

**BIOLOGY AND MANAGEMENT OF MACROPHOMINA
STEM CANKER OF PIGEONPEA CAUSED BY
Macrophomina phaseolina (Tassi) Goid.**

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**BIOLOGY AND MANAGEMENT OF MACROPHOMINA
STEM CANKER OF PIGEONPEA CAUSED BY
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By

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CERTIFICATE

This is to certify that the thesis entitled “**BIOLOGY AND MANAGEMENT OF MACROPHOMINA STEM CANKER OF PIGEONPEA CAUSED BY *Macrophomina phaseolina* (Tassi) Goid.**” submitted by **Mr. CHIKKANNA SWAMY** for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** to the University of Agricultural Sciences, Raichur is a record of research work carried out by him during the period of his study in this University, under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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TO

MY BELOVED PARENTS, TEACHERS,

SENIORS, JUNIORS AND FRIENDS

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With Regardful memories....

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LIST OF ABBREVIATIONS

%	:	per cent
±	:	plus or minus
µm	:	micrometer
>	:	greater than
°	:	degree
C	:	centigrade
CD	:	Critical Difference
CRD	:	Completely Randomized Design
cm	:	centimeter/s
DAS	:	Days After Sowing
DAI	:	Days After Inoculation
<i>et al.</i> ,	:	and others
Fig.	:	figure
g	:	gram/s
ha	:	hectare
h	:	hour/s
<i>i.e.</i> ,	:	that is
kg	:	kilogram/s
mha	:	million hectare
mt	:	million tones
mm	:	mili meter/s
mM	:	mili molar
µm	:	micro meter/s
µg	:	micro gram/s
ml	:	mili liter
no.	:	number
RBD	:	Randomized Block Design
S.Em	:	Standard Error mean
<i>viz.</i> ,	:	namely

l	:	liter/s
km	:	kilo meter/s
x	:	magnification
NCBI	:	National Center for Biological Information
BLAST	:	Basic Local Alignment Tool
ITS	:	Internal Transcribed Spacer
PDI	:	Per cent Disease Index
MSC	:	Macrophomina Stem Canker

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INTRODUCTION

I. INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Mill spaugh] is one of the major legume crops grown in the tropics and sub tropics which accounts for about 5 per cent of world legume production with India being the largest producer. It is hardy, widely adopted and drought tolerant crop with a large temporal variation (90-300 days) for maturity. Pigeonpea is grown in over 50 tropical countries of the world especially in semi-arid regions of Africa, Asia and America. In India, it is cultivated as an annual crop, but in other countries it is grown as perennial crop, where pods are harvested at regular intervals. In some countries, it is mostly grown as a kitchen garden crop for vegetable purpose.

Pigeonpea is commonly called as red gram or arhar. Seeds are rich in protein, iron, iodine and essential amino acids like lysine, cystine and arginine. Nutritional values of 100 g of dry seeds contain 7 to 10.3 g water, 14 to 30 g protein, 1 to 9 g fat, 36 to 65.8 g carbohydrates, 5 to 9.4 g fiber and 3.8 g ash. The energy content averages 1450 kj/100 g (Orwa *et al.*, 2009).

The pod husk and leaves after threshing serves as a valuable fodder for cattle. Woody stems are used as a fuel, pigeonpea being a legume possesses valuable property since it restores nitrogen in soil (40 kg/ha). The plants are also used to culture the lac producing insect in China. The young leaves are applied to sores, herpes and itches as medicine. Perennial pigeonpea is also used for agro forestry system. The traditional pigeonpea cultivars and land races are long duration types grown as intercrops with other more early maturing cereals and legumes with so much benefit at low cost. Pigeonpea has become an ideal crop for sustainable agriculture system in rain-dependent areas. Pigeonpea belongs to Fabaceae family, having chromosome number $2n=22$, with two centers of diversity; Eastern Africa and India, it is now generally accepted that pigeonpea originated in India (Vavilov, 1951), where there are several wild relatives with most diverse crop gene pool. India and Myanmar account for 16 wild species one of which *Cajanus cajanifolious* (Hainer) could be regarded as progenitor (Maesen, 1990).

In India, it is one of the very important grain legumes and occupies second position in area and production next to chickpea. It is mainly grown in the states of Maharashtra, Karnataka, Uttar Pradesh, Madhya Pradesh and Gujarat. It is grown on an area of 4.04 m ha with an annual production of 2.65 million tons with productivity of 656

kg ha⁻¹. Its area, production and productivity trends in India for the last five decades showed that there was about two per cent increase in area per year but the yield levels are stagnant around 600-700 kg ha⁻¹ (Anon., 2012).

In Karnataka, pigeonpea occupies second place in area (0.77 mha) and ranks second in production (0.36 mt) with a productivity of 466 kg ha⁻¹. Gulbarga is a very potential district in the country for extensive cultivation of pigeonpea. It is also grown in Bidar, Bijapur, Dharwad, Bellary and Belgaum districts of Northern Karnataka. The average productivity of pigeonpea in Karnataka accounts for 700 kg ha⁻¹ and its potential yield marked up to 3.5 tons ha⁻¹ (Anon., 2012).

Pigeonpea is affected by several abiotic (water-logging, drought, temperature, photoperiodism, mineral deficiency) and biotic stresses (fungal pathogens, insects and storage pests) that limit the realization of true potential yield of pigeonpea. The International Crops Research Institute for the Semi- Arid Tropics (ICRISAT), Patancheru, India has a world mandate to improve pigeonpea in order to study the prevalence of various diseases and their relative importance in countries of the semi arid tropics. The most widespread and destructive of which is Fusarium wilt (*Fusarium udum* Butler), sterility mosaic and Phytophthora blight (*Phytophthora drechsleri* f. sp. *cajani*) which are important in India and Cercospora leaf spot can cause serious losses under humid conditions in Asia and Africa (Hillocks *et al.*, 2000).

The first record of pathogen of dry root rot seems to be from India as *Macrophomina cajani* (Syndow and Butler, 1916). It was reported to infect living stems of pigeonpea in India by Asby (1927). It is synonym of *Macrophomina phaseoli*. The disease is also reported from Australia, Myanmar, Sri Lanka, Vietnam and Zambia (Nene *et al.*, 1996).

Macrophomina is primarily a soil and seed-borne fungal pathogen that incites the disease by producing microsclerotia/pycnidia (Farr *et al.*, 1995 and Pun *et al.*, 1998). It has a wide host range of more than 500 species from 75 families with heterogeneous host specificity i.e. the ability to infect monocots as well as dicots and can exhibit non-uniform distribution in the soil (Mayek-Perez *et al.*, 2001 and Su *et al.*, 2001). The disease is often referred to as charcoal rot, due to the charcoal type discoloration imparted to the colonized plant tissues. *Macrophomina* infection causes both pre and post-emergence plant mortality. Characteristic post-emergence symptoms include the development of

spindle shaped lesions with dark border and light grey centre covered with small pin head sized microsclerotia and sometimes pycnidia. Sclerotia allow the fungus to survive for a prolonged periods of time in the soil (Baird *et al.*, 2003).

In India, the first report of stem canker occurrence was reported by Kannaiyan *et al.* (1979) in part of Eastern Uttar Pradesh (Varanasi and Mirzapur districts). Recently, the stem canker incited by *Macrophomina phaseolina* has emerged as one of the important pathogen of different agricultural crops, including pigeonpea (Kaur *et al.*, 2012a). *Macrophomina* Stem Canker disease (MSC), caused by *M. phaseolina* is potentially a serious disease in pigeonpea that occurs from flowers to physiological maturity. The fungus incites necrotic lesions on stem and girdles the plant at the base leading to premature flower drop leading to complete wilting and finally death of the entire plant. The incidence of *Macrophomina* Stem Canker has increased gradually in recent years to reach an alarming proportion. The disease also occurs in states of Bihar, Madhya Pradesh, Maharashtra, Orissa, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Gujarat and Rajasthan (Kannaiyan *et al.*, 1981).

Considering the above facts a survey on assessing the incidence of stem canker caused by *M. phaseolina* was the need of the hour particularly in pulse growing districts of North Eastern Karnataka. That would also give an idea of prevalence and “hot spot” areas of emerging disease within the ambient of its cultivation domain of North Eastern Karnataka. It was also proposed to collect the isolates from different parts of the surveyed areas for assessing the variability with respect to cultural, morphological and molecular aspects. It equally pertinent to devise the strategies for the management of stem canker an emerging diseases problem. In view of the above facts, attempts have been made to carryout investigations on the following objectives.

- 1) To undertake survey on incidence and collection of the isolates of *M. phaseolina* causing stem canker of pigeonpea.
- 2) To assess the cultural, morphological and molecular characterization of the isolates of *M. phaseolina* causing stem canker of pigeonpea.
- 3) To undertake management of *M. phaseolina* causing stem canker of pigeonpea.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Pigeonpea stem canker/Macrophomina Stem Canker (MSC) caused by *Macrophomina phaseolina* is an emerging disease in North Eastern Karnataka causing considerable economic losses. This may probably be attributed to an increased environmental temperature and drought conditions prevailing during past few crop seasons. The present investigation included different aspects on the biology and management of the pathogen. The literature pertaining to studies on symptomatology, cultural characteristics, morphological and molecular variability, identification of source of resistance and efficacy of bio-agents and fungicides in management are reviewed here under.

2.1 The Pathogen and Symptomatology

Pycnidial state of the fungus was originally named *Macrophoma phaseolina* by Tassi (1901) and *Macrophoma phaseoli* by Maublanc (1905). Halsted (1890) described the sclerotial state as *Rhizoctonia bataticola* (Taub.) Butler on sweet potato (*Ipomoea batatas*). Finally, Ashby (1927) critically examined and compared the type specimens of the fungus from beans with other related genera and established the binomial species *Macrophomina phaseoli* (Maubl.) (Ashby, 1927). Later, Goidanich (1947) changed the binomial *Macrophomina phaseoli* to *Macrophomina phaseolina* (Tassi.) Goid., since the original specimen of *Macrophomina* was collected by Tassi in 1901. Hence, the two names, that is, *Macrophomina phaseoli* (Maubl.) Ashby and *Macrophomina phaseolina* (Tassi) Goid. became widely accepted in literature.

Macrophomina is primarily soil and seed-borne fungal pathogen that incites the disease by producing microsclerotia/pycnidia (Farr *et al.*, 1995; Pun *et al.*, 1998). It has a wide host range with more than 500 species from 75 families with heterogeneous host specificity. They have the ability to infect monocots as well as dicots and exhibit non-uniform distribution in the soil (Mayek-Perez *et al.*, 2001 and Su *et al.*, 2001).

Two asexual sub-phases of *Macrophomina* are known as: (1) a saprophytic phase (*R. bataticola*) that forms microsclerotia and mycelia (2) a pathogenic phase (*M. phaseolina*) present in host tissues that forms microsclerotia, mycelia and pycnidia. In spite of being a mono-specific genus, *M. phaseolina* exhibits a high degree of

morphological (Mahdizadeh *et al.*, 2011), physiological (Mihail and Taylor, 1995), pathogenic (Su *et al.*, 2001) and genetic variability (Babu *et al.*, 2007).

Macrophomina infection causes both pre and post-emergence plant mortality. Characteristic post-emergence symptoms include the development of spindle shaped lesions with dark border and light grey centre covered with small pin head sized microsclerotia and sometimes pycnidia. Sclerotia allow the fungus to survive for prolonged periods of time in the soil (Baird *et al.*, 2003). Deep and irregular necrotic lesions extending toward hypocotyls and root surfaces were observed in soybean (Ammon and Wyllie, 1972), chickpea (Singh and Mehrotra, 1982) and sorghum (Pedgaonkar and Mayee, 1990). As lesions coalesced, they formed larger patches on branches or entire plant, leading to premature senescence and plant death (Dhingra and Sinclair, 1978).

Stem-split was observed in 30 day old safflower *M. phaseolina* infected plants as minute cracks approximately 2-3 cm above the soil surface, which over time extended to both upward and downward directions, resulting in the formation of a wider split. The split portion was hollow and brown with a white to grey mycelial mat of the fungus on the inner surface (Govindappa *et al.*, 2005). The spindle-shaped lesions with light gray centers and brown margins with scattered pycnidial bodies are the early symptoms of *Macrophomina* infection in woody plants. Generally, *Macrophomina* can cause a range of symptoms after a successful infection from restricted spindle-shaped lesions on the stem to extended lesions that result in wilting of the plant in pigeonpea (Kaur *et al.*, 2013). During the early stages of disease development several black dots like pycnidia are evident on these lesions. Later on, several pycnidia were observed on most of the infected parts of the plant.

2.2 Survey and distribution of *M. phaseolina*

Occurrence of disease at various places indicates wide geographical distribution of *Macrophomina* Stem Canker in the area covered.

The disease incidence of charcoal rot of sunflower caused by *M. phaseolina* was recorded in Spain during 1976, 1977 and 1978. Higher disease prevalence was recorded in Andalusia 50 per cent during 1976, Extremadura 25 per cent in 1977 and Centro 25 per cent in 1978 at low rainfall high temperature coincided during flowering stage

(Diaz *et al.*, 1983). A periodic survey conducted by Sharma *et al.* (1983) in Gwalior, indicated that the incidence of dry root rot in all five major districts of Madhya Pradesh causing yield loss ranged from 3.58-20.63 per cent.

M. phaseolina (charcoal rot) and *Rhizopus* spp. were reported as most destructive on pathogens of sunflower. Mirza (1984) reported yield losses due to *M. phaseolina* up to 90 per cent in Pakistan on sunflower. The occurrence of severe outbreaks of Macrophomina Stem Canker of pigeonpea caused by *M. phaseolina* in Central and Western Nepal during March 1988 is a new record for the country. The disease was favoured by high temperature and relative humidity (Reddy *et al.*, 1989).

Singh *et al.* (1990) identified eleven new hosts in addition to ten species which were identified as new pycnidial hosts. It was concluded that *M. phaseolina* was prevalent in all commonly cultivated crops in the semi-arid tropics. Pandey and Singh (1990) conducted the survey of chickpea diseases with reference to dry root rot in Allahabad region and reported that the chickpea is infected by *Rhizoctonia bataticola*, *Fusarium oxysporum* f. sp. *ciceri*, and 83 per cent of villages showed less than 25 per cent disease intensity and remaining ones recorded 26-50 per cent disease intensity.

Macrophomina Stem Canker was a serious problem in late sown or summer crops and in perennial or ratooned pigeonpea. Hot (>30 °C) and dry weather encourage disease development which is more prevalent on vertisols than alfisols. Rain after a prolonged dry spell predisposes plants to the disease. The pathogen poses greater problem in pigeonpea cultivation and causes considerable loss (Bajpal *et al.*, 1999). Macrophomina Stem Canker is ranked among five most important soybean diseases causing huge annual economic losses in the top-ten soybean-producing countries (United States, Brazil, Argentina, China, India, Paraguay, Canada, Indonesia, Bolivia and Italy in 1998 (Wrather *et al.*, 2001).

A systematic survey of *M. phaseolina*, the causal agent of soybean charcoal rot was conducted between late August and early September 2003. The disease was observed in 60 per cent of fields surveyed in Northern Iowa, 90 per cent in Central Iowa and 20 per cent in Southern Iowa Yang and Navi (2005). In some crops such as sunflower losses reached 60-90 per cent in environments that favored infection (Khan, 2007). Yield losses and epidemic outbreaks ranging from 46 to 70 per cent due to charcoal rot of cowpea caused by *M. phaseolina* was observed in many regions of Nigeria (Amusa *et al.*, 2007).

The disease incidence of *Macrophomina* Stem Canker of pigeonpea varied from 2 to 70 per cent in most fields and its severity ranged from 17 to 55 per cent in the aforesaid regions of Eastern Uttar Pradesh (Kaur *et al.*, 2009).

Intensive roving survey was conducted by Manjunatha *et al.* (2011) during November to December 2008 in the farmers field at Raichur, Gulbarga and Bidar districts. The mean maximum dry root rot incidence in chickpea was noticed in Gulbarga district (9.8 %) followed by Raichur (7.6 %) and the least (6.18 %) in Bidar. This was mainly attributed to variation in soil temperature (35-37 °C) and moisture.

The field survey was conducted (Mohan and Balabaskar, 2012) to assess the extent of root rot of groundnut in Cuddalore district of Tamil Nadu State. Among the different locations of Cuddalore district Vengatakuppam (MP18) recorded the maximum incidence of the disease (31.68 %) followed by Keelpuvanikuppam (MP13) with 29 per cent, Aduragaram (MP1) with 26.33 per cent and Nellikuppam (MP11) with 24.87 per cent, more in sandy loam soils and rainfed conditions. The local cultivars of groundnut recorded comparatively lower (14 to 20 %) root rot incidence than the improved cultivars *viz.*, VRI2 and JL24 (15.87 to 31.68 % and 18.45 to 26.75 % of disease incidence respectively). The survey for the occurrence and severity of chickpea dry root rot was made during crop season 2010-11 in Jammu and Kashmir. The highest incidence recorded was up to 40 per cent in Shangus and lowest was up to 4.11 per cent in Nawhar of Jammu and Kashmir (Khan *et al.*, 2012).

A roving survey of *Macrophomina* Stem Canker (MSC) of pigeonpea at various places in Varanasi and Mirzapur districts of Uttar Pradesh was conducted during the years 2008-09 and 2009-10 near maturity growth stage of the crop. The incidence of MSC recorded in the range of 1 to 60 per cent in 2008-2009 and 0 to 19.5 per cent in 2009-2010. The incidence during 2008-2009 was highest at Dhaurahara (60 %) followed by Chilbilla (25%), Dugmugpur, Arjunpur and Basani (20 %), Lalpurchatti (10.25 %), Fulwaria (10 %) and Sagarpur (9.5 %). During 2008-09 hundred per cent disease prevailed in both the districts, while in 2009-10 disease prevalence was 52.63 per cent and 75 per cent in Mirzapur and Varanasi district respectively (Kaur *et al.*, 2012b).

A survey was conducted in 2010-2011 *rabi* cropping season to obtain information on the distribution and incidence of chickpea diseases with respect to soil type, cultivar used, seed treatment in Central and Southern parts of India. Dry root rot and collar rot

diseases were found at all the sites and incidence ranged from 8.9-10.3 per cent and 7.1-10.5 per cent respectively irrespective of cultivar type and locations (Ghosh *et al.*, 2013).

2.3 Cultural, morphological and molecular characterization of *M. phaseolina*

2.3.1 Growth on solid media

Fungal growth could be quantified easily in linear dimensions on an agar medium, that first the mycelia start to growth and then follows the formation of microsclerotia/sclerotia; different media helps to reveal the cultural studies of the *M. phaseolina*.

Uppal *et al.* (1936) reported variation in growth rate and colony character for two sorghum strains of *M. phaseolina* from two different regions, one from Broach farm and another from Mohol farm in Maharashtra. Cultures of Broach strain showed concentric rings on PDA, while that of Mohol strain produced aerial mycelium. Thomas (1938) observed that the pycnidia of *M. phaseolina* developed in cultures on several solid media.

Adam and Stokes (1942) reported that mean diameter of sclerotia varied from 63-165 μm on Czapeks Dox's agar and 18-50 μm on potato dextrose agar. Goidanich and Camici (1947) observed similar cultural and morphological characters among the strains of *M. phaseoli* isolated from bean (*Phaseolus vulgaris* Linn.) and lavender.

Jain *et al.* (1973) isolated *M. phaseolina* from urd bean plant parts from root, stem, leaf, pod and seeds and an isolate from the soil, wherever, urd bean crop was taken. In the study for growth on different media, the isolates differed from each other in the growth pattern either in the diameter of colony or nature of aerial mycelium or the type of mycelial growth. The pycnidial stage was never produced in culture (Grover and Sakhuja, 1981). Waseer *et al.* (1990) isolated *M. phaseolina* from soybean and reported that the pathogen grew better on potato dextrose agar or potato dextrose juice at 35 °C and little growth occurred at either 20 °C or 40 °C.

Five culture media were tested for mycelial growth and sclerotia production of 25 geographical isolates of *M. phaseolina* causing charcoal rot of sunflower. Among the media tested, PDA supported the best growth and the sclerotia production of isolates of pathogen requiring the lowest period. The most virulent isolate (MP8) recorded the highest mycelial growth (87.1 mm) of all the media tested and it produced abundant

sclerotia in all the media within a short period of 48.8 h (Wasser *et al.*, 1991; Sahi *et al.*, 1992; Singh and Kasir, 1994 and Suriachandraselvan and Seetharaman, 2003).

Chowdary and Govindaiah (2007) reported that the radial growth was found maximum after 24 h of incubation on PDA (40.3 mm), followed by potato sucrose agar (39.0 mm), Czapeks Dox agar (36.0 mm) and oatmeal agar (35.5 mm). Likewise after 36 h of incubation period also, the radial growth was found maximum on PDA (81.3 mm) followed by Czapeks Dox agar (78.0 mm). After 48 hours of incubation, maximum radial growth of the fungus was recorded on PDA (90.0 mm) and minimum was noticed in oatmeal agar (80.0 mm).

2.3.2 Liquid media

Variation in *M. phaseolina* biomass accumulation due to change in differential liquid media. Shanmugam and Govindswamy (1973) reported that Richards' medium gave the maximum growth of *M. phaseolina* of groundnut. Satishchandra (1977) reported that the fungus attained maximum growth after 11 days of incubation in Richards' medium. Lokesha and Benagi, (2004) reported on fungus of (*M. phaseolina*) maximum mean dry mycelial weight of 231.0 mg was recorded on 18th day of incubation and the isolate ICRISAT recorded significantly highest dry mycelial weight (190.9 mg). PDA and Czapeks Dox agar media were found best for the growth of all the isolates (88.4 mm each). The sclerotia production was maximum on PDA (123.3/microscopic field of 10x). The ICRISAT isolate recorded maximum sclerotia production (71.2/microscopic field of 10x) followed by Bidar isolate (68.68/microscopic field of 10x).

Chowdary and Govindaiah (2007) reported maximum mycelial dry weight of *M. phaseolina* in potato dextrose broth (998.3 mg) and minimum in oatmeal broth (439.3 mg), whereas 823.3, 811.6 and 806.0 mg of mycelial dry weight were recorded in Czapeks Dox, Richards and potato sucrose broth, respectively.

The growth of *M. phaseolina* was tested on eight different solid and liquid media. Among them growth was significantly more on potato dextrose agar (89.67 mm) as compared to the rest but was at par with potato carrot sucrose agar (86.67 mm). The sclerotial formation of *M. phaseolina* was high on potato dextrose agar, potato carrot sucrose agar and Richard's agar. Among the various liquid media tested, significantly higher dry mycelial weight was observed in Richards' broth (776.25 mg) as compared to

the rest of the liquid media but was at par with Czapek's Dox solution (758.68 mg). The sclerotial formation was found high level on Richard's solution, Czapek's Dox solution, potato dextrose broth and potato carrot sucrose broth (Jha and Dubey, 2000; Suriachandraselvan and Seetharaman, 2000 and Tandel *et al.*, 2012).

2.3.3 Morphological variability

Exhibition of morphological variability by *M. phaseolina* reflects its heterokaryotic behaviour. Phenomenon of heterokaryosis was observed in *Macrophomina* during hyphal fusion, mitotic crossing-over and recombination (Punithalingam, 1983). Therefore, in the absence of sexual reproduction parasexual mechanism might be one of the causes for cultural variability in *M. phaseolina*.

Dhingra and Sinclair (1978) noticed variation in growth rate among the isolates from the same plant and between isolates from different soybean plants on different media. The stem isolates produced fluffy growth, root isolates showed partially fluffy growth and all other isolates produced appressed growth. They also collected nine isolates of *M. phaseolina* from various part of United States and reported variation in growth rate and colony characters. Colony characteristics of isolates varied at 35 °C on PDA. Isolates I-2, M-1 had completely appressed with margin, MP-3 producing irregular margin turning dark green with age with no sclerotia, M-2 was white and fluffy (Dhingra and Sinclair, 1973).

Gosh and Sen (1973) recorded sclerotial diameter of justica, soyabean, bengal gram, jute, kochia, groundnut and pigeonpea isolates, which were 64.6-114.0 µm, 45.2-81.6 µm, 98.1-119.2 µm, 40.2-85.0 µm, 56.6-80.1 µm, 10-114 µm and 89.1-116.3 µm respectively. Further Smits and Nogurea (1988) used scanning electron microscope and light microscope to observe the sclerotial formation, which began with branching and intertwining of adjacent hyphal filaments and an increase in size of associated cells and compaction of the sclerotial mats. The pycnidial state began with the merging of hyphal filaments towards a common point, followed by the development of primordial and finally pycnidia.

Gupta and Kolte (1982) collected isolates of *M. phaseolina* from leaf and root of groundnut which showed differences in both morphology and pathogenicity. Sclerotia, pycnidia and pycnidiospores produced by the leaf isolate were bigger in size than the root

isolate. However, the root isolate was more pathogenic to ground nut seedlings causing 33.3 per cent seed mortality and 23.8 per cent post emergence mortality, while the leaf isolate recorded only 10 per cent seed mortality and 17.8 per cent post emergence mortality under similar conditions.

Thirty six isolates of *M. phaseolina* of mung bean collected from different region of North, South, North East and central India were studied for morphological variability. Representative isolate from each region selected for morphological study viz., MP-1, MP-2 and MP-3. MP-1 isolate had cottony mycelial growth with pale grey colonies. The colony colour of the isolate was brown in MP-2 and showed 5 μ appressed mycelial growth. The sclerotia were bigger in size in isolate MP-2 (400 x 280 μ m). Typical right-angled branching of mycelium was found in isolate MP-1 and it was acute to right angled in isolates MP-2 and MP 3. The mean diameter of the pycnidia produced was 186 μ m and acute to right angled in isolates MP-2 and MP-3 (Devi and Singh, 1998).

Sharma *et al.* (2004) reported on *M. phaseolina* from sesame and horse gram isolates were flat, compact and white to black in colour, while moth bean isolates exhibited black centred fluffy growth. *M. phaseolina* isolates from pearl millet exhibited fluffy, fast-growing, dull-white growth. Such variations have also been observed earlier in *M. phaseolina* isolates of *Phaseolus vulgaris* (Fernandez *et al.*, 2006).

Shekhar *et al.* (2006) collected seven isolates of *M. phaseolina*, incitant of charcoal rot of maize, obtained from different agro climatic zones of India. On the basis of colony colour, isolates were divided in to four groups i.e. grayish white, blackish white, blackish gray, dark black in the centre periphery creamish and cottony white. Hyderabad isolate produced highest number of sclerotia (180.3 sclerotia/9 mm disc) of bigger size (95.7 μ m) compared to other isolates. On the basis of sclerotial morphology, isolates were divided in to oblong shape having a regular edges and round with regular edges. On the basis of disease expression Hyderabad isolate was more virulent compared to other isolates.

The diversity patterns of 30 isolates of *M. phaseolina* of beans 15 from Mexico and 15 from other countries were analyzed based on morphological characteristics such as colour of colony (gray and black), presence/absence of aerial mycelium, shape (round, ovoid and irregular) and color (brown) of microsclerotia, Production of microsclerotia was higher in most Mexican isolates than in non-Mexican isolates. However, no

significant differences between Mexican and non-Mexican isolates were found in length, width and length/width ratios of microsclerotia (Fernandez *et al.*, 2006).

Aboshosha *et al.* (2007) *Macrophomina* isolates of sunflower charcoal rot colonies were mostly grey, dark green, black, or brown for the tested fourteen stem isolates on PDA and Czapeks Dox Agar. In root isolates of chlorate selective medium was not much variation as the isolates were mostly either white or grey isolates of the brown colony phenotype, 4 of dark green and 3 of the grey colony phenotype out of 19 isolates.

Rayatpanah *et al.* (2009) collected sunflower, soybean, sesame isolates of *M. phaseolina* which showed variability on with or without potassium chlorate minimal medium and grouping based on growing pattern such as feathery, grew sparse, completely restricted radial growth and dense growth. Among soybean isolates, feathery isolates were much more abundant than either restricted or dense isolates whereas dense isolates predominated in sesame roots, isolates from sesame grew more rapidly on the defined medium containing chlorate than isolates from soybean or sunflower.

Edraki and Banishashemi (2010) studied on sixty isolates of *M. phaseolina* isolated different parts of Iran on various crops like soybean, cucumber and sesame, phenotypic characteristics of the isolates were compared by growing on PDA at 35 °C. Isolates were grouped in to four phenotypes: fluffy with abundant sclerotia, fluffy with few sclerotia, partially fluffy and appressed growth. The results showed that the morphological characteristics, including density of aerial mycelia, sclerotia quantity, sclerotium size (0.13 mm) and growth rate of colony 35 isolates ranged from 0.60mm/h in average (Linhai *et al.*, 2011).

Prasad *et al.* (2011) collected isolates of *M. phaseolina* that causes root rot in safflower from semi arid regions of Maharashtra, Andhra Pradesh and Karnataka states of India. Color of the mycelium was grey in all isolates except in the isolate, *M. phaseolina* AP3, which showed black color of microsclerotia ranging from brown to black. Among all the cultural characters studied variations were prominent in microsclerotia (shape, length and width) dimensions no significant differences were found among the isolates in morphological characters like length, width and length/width ratios of microsclerotia except the isolate, AP 21 that vary in length/width ratio (483.3/ 200.0 µm).

Mohan and Balabaskar (2012) groundnut root rot pathogen *M. phaseolina*, 13 isolates showed variability on PDA medium. The isolates MP1, MP11, MP13 and MP18 significantly recorded the maximum (90 mm) mycelial growth with cottony white colour. Maximum sclerotial number of 185.30 per nine mm culture disc was obtained from the most virulent isolate MP18. The minimum number of sclerotial production of 171.70 was recorded by the least virulent isolate MP13. Biggest (105.10 μm) and the smallest (85.70 μm) sclerotia were produced by MP11 and MP5, respectively. Similar variation in the sclerotial size was observed by several workers (Raut and Ingle, 1989 and Sobti and Sharma, 1992).

Twenty-four samples of *M. phaseolina* were collected from infected stems and roots of soybean, sunflower and sesame plants from Mazandaran Province in Northern Iran. Three various growth patterns (feathery spreading growth, restricted growth and dense growth) were observed on the minimal medium containing 120 mM potassium chlorate, among soybean isolates, feathery isolates were much more abundant than restricted, whereas dense isolates predominated in sesame. Sclerotia production on chlorate medium by the sensitive isolates was too low compared to resistant ones. All isolates had dense growth when they were grown on the minimal medium without chlorate and could not be differentiated (Rayatpanah and Dalili, 2012). Similar results were also observed in cotton by Omar *et al.* (2007).

Almomani *et al.* (2013) reported 25 isolates of *M. phaseolina* which were classified into four growth types: thin upraised, thin aerial, dense aerial and dense upraised with the following numbers 18, 3, 3 and 1, respectively. Morphological appearance of the microsclerotia distribution on PDA medium was found in three forms: scattered irregular, scattered round and uniform with the following numbers 19, 3 and 3, respectively. Pycnidia formation was tested in two media which are PDA and SBA. Most of the isolates (22/25) formed pycnidia on SBA, whereas 11/25 formed pycnidia on PDA and 10/25 were able to form pycnidia on both media.

Kaur *et al.* (2013) studied sixty isolates of pigeonpea stem canker caused by *M. phaseolina* were characterised for microsclerotia (present/absent), type of mycelium (aerial/immersed), mycelium colour (greyish black/black) and shape of the colony (regular/irregular). Colour of the isolates and the presence of the microsclerotia have a significant effect on the area under disease progress curve and also more aggressive

compared to isolates with no production of microsclerotia. Lowest frequencies (11.5 %) of isolates were found have microsclerotia and highest frequency (72.1 %) was of grey-black isolates as compared to black coloured isolates (27.9 %).

Iqbal and Mukhtar (2014) collected 65 isolates of charcoal rot caused by *M. phaseolina* from Punjab and Khyber Pakhtunkhwa provinces of Pakistan and were analyzed for morphological and pathogenic variability. Significant differences were noticed among 65 isolates with sixteen isolate rated as fast growing, 11 as slow growing, and the rest of the isolates as medium growing. Nine isolates were classified as large sized, 26 as small sized, and the remaining 30 isolates as medium sized. Thirty five isolates were ranked as heavy weight, 12 as low weight, and the rest of isolates were grouped as medium weight. Ten fungal isolates appeared to be least virulent, whereas eight isolates of diverse origin were proved to be highly virulent against mungbean cultivars. The remaining isolates were regarded as moderately virulent.

2.3.4 Molecular characterization

Nuclear rDNAs, particularly in the internal transcribed spacer (ITS) region, are good targets for phylogenetic analysis in fungi (Bruns *et al.*, 1991). High degree of polymorphism was observed among the isolates of *M. phaseolina* isolated from the same geographical location which confirms the existence of high genetic variability (Rajkumar and Kuruvishetti, 2007).

Eight isolates of *M. phaseolina* collected from different host and diverse ecological conditions were selected for sequencing of ITS region. Sequencing and analysis for identifying the conserved and variable motifs of *M. phaseolina* genomic DNA amplification of ITS region of different isolates yielded a fragment of approximately 650 bp (Babu *et al.*, 2007).

RAPD-PCR analysis using eight random oligonucleotide primers revealed DNA fingerprints 2-9 bands ranging from 350 bp to 3 kbp. There is no variations were revealed for number of bands between isolates with primers OPS-16 or OPA-2, while more variations were revealed with OPA-1, OPA-4, OPA-5, OPA-8, OPA-13 and OPR-14 primers. The RAPD-PCR phylogenetic analysis did not reveal known genetic entities for the stem type and the root type of isolates, since it revealed two clusters for the high pathogenic isolates and the low pathogenic isolates in the dendrogram tested *M. phaseolina* isolates (Aboshosha *et al.*, 2007).

PCR-RAPD markers proved to be informative and useful in detecting the genetic variability among ten isolates of *M. phaseolina*. The lowest genetic similarity coefficient of 0.06 was observed between isolates D5 and B1; D7 and B1, while the highest genetic similarity coefficient of 0.71 was between D1 and D3 which was closely (0.70) followed by D2 and D5. Two primer kits (20 of kit A and 20 of kit C) were employed for this study (Rajkumar and Kuruvinashetti, 2007). Close genetic relationship was found among the isolates even when isolated from the same locations (Das *et al.*, 2008).

Thirty isolates of *Rhizoctonia bataticola* from chickpea were studied for molecular variability by using three primers OPO-10, OPO-12 and OPN-12. There were four isolates in Group-I, 16 isolates in Group-II and 10 isolates in Group-III. However, in Group-II, three isolates (RG-18, RG-19 and RG-20) exhibited 100 per cent similarity. RB-29, RB-25 and RG-16 isolates from Bidar and Gulbarga respectively showed genetically divergent among thirty isolates (Manjunatha, 2009).

Weems *et al.* (2011) analyzed sunflower charcoal rot caused by *M. phaseolina*. PCR amplification of a subunit rDNA and internal transcribed spacer (ITS) region with primers EF3RCNL and ITS-4 was performed. The resulting nucleotide sequence shared the highest homology (99%) with sequences of *M. phaseolina* when compared with the subunit rDNA and ITS sequences in the nucleotide database (<http://www.ncbi.nlm.nih.gov>).

Pathogenic and genetic variability among the eleven isolates of *R. bataticola* from different pulse crops, were categorized into three groups *viz.*, linear, fluffy and linear at the end with fluffy growth at the centre. Isolates also showed variability in sclerotial characters (intensity and shape) and intensity of pigment synthesis. All isolates were more aggressive on the original host from which it was isolated, which was shown by the variability in pathogenic characters. RAPD-PCR analysis has shown that genetic clustering agreed with the dendrogram analysis (2 clusters A and B). The black gram root isolates showed a maximum genetic similarity of 73 per cent with soybean shoot isolate. Red gram shoot isolate showed 61 per cent genetic similarity with green gram isolates (Sundravadana *et al.*, 2011).

RAPD-PCR amplification profiles, by using of six random OPA primers (kit A) showed polymorphisms among the isolates. The primer OPA-13 (5'_CAGCACCCAC_3') amplified the genomic DNA of all isolates of *M. phaseolina*

producing 4-12 bands of sizes between 0.25 and 2.3 kb (Almeida *et al.*, 2003 and Rayatpanah *et al.*, 2012).

Kaur *et al.* (2012a) reported the species-specific primers and probes which are used for the identification and detection of *M. phaseolina* at molecular level by exploiting rDNA gene cluster as a target. The primers, MpKF1 and MpKR1 designed from the conserved sequences of the ITS region was highly specific and yielded a specific 350 bp products.

Mahdizadeh *et al.* (2012) assayed the RAPD and ISSR to find the genetic diversity of sesame isolates of *M. phaseolina*. A high level of polymorphism was found with RAPD and ISSR markers and PIC values were 0.3 and 0.305 for RAPD and ISSR markers respectively. In RAPD analyses 96 out of 118 bands (79.81 %) were polymorphic. In ISSR analyses, a total of 55 bands were detected, among which 38 bands (66.61%) were polymorphic. Same variability was revealed by Andrea *et al.*, (2013) for growth velocity (optical density ranged between 1.69 to 2.32 at 96 h of growing) and microsclerotia production (18-56 in 100 μ l). Ten primers used were able to amplify the DNA, generating 81 bands (100% polymorphic).

Prabhu *et al.* (2012) studied thirty five isolates of *M. phaseolina*, causal organism of charcoal rot of sorghum from Karnataka, Maharashtra and Andhra Pradesh. Variation among different isolates was studied using PCR-RAPD markers. From a set of 20 random primers tested, a total of 142 amplicon levels were available for analysis. Primers, OPA-03 and OPA-08 showed cent per cent polymorphism. It was possible to discriminate all the isolates with any of the primers employed. Maximum genetic similarity of 97 per cent was found between Solapur and Mulegaon. Least genetic similarity was observed between Ahmednagar and Mehbubnagar isolates.

Dry root rot caused by *R. bataticola*. [Pycnidial stage: *M. phaseolina* is emerging as a serious biotic constraint for chickpea production. Total of 94 isolates collected from *R. bataticola* infected chickpea plants from different agro climatic regions of India were analyzed with amplified fragment length polymorphism (AFLP) and different morphological properties. *R. bataticola* populations collected from different agro-ecological zones were very diverse with respect to their different cultural and morphological parameters like colony colour, growth pattern, growth rate, mycelial characters, sclerotial initiation time, sclerotial intensity and morphology of the

sclerotia. The dendrogram based on AFLP analysis showed that the maximum number of *R. bataticola* isolates were very diverse and did not depend on geographical origin (Vandermark *et al.*, 2000; Reyes-Franco *et al.*, 2006 and Sharma *et al.*, 2012).

Almomani *et al.* (2013) amplification of the isolated DNA from the 25 pathogenic fungal cultures using ITS specific primers (ITS 1 + ITS 4) showed a single band of 580 bp. There was a significant variation of their mycelial linear growth rate on PDA medium. Twenty five *M. phaseolina* isolates showed a wide heterogeneity in their mycelium colour, microsclerotia distribution, pycnidia formation and chlorate phenotypes. The overall means similarity matrix of the 25 *M. phaseolina* recovered isolates were 0.58. The means of similarity matrix of the 25 *M. phaseolina* was in between 0.83 and 0.14. The similarity coefficient between the 25 isolates varied between 0.27 and 1.0.

2.4 Interconnectivity studies between sclerotia and pycnidia

Occurrence of pycnidial stage *Botrydiploida phaseoli* of charcoal rot fungus *M. phaseolina* on maize stalk has been reported for the first time in India. Isolate from sclerotia of the fungus occurring on maize stalk produced only sclerotia on PDA, cornmeal agar and Czapek's Dox agar while the isolate from pycnidiospore produced both sclerotia and pycnidia on PDA and cornmeal agar, but it failed to produce pycnidia on Czapek's Dox agar. Cross inoculation experiment showed that the isolates from both sclerotia and pycnidiospore were capable of producing pycnidia as well as sclerotia on the stalks of maize and sorghum (Singh and Kasir, 1994).

Macrophomina Stem Canker disease (MSC), caused by *M. phaseolina* is a potentially serious disease in pigeonpea that occurs when it reaches physiological maturity *i.e.*, during flowering. Histopathology of the infected plant parts showed the presence of intercellular mycelia and microsclerotia in the cortex and vascular tissues. The germ tube colonized the plant with growth of seedlings following seed coat, cotyledon, stem, root and leaves. According to the results, the pathogen can penetrate and invade the seeds within 24 h post inoculation but fungal growth in roots after 12 days suggests no fungal invasion during early growth stages of root development (Maurya *et al.*, 2013).

2.5 Management studies of Macrophomina Stem Canker of pigeonpea

2.5.1 Screening the genotypes against the Macrophomina Stem Canker

The first step in a resistance breeding programme is to rapidly screen all the available genetic stocks, including the local land races, improved cuttings and exotic germplasms using empirical techniques in field. An escape from disease is a hazard of breeding for resistant varieties. Dry root rot caused by *R. bataticola* (*M. phaseolina*) is widely spread in pigeonpea growing countries. Extra duration genotypes are more susceptible than medium and long duration genotypes. Field evaluations showed ICPX860087 and ICPX860200 genotypes were resistant to dry root rot compared to ICPL87019 and ICPH149 and disease incidence was more in vertisols than alfisols. That was the first report on lines resistance to dry root rot (Reddy *et al.*, 1993).

Pande *et al.* (2004) screened different lines against dry root rot caused by *M. phaseolina* chickpea under field condition. Then ICRISAT again confirmed resistance using paper towel method (Nene *et al.*, 1981). The dry root rot severity was scored on a 1-9 rating scale. ICC 14395 and ICCV-2 showed resistance.

Genotypes were screened against dry root rot of pigeonpea by sick pot technique. The fungus was multiplied on sand corn meal media and applied to sterilized soil. Among twenty-four pigeonpea genotypes screened for the two consecutive years (2001 and 2002), none of the genotypes showed immune reaction. However, two genotypes PT-221 and ICPL-90097 had resistant reaction and DEPS- 9, Gulyal local, GS-1, ICPL-89049, Phy-K-2, TAT-9621 and V-50 recorded moderately resistant reaction (Lokesha and Benagi, 2006).

2.5.2 Chemical control

Fungicides, which have been reported to be effective against *M. phaseolina*, are captan, thiram and agallol (Grewal and Vir, 1958; Clinton, 1960; Bhargava, 1965; Sahai, 1969 and Masih *et al.*, 1970). The efficacy of various fungicides and observed that carbendazim, quintozone and mancozeb reduced *M. phaseolina* population under laboratory conditions (Ilyas *et al.*, 1975).

Hooda and Grover (1988) studied the effect of seed treatment of various chemicals including carbendazim against seedling of mung bean caused by *M. phaseolina*. They reported carbendazim @ 2.0 g/kg seed treatment, mean seedling

mortality was minimum (8.2 %) while thiram recorded 19.7 per cent seedling mortality. Thiophanate methyl recorded 11.6 per cent mortality. Carbendazim and carboxin at 0.2 per cent used as seed treatment, soil drench and seed treatment along with soil drench significantly reduced the incidence of dry root rot of chickpea, with a corresponding increase in phenolics and carbohydrates in plants, followed by captan and thiram at 0.2 per cent (Singh *et al.*, 1998). Bavistin, calixin and antracol were more effective in that order in reducing sclerotia formation of *M. phaseolina* (Ahmad *et al.*, 1995).

Kulkarni (2000) screened various chemicals against safflower root rot caused by *M. phaseolina* and concluded that carbendazim and propiconazole were the most effective fungicides. Captan and ridomil showed 100 per cent inhibition over the control (Iqbal *et al.*, 2004). Seed treatment with carbendazim at 1 g/kg seeds of chickpea showed minimum root rot incidence (17.31-20.71 %) compared with control (38.56-41.11 %) in two years respectively, and the grain yield in both years increased significantly over control (Sharma and Gupta, 2004).

Khan *et al.* (2012) and Iqbal *et al.* (2004) revealed the efficacy of eight fungicides against the *R. bataticola* depending upon their inhibitory effect on the growth of the fungus. Among eight fungicides, indofil M-45, bavistin, companion, copper-oxy-chloride and benlate completely inhibited the growth of the fungus compared to control. Nagamma *et al.* (2012) reported that thiophanate methyl, hexaconazole, carbendazim, difenoconazole, propiconazole and mancozeb showed 100 per cent inhibition at 500 ppm.

2.5.3 Biological control

The role of antagonists in suppressing the growth of soil-borne pathogens was well documented (Garret, 1980). Biological control of plant pathogens using antagonistic fungi and bacteria is a distinct possibility for the future. Use of antagonistic organisms against *Macrophomina* root rot was well documented in several crops (Mukhopadhyay, 1987).

Elad *et al.* (1986) showed that isolates of *T. harzianum* coated on melon seeds, reduced disease caused by *M. phaseolina* by 37-46 per cent in green house experiment. Naik *et al.* (2000) reported that among the several antagonists isolated from the healthy rhizosphere of chilli, *T. viride*, *T. harzianum*, *Aspergillus niger*, *Penicillium pinophilum* and *Pseudomonas fluorescens* gave distinct antagonistic reactions on *F. oxysporum* f. sp.

capsici. In dual culture, antagonists induced suppression and lysis of white fluffy mycelial growth of pathogen. Consequently, the mycelium of pathogen became thinner resulting in deshaping and breaks down of hyphal cell walls when observed under Scanning Electron Microscope (SEM).

Jana *et al.* (2000) studied the agglutination potential of *P. fluorescens* in relation to energy stress and colonization of *M. phaseolina*. Among 172 isolates of fluorescent pseudomonads tested, 12 isolates showed maximum (73 %) agglutination and 30 isolates showed minimum (10 %) and the remaining were in the range of 10-73 per cent agglutination. Ebenzer and Wesely (2000) reported that among six fungicides studied under *in vitro* condition for their inhibitory effect on growth of *M. phaseolina*. Carbendazim completely inhibited the growth of *M. phaseolina* at 0.1 per cent followed by copper oxy chloride and thiram. Kumar *et al.* (2000) observed that pigeonpea seeds treated with *T. viride* reduced seed borne infection of *R. bataticola* and *R. solani* with significant increase in seedling emergence, root, shoot length and fresh weight of chickpea seedling when compared to *Chaetomium globosum* treated and untreated seeds as control.

Karunanithi *et al.* (2000) observed the production of antibiotic components *i.e.* pyrrolnitrin by *P. fluorescens* which inhibited the mycelial growth of *M. phaseolina* in dual culture by producing an inhibition zone of 12 mm. Similarly, *P. fluorescens* isolate 4 induced the systemic resistance against charcoal rot pathogen in chickpea by accumulation of pathogenesis related proteins such as chitinases and glucanases (Srivastava *et al.*, 2001). Nagamma *et al.* (2012) showed *T. viride* and *T. harzianum* both suppressed the growth of *M. phaseolina*. Same results were supported by Indira *et al.* (2003) and Ammajamma *et al.* (2009).

Gayathri *et al.* (2003) while working with dry root rot of sesame (*M. phaseolina*) reported that the combination of *Azospirillum* with other bio-control agents like *T. viride*, *P. fluorescens* showing a moderate level of disease control. Suriachandraselvan *et al.* (2004a) evaluated known fungal antagonists against, *M. phaseolina*. Among them, *T. harzianum* recorded the maximum growth inhibition (74.0%) followed by *T. virens* (73.0 %). They also reported that both bio-agents produced non-volatile antibiotics in culture filtrate which showed the maximum fungi toxic effect against the pathogen.

Dhoke and Kurundkar (2006) observed that the combined effect of *T. viride* and fluorescent *Pseudomonas* isolates were found inhibitory against *M. phaseolina* causing root rot of chickpea under *in vitro* conditions. Frequently studied fungi in relation to biological control are mostly species of *Trichoderma*. The efficacy of bio-control agents on *M. phaseolina* which caused dry root rot of pigeonpea was studied. In dual culture technique both *T. virens* (PDBC TVS-2) and *P. fluorescens* (PDBC Pf1) at the rate of (6×10^7 cfu), showed significantly inhibition of the mycelial growth of *M. phaseolina* by 78.22 per cent and 76.66 per cent respectively (Loksha and Benagi, 2007).

Paramasivan *et al.* (2007) tested the fungal and bacterial antagonists against dry root rot pathogen in which *T. virens* (Isolate 4) resulted in maximum growth of inhibition followed by *T. harzianum* and least growth of inhibition by *P. fluorescens*. The per cent inhibition by all other fungal and bacterial antagonists ranged between 41.0 and 56.0 per cent respectively.

The effectiveness of plant growth promoting rhizobacteria especially *P. fluorescens* isolates were tested against charcoal rot of chickpea both in green house and in field conditions. Among all the *P. fluorescens* isolates, Pf4-99 was found most effective in the improvement of chickpea crop in green house as well as in field. This isolate also inhibited the mycelial growth of the *M. phaseolina* under *in vitro* conditions and reduced the disease severity, and also increased seed yields significantly (Kumar *et al.*, 2007).

Surender Singh *et al.* (2007) reported the antagonistic activity of *T. harzianum* against *M. phaseolina* showing maximum growth inhibition (75.5 %) followed by *T. virens* (65.93 %) and *G. virens* (57.77 %). The least inhibition was 45.74 per cent by *Aspergillus* spp. Ramezani (2008) evaluated the efficacy of four fungal bio-agents *viz.*, *T. hamatum*, *T. harzianum*, *T. polysporum* and *T. viride* under *in vitro* condition against the brinjal root - rot pathogen *M. phaseolina*. Among the bio-agents, *T. harzianum* produced the maximum inhibition zone of 18.20 per cent compared to the minimum of 7.30 per cent by *T. hamatum*.

Biological control of safflower root rot caused by *M. phaseolina* the pycnidial stage of this fungus. Among the 13 isolates assayed, all the seven fungi and six bacteria significantly inhibited colony growth. *T. viride* (F-2) and *T. harzianum* (F-6) and two bacteria *viz.*, *Bacillus subtilis* (B-4) and *P. fluorescens* (B-6), two each of the fungal and

bacterial isolates were found to be especially effective against the pathogen *M. phaseolina* (Singh *et al.*, 2008).

Soil application of *T. viride* thrice, *i.e.*, 7, 15 and 30 DAS was found best in controlling seedling blight, collar rot, stem rot and root rot diseases giving minimum per cent disease incidence (1.45, 3.07, 4.70 and 4.92, respectively) as compared to control (16.12, 9.47, 16.67 and 16.34, respectively). Soil application with *T. viride* twice (7 and 15 DAS) showed 2.05, 4.42, 6.14 and 6.15 per cent incidence of the same diseases and ranked second. The treatment with carbendazim 50WP @ 2g/kg, seed showed 8.52 per cent seedling blight 5.24 per cent collar rot, 6.93 per cent stem rot and 8.56 per cent root rot incidence (Srivastava *et al.*, 2010).

Six isolates of *T. harzianum* were tested as potential bio-control agents under dual culture plate technique for their efficacy against *M. phaseolina*, a charcoal rot pathogen of maize. Hyderabad isolate of *T. harzianum* inhibited 62.3 per cent radial growth of *M. phaseolina* whereas isolates from Coimbatore, Dharwad, Delhi and Pantnagar inhibited 58, 56, 53, and 51 per cent of radial growth, respectively, over control (Shekar and Kumar, 2010).

The spray combination of chemicals and botanicals and micronutrients treatments under pot culture studies *viz.*, mancozeb (0.2 %) + *Allium* spp. (10 %), mancozeb (0.2%) + zinc sulphate (0.5 %), *Allium* spp. (10 %) + zinc sulphate (0.5 %) were significantly superior and respectively recorded 94.1, 90.6 and 88.2 per cent disease reduction over the control in green house (Murugapriya *et al.*, 2011).

Manjunatha *et al.* (2013) evaluated several isolates of *Trichoderma* spp. and *P. fluorescens* against the dry root rot of chickpea caused by *M. phaseolina* both under field and laboratory. *T. viride* and *T. harzianum* isolates were found to show significant differences in their ability to reduce mycelial growth of *M. phaseolina*. Over all, *T. viride* isolate, (Tv-R) was found to be the most effective and the isolate *P. fluorescens* (Pf4) was found to be significantly more effective than the other isolates. *T. viride* and *P. fluorescens* were applied as seed treatments alone and in combination with soil treatment and also along with chemical seed treatments with carbendazim and hexaconazole under field condition. *T. viride* seed treatment followed by soil application of FYM enriched with *T. viride* was found to have the highest germination (97.6 %) percentage and lowest root rot (2.67 %) incidence over the control (19 to 32 %).

Ten different strains of *P. fluorescens* were isolated from coleus rhizosphere. These strains were screened against *M. phaseolina* the causal organism of coleus root rot. Pf1 strains recorded maximum inhibition of mycelial growth against control. The mechanism of *Pseudomonas* strain such as iron-chelating agent (siderophore), volatiles (HCN) and antibiotic (Fluorescein and Pyocyanin) production tests were studied for reacted for siderophore, antibiotic and HCN production (Vanitha and Ramjegathesh, 2014). Among the 219 isolates 43 strains were showed antagonistic activity against pathogen but one isolate (cf60) was showed maximum inhibition (52.22 %) against mycelial growth of the pathogen by dual culture plate technique (Malleswari, 2014).

2.5.4 Integrated disease management

Soil-borne pathogens can be managed to reduce but not eliminate damage. Integrated and carefully implemented approaches to crop production reduce disease pressure and plant stress. This enables vigorous plants to more successfully obtain nutrients and moisture during critical vegetative and reproductive periods.

Charcoal rot of sesame caused by *M. phaseolina*. Combination of mancozeb (100 ppm), soil application of bio-agents *viz.*, native isolate of *T. viride* (10 g/kg of soil) and *P. fluorescens* (20 g/kg of soil) significantly reduced disease incidence (10.67 %) and enhanced plant growth components (Durai *et al.*, 2006).

Root rot of Jatropha caused by *R. bataticola* reduced the disease using fungicides, plant extracts and bio control agents. Results revealed that bavistin and vitavax were effective in inhibiting the growth of *R. bataticola* and reducing the incidence of Jatropha root rot. These fungicides resulted in 100 per cent inhibition of mycelial growth at 50 ppm. Seed treatment with bavistin and its soil drenching caused the least pre-emergence (16.7 %) and post-emergence mortalities (10.1 %). Under *in vitro* studies, neem extract at 20 per cent concentration provided 55.6 per cent inhibition. Integrated methods showed higher disease reduction compared with single method. Bavistin (2 g kg⁻¹ seed) + neem extract (20 %) was the most effective treatment with 67.3 per cent reduction followed by *T. harzianum* (15 g kg⁻¹ seed) + Bavistin (54.2 %) and neem extract + *T. harzianum* (44.0%) (Kumar *et al.*, 2011).

Groundnut dry root rot disease recorded 69.4 per cent in control with lowest disease of 11.1 per cent in the seed treatment with vitavax + soil application of *T. viride*

isolate I with neem cake followed by seed treatment of raxil + soil application of *T. viride* isolate I with neem cake and seed and soil application of *T. viride* isolate I with neem cake. The pod yield was highest (1427 kg/ha) in the treatment combination of seed treatment with vitavax + soil application of *T. viride* isolate I with neem cake where pod yield was increased by 35.1 per cent followed by seed and soil application of *T. viride* isolate I with neem or castor cake (Moradia and Khandar, 2011).

Integrated management of the root rot disease of mungbean caused by *M. phaseolina*, bio-control agents, fungicides, herbal oils, plant extracts and organic manure as well as their combinations. Among the bio-control agents *T. harzianum* was found the most effective against the fungus under *in vitro* and in pots conditions followed by *T. viride* and *T. polysporum*. *P. fluorescens* was the least effective in reducing root rot incidence. All the seven fungicides were tested *in vitro* by poisoned food technique and in pots (*in vivo*), bavistin was considered to be the most effective to inhibit mycelial growth of pathogen as well as reducing root rot incidence, followed by captan or thiram, indofil M-45 and vitavax or raxil, while copper sulphate was the least effective treatment in both conditions. In case of organic manures, vermicompost was the most effective in reducing the root rot incidence under pots conditions. Integrated management approach showed that vermicompost and bavistin in combination was more effective in reducing the root rot incidence in pots conditions (Kumari *et al.*, 2012). Same result was observed in apple root rot (Upmanyu *et al.*, 2002 and Gupta and Sharma, 2007) and green gram root rot (Rathore, 2006 and Rathore, 2012).

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The present investigation on *Macrophomina* Stem Canker caused by *Macrophomina phaseolina* was carried out during 2012-13 at Department of Plant Pathology, Agriculture College Raichur and ARS, Gulbarga. Raichur which is situated in North-Eastern Dry Zone (Zone 2) of Karnataka state at 16° 12' N longitude, 77° 21' E latitude and at an altitude of 389.37 m above mean sea level. The details of the material used and the methodology followed are described under this chapter.

3.1 General laboratory procedure

3.1.1 Glassware and cleaning

Borosil, Qualigens and Technico glassware were used for all experiments. The glassware were kept in the cleaning solution containing Potassium dichromate ($K_2Cr_2O_7$), concentrated Sulphuric acid (H_2SO_4) @ 60 g and 60 ml respectively in one liter of water. They were washed with detergent powder followed by washing in running tap water and rinsing in distilled water and kept in hot air oven for few minute for complete drying of moisture.

3.1.2 Sterilization

All glassware's were sterilized in hot air oven at 160 °C for two hours. Both solid and liquid culture media were sterilized by autoclaving at 1.1 kg pressure/sq. cm (121.6 °C) for 20 minutes. Soil used for experiments was sterilized for two hours at 1.33 kg pressure/sq. cm in an autoclave. The seeds and plant tissues were surface sterilized in 1: 1000 mercuric chloride solution followed by three changes in sterile water. All cultural studies were conducted in aseptic conditions under laminar flow. The tip of inoculation needle and forceps were sterilized by autoclaving and using flame.

3.2 Survey on incidence of *Macrophomina* Stem Canker of pigeonpea caused by *M. phaseolina*

An intensive roving survey was conducted during *Kharif* season 2013-2014; at near maturity growth stage of the crop to know the incidence of *Macrophomina* Stem Canker and Dry root rot of pigeonpea in the farmer's field at Raichur, Gulbarga, Yadgir and Bidar districts. The pigeonpea fields were randomly selected at the interval of 5-10 km along the roadside depending upon the topography and the cultivation of pigeonpea in

vertisols and alfisols. In each district a selected three major pigeonpea growing taluks and in each taluk four fields were surveyed and then the average incidence was calculated by using formula given below and based on disease rating scale (Table1) and expressed in percentage.

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

Table 1. Disease rating scale for Macrophomina Stem Canker disease of pigeonpea (modified from Abawi and Pastor-Corrales, 1990) reported by Kaur *et al.* (2013)

Score	Symptoms
1	No visible symptoms developed on the stem
3	Slight browning of the stem circumference at the collar region and the appearance of few lesions on the stem
5	Increase in the number of spindle shaped lesions, slight cracking in the centre of the spots and slight girdling of the stem but firm
7	Stem girdled, weak or breaks from the spots or collar region
9	Severe girdling of the stem, plants breaks and complete drying of the plant

3.2.1 Collection, isolation, identification, purification and maintenance of *M. phaseolina*

The symptomatic parts of Macrophomina Stem Canker diseased plants collected from different places *viz.*, Raichur, Gulbarga, Yadgiri and Bidar during survey were thoroughly washed under running tap water and blotted to dry on previously sterilized blotting paper. Upper surface of infected stem was peeled off and cut into pieces of 5-6 mm size and were surface sterilized by dipping in 0.1 per cent mercuric chloride. After thorough washing in sterile distilled water, the pieces were then transferred aseptically on to sterilized 1 per cent (w/v) agar and incubated for 72 h at 30 ±1 °C.

The mycelium growth started appearing, hyphal tips of the growing mycelium were marked on the underside of the Petri dish with glass marker under the light microscope. The hyphal tips from margins of resulting colonies were cut with sterilized cork borer and transferred to Petri dish containing fresh potato dextrose agar (PDA). The isolates were transferred to PDA slants and stored at 4 ± 1 °C for further studies. The culture of each isolate was regularly sub-cultured at three months interval. Such culture was used throughout the study.

3.2.2 Identification of the fungus

The morphology cultural characters, formation of sclerotia and branching of mycelium were the principal characters considered for identification of the fungus *Macrophomina*. The identity was also confirmed by sequencing and NCBI, BLAST.

3.2.3 Proving Koch's postulate

Pigeonpea plants of TS-3R variety was selected during flowering stage. Stems were superficially wounded by peeling 1 mm deep and 0.5 cm long to the epidermis with razor blade. The wounded area was inserted with *M. phaseolina* culture using a sterilized needle and smeared by 2 per cent sugar solution and covered with cellophane tape (Mayee and Garud, 1979; Besset *et al.*, 1996). This method was replicated for thrice under glass house condition. Water stress was given after 15 days of inoculation to create favourable condition.

3.2.4 Detached stem technique

Five cm length of stem at flowering stage/nearly maturity were collected from field. Collected stems were dipped in 0.1 per cent mercuric chloride followed by sterile distilled water then dried for few minutes such stems bits were superficially wounded to 1mm deep and 0.5 cm length peeling the epidermis with a razor blade and then inserted with *M. phaseolina* culture using sterilized needle and also smeared by 2 per cent sugar solution and covered with cellophane tape (Mayee and Garud, 1979 and Besset *et al.*, 1996). They were incubate in moist chamber at 30 ± 1 °C. The stress condition was created in intermediate days. At the end of 12 days after incubation symptoms were expressed. The stems were completely infected with pycnidia with spindle shaped lesion.

3.3 Studies on cultural, morphological variability and molecular characterization among the isolates of *M. phaseolina*

The pathogenic variation in 52 isolates of *M. phaseolina* was studied by employing the following techniques. For the study of culture characteristics one isolate (MP-GB-25) from among 52 isolates collected from four districts of North Eastern Karnataka was used and all isolates were used for morphological characteristics.

3.3.1 Cultural characters of *M. phaseolina* (MP-GB-25) on solid media

The cultural characters of *M. phaseolina* were studied on the following fourteen different solid media.

1. Potato Dextrose Agar
2. Potato carrot dextrose agar
3. Carrot agar
4. Oat meal agar
5. Malt extract agar
6. Richard's agar
7. Asthana and Hawkers Agar
8. Czapek's agar
9. Sabouraud's dextrose agar
10. Host stem extract agar
11. Host stem dextrose agar
12. Host leaves extract agar
13. Host leaves dextrose agar
14. Water agar

The composition and preparation of the above mentioned synthetic and non synthetic/semi- synthetic media were obtained from Ainsworth and Bisby's "Dictionary of the Fungi" by Hawksworth *et al.* (1983). The composition and preparation of the media are given in Appendix 1.

Twenty ml of each medium listed above was poured in to the Petri dishes for solidification. Five mm discs of *M. phaseolina* were placed at the centre of the plate. Each set of experiment was replicated thrice and plates were incubated at 30 ± 1 °C for 9 days. Observations were taken on parameters such as colony diameter, colony colour, presence or absence of sclerotia, pycnidia and its size. The results were analyzed statistically.

3.3.2 Growth on liquid media

Twenty ml of PDB was poured into each of the 150 ml conical flasks and the flasks were sterilized at 1.1 kg/cm^2 for 15 min. Inoculum disc of five mm diameter of fungus was taken from periphