(1): 54-56.
- Malathi, P. and Sabitha, D. (2003). Compatibility of *Trichoderma harzianum* with fungicides against. *Macrophomina phaseolina*. *Pl. Dis. Res.*, **18**(2): 139-143.

- Martin, S. Bruce, Lean, Lucas T. and Canbell C. Lee (1984). Comparative sensitivity of *Rhizoctonia solani* and *Rhizoctonia* like fungi to selected fungicides *in vitro*. *Phytopathology*, **74**(4): 778-781.
- Mathur, S.B. and Sinha, S. (1970). Role of manuring in control of root rot of guar (*Cyamopsis psorabides*) and wilt of gram caused by *S. rolfsii*. *Mycopath. Mycol. Appl.*, **40**:155-159.
- Mayer, W.A.; Sinclair, J.B. and Kher, M.N. (1974). Factor affecting charcoal rot of soybean seedling. *Phytopath.* **64**(845-849).
- Mehetre, N.M. (2000). Screening of safflower genotypes against *Fusarium oxysporum* f.sp. *carthami* causing wilt, M.Sc. (Agri.) thesis submitted to MAU, Parbhani pp :56.
- Miller, D.A. and Cooper, W.C. (1967). Green house technique too studying fusarium wilt of cotton. *Crop Sci.*, **7**: 75-76.
- Moniz, L. and Bhide, V.P. (1963). Root rot of cotton in Maharashtra and Gujrat states caused by *Macrophomina phaseolina* (Maudl) var. *Indian Cotton. Rev.*, **17**: 285-293.
- Moulin, F.; Lemanceau P. and Albouvette C. (1994). Pathogenicity of *Phythium* spp. on cucumber in peat-sand, rock wool and hydroponics. *European, J. Pl Path.*, **100**(1); 3-17.
- Nene, Y.L.; Kannaiyan, J. and Reddy, M.V (1981). Pigeonpea disease resistance screening techniques. *ICRISAT Information Bull.*, No. 9-14.
- Nirmal, D.D. (1985). Studies on wilt disease of safflower. Annual progress report of research work carried out by Dept. of plant pathology, MAU, Parbhani for the year 1984-85, pp :46.

- Pahlavani, M.H., Razavi S.E., Mirizadeh I., and Vakili S. (2007). Field screening of safflower genotypes for resistance to charcoal rot disease. *International J. of Pl. Prod.*, 1(1): 45-52.
- Pathak, D.V. Sharma, S. and Kumar, S. (2003). Comparative performance of biological agents in clusterbean root rot control. *J. Mycol. Pl. Pathol.*, 33(3): 482.
- Patil, B.C. (1962). Studies on *Macrophomina phaseolina* (Mauhl) Ashby. M.Sc. thesis submitted to Poona University. Poona.
- Patil, S.K. and Wangikar P.D. (1984). Effect of fungicides and different plant extracts on the growth of certain pathogenic fungi. Dr. PDKV, Akola. Thesis Abstr., 74: 334.
- Prameela, D.T. and Singh, R.H. (1998). Studies on virulence of *Macrophomina phaseolina* isolates from blackgram and greengram. *J. Mycol. Pl. Path.*, 28(2): 196-201.
- Raghuchander, T.; Rajappan, K. and Samippan, R. (1997). Evaluating methods of application of biological agents in the control of mungbean root rot. *Indian Phytopath.*, 50(2):229-234.
- Rao, C.H. and Narayana, Y.D. (2005). *In vitro* evaluation of fungicides, plant extracts and biocontrol agents against *C. dematium* the causal organisms of chickpea blight. In national symposium on crop disease management in dryland Agril. And 57th annual meeting IPS, MAU, Parbhani.
- Raut, J.G. and Bhombe B.B. (1977). Physiological studies of *Rhizoctonia bataticola* on sorghum. *J. Maharashtra Agric. Univ.*, 1: 264-267.

- Ray, A.; Kumar, P. and Tripathi, H.S. (2007). Evaluation of bioagent against *Rhizoctonia solani* Kuhn. the cause of aerial blight of soybean. Indian Pytopath., **64**(4): 532-534.
- Reichert, I. and Hallinger E. (1997). On the occurrence, morphology and paracitism of *Sclerotium bataticola*. Palost. J. Bot. Res., **6**: 107-147.
- Rodrigues, V.J.L.B.; Menezes M. and Coelho R.S.B. (1997). Effect of temperature, filter paper and culture media on physiology of *Macrophomina phaseolina*. Arquivos de Biologiae Technologia, **40**(1): 197-203.
- Sahi, S.T.; Shakri A.S., Bajwa M.N. and Intizar-ul-Hassan M. (1992). Physiological studies on *Macrophomina phaseolina* dry rot of mungbean. J. Agri. Res. Lahore, **30**(3): 409-413.
- Sajeena, A.; Salatrajan, F., Seetahraman, K., Erajvan, K. and Mohanbabu, R. (2003). Biological controls of root rot of blackgram caused by *M. phaseolina* (Tassi) Goid. National Seminar on IPDMSA, Annamalai (Abstract), University, pp 30.
- Saksena, H.K. (1979). Epidemiology of disease caused by *Rhizoctonia* species. In proceeding of the consultants group discussion on the resistance to soil born disease of legume 8-11 January, ICRISAT, Hyderabad, India.
- Sandhu, A. and Singh, R.D. (1999). Factors influencing susceptibility of cow pea to *M. phaseolina*. J. mycol. Pl. Pathol., **29**(3): 421-424.
- Satishlodha, Mathur, B.K. and Solanki, K.R. (1990). Factors influencing population dynamics of *Macrophomina phaseolina* in arid soils. Plant and Soi., **125**(1): 75-80.

Sengupta and Das, P.K. (2003). Cultivated annual oilseed crops of Indian/K. Kolkata, Naya Udyog, XII, 296 p., tables, figs., ISBN 81-85971-34-X.

Sethuraman, K.; Revathy N. and Manivappan M. (2003). Efficacy of biocontrol micro-organisms on root rot of black gram caused by *Macrophomina phaseolina*. Legumes Res., 26(3): 218-220.

Shambharkar, D.A. and Indi, D.V. (1987). Causal organism of safflower disease and their importance, Patil P.S. (Ed.). All India co-ordinated. Res. Project on oilseed (safflower). Nilama printing press Solapur pp: 181.

Shanmugam, V., Renukadevi, P., Senthids, N., Raguchander, T. and Samiyappan (2001). Interaction of *Pseudomonas fluorescens* with rhizome in management of blackgram root to incited by *M. phaseolina*. Pl. Dis. Res., 16(1):23-29.

Sharma, S.; Singh, S.D., Rawal, P and Lodha, P.C. (2002). Antifungi activities of plant extracts and oils against seed borne pathogen of pea. J. Mycol. Pl. Pathol., 32(1): 151.

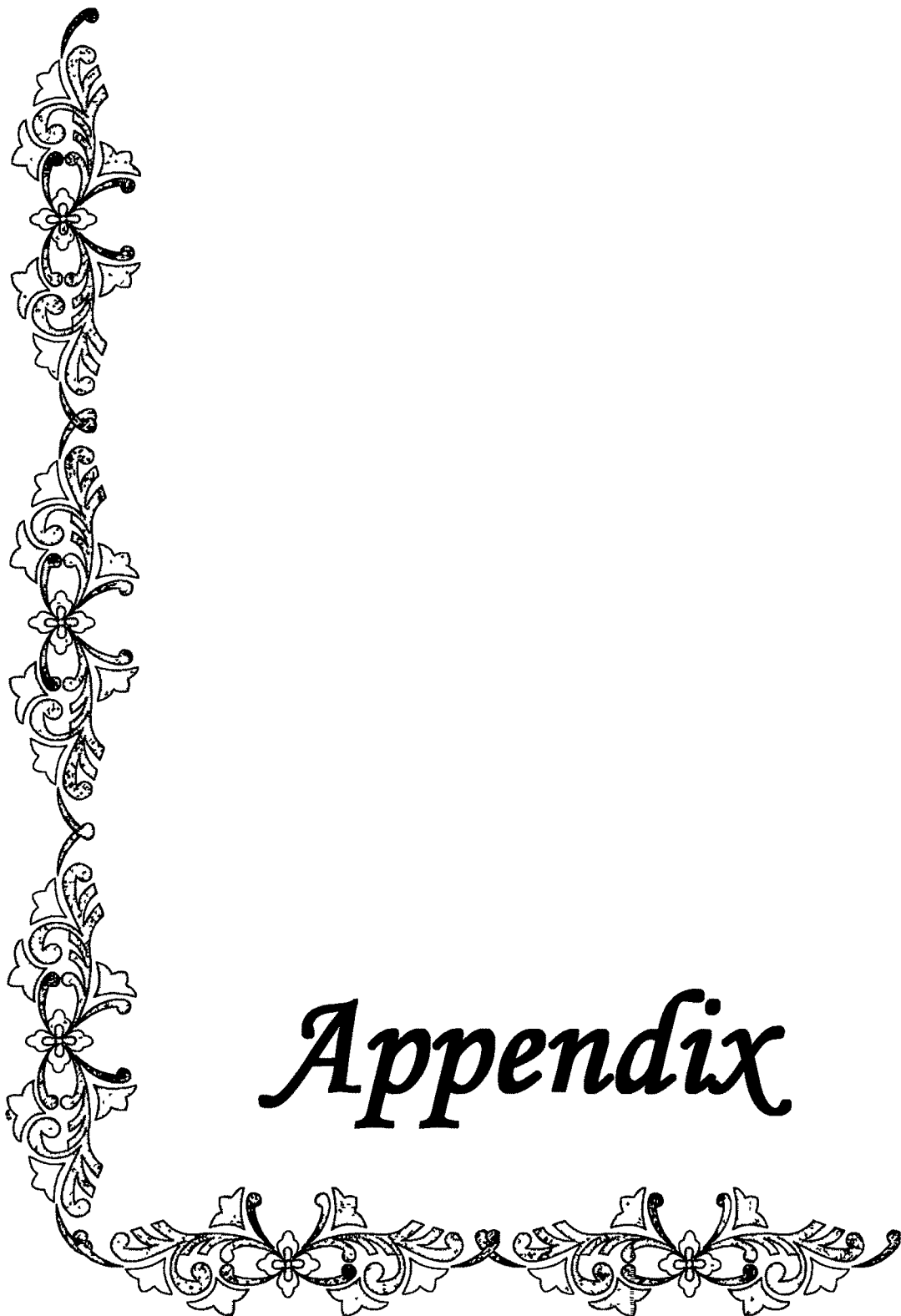
Sheela, J. and Packiaraj, D. (1999). Biological controls of root rot of groundnut. Madras Agric. J., 86(10-12):599-601.

Singh, R.D.N and Kaiser S.A.K.M. (1994). Effect of different culture media and pH levels on growth and cultural characteristics of charcoal rot pathogen (*Macrophomina phaseolina*) infecting maize. Crop Res. Hisar, 7(2): 282-287.

Singh, R.S. and Chohan J.S. (1982). Physio-pathological studies of *Macrophomina phaseolina* causing charcoal rot of muskmelon. Indian J. Mycol. Pl. Path., 12(1): 81-82.

- Sundravandana, S. and Alice D. (2006). Detence response induced by nutrients and biogents in blackgram roots infected by *Macrophomina Phaseolina*. Pl. Dis. Res., **2**(1): 26-29.
- Suriachandraselvan, M. and Seetharaman K. (2000a). Relationship among pigment synthesis, culture media, growth and virulence of the geographical isolates of *Macrophomina phaseolina* causing charcoal rot of sunflower. J. mycol.Pl. Pathol., **30**(3): 370-374.
- Suriachandraselvan, M. and Seetharaman K. (2000b). Survival of *Macrophomina phaseolina*, the causal agent of charcoal rot of sunflower in soil, seed and plant detris. J. Mycol. Pl. Pathol. **3**(3): 402-405.
- Suriachandraselvan, M. and Seetharaman K. (2002). Relationship between mycelia growth rate and virulence of the isolates. Madras. Agri. J. **89**(1-3): 103-106.
- Suriachandraselvan, M.; Salairajan F. and Aiyyanahan K.E.A. (2005). Effect of organic amendmets against characoal rot in sunflower. Madras Agri. J., **92**(10-12): 705-708.
- Surichandraselvan. M.; Aiyyanathan K.E.A. and Vimala R. (2005). Host range and cross inoculation studies on *Macrophomina phaseolina* from sunflower. Madras Agri. J. **92**(4-6): 238-240.
- Tetarwal, M.L. and Rai, P.K. (2007). Management of *Alternaria* blight of senna (*Cassia angustifolia*) through fungicides and plant extracts. Annl. Pl. Prot. Sci., **15**(2): 165-169.

- Thakre, R.H. and Gahukar K.B. (1989). Studies on leaf and stem blight of greengram caused by *Macrophomina phaseolina* (Tassi) Goid. thesis Abstr. pp. 341.
- Uppal, B.N.; Klathkar G.G. and Patel M.K. (1936). Blight and hallow stem of sorghum Indian J. Agric. Sci., 2 : 667-678.
- Uppal, B.N.; Kolhatkar, K.G. and Patel, M.K. (1931). Blight and hallow stem of sorghum. India J. Agric. Sci., 6(6): 1323-1324.
- Vasudeva, R.S. (1936). Some studies in the physiology of causal fungi of cotton rot. Ind. J. Agric. Sci., 6(4): 904-916.
- Wyllie, T.D. and Fry G. (1973). Liquid nitrogen storage of *Macrophomina phaseolina*. Plant Dis. Repr., 57: 478-480.
- Zote, K.K.; and Dhutraj, D.N. (2005). BPMR-14 multiple disease resistant mungbean variety. In national symposium on crop disease management in dryland agril., and 57th annual meeting IPS, MAU, Parbhani pp:11.
- Zote, K.K.; Dandnaik, B.P. and Khalikar, P.V. (1983). Reaction of mungbean cultivars to *Macrophomina* blight. J. Maha. Agric. Univ., 8(2): 146-147.



Appendix

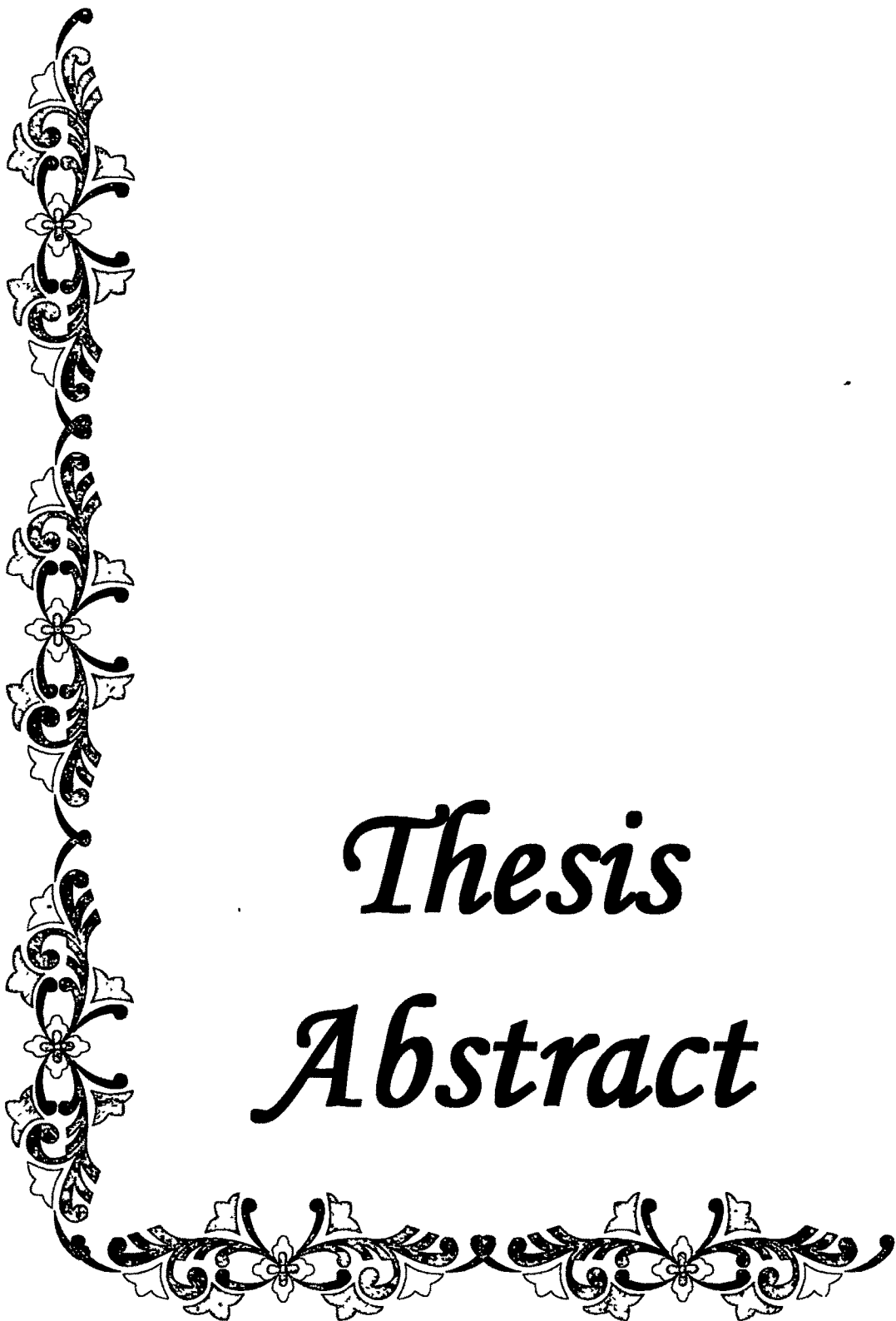
APPENDIX

1] POTATO DEXTROSE AGAR:

Peeled potatoes	:	200 gms
Dextrose	:	20 gms
Agar	:	20 gms
Distilled Water	:	1000ml

2] POTATO LIQUID BROTH:

Peeled potatoes	:	200gms
Dextrose	:	20 gms
Distilled Water	:	1000ml

A decorative floral border in black ink, featuring intricate scrollwork, leaves, and small flowers. It runs vertically along the left side of the page and horizontally across the bottom, framing the central text.

Thesis Abstract

a) Title of the thesis : **STUDIES ON ROOT ROT OF SAFFLOWER
INCITED BY *Macrophomina phaseolina*
(Tassi) Goid**

b) Name of the student : **ADSUL AMARCHAND KESHAV**

c) Degree to be awarded : M.Sc. (Agriculture)

d) Year of award of degree : 2010

e) Major subject : Plant Pathology

f) Total number of pages : 72
in the thesis

g) Number of words in : 281
thesis abstract

h) Signature of the student : 
(Adsul A.K.)

i) Signature, name and : 
address of major advisor : **Prof. P.V. Khalikar**
Associate Professor
Dept. of Plant Pathology
Marathwada Agricultural University,
Parbhani - 431 402 (M.S.).

i) Signature, name and : 
address of forwarding : **(Dr. G.D. Deshpande)**
authority : Head
Department of Plant Pathology
MAU, Parbhani.

Thesis title: Studies on Root Rot of Safflower incited by
Nectophomine phaseolina (Tassi) Goid
ADSUL, AMARCHAND KESHAV
Reg. No. 94M/2005A
Major Advisor: P. V. Kheliker

ABSTRACT

Safflower (*Carthamus tinctorius* L.) is one of the important oilseed crops of the world valued for its highly nutritious edible oil. It belongs to family *Compositae* and believed to be native of Afghanistan and Abyssinia. Safflower occupied 771.5 thousand ha area in India with production of 418.8 thousand tonnes with average productivity of 465 kg/ha. Maharashtra ranks first in area and production accounting as 67% and 63%, respectively, in India. The crop is known to be infected by several disease. Root rot of safflower disease is of economic importance in India including Maharashtra. Pathogenicity of the pathogen was tested by proving Koch's postulate on safflower seedlings of cv. Nira, Mangira, Parbhani-12 and Sharda by water culture technique and sick soil technique (pot culture). Symptoms were initiated after 12 hours incubation with water culture and 25 DAS by sick soil technique. Parbhani-12 and Mangira varieties recorded least mortality in both the methods. Amongst antagonist tested *Trichoderma harzianum* was significantly superior in percent reduction of mycelial colony diameter (mm) of pathogen at all incubation periods tested. Maximum inhibition of per cent mycelial colony diameter (mm) and average sclerotial production of the pathogen were observed due to carbandazim, chlorothalonil, hexaconazole and captan at different concentrations. Of the plant extracts (botanicals) tested, Neem was found highly fungistatic against the test pathogen. Neem extract recorded highest average mycelial inhibition (44.74 %) and sclerotial inhibition 76.09 per cent, followed by ginger (average mycelial inhibition 22.96% and average sclerotial production 26.03%). Screening of safflower material under natural condition of infection revealed that none of the entry was found completely free from disease, however, variety AVT-09-01 showed resistant disease reaction and rest of the advanced varieties were moderately susceptible.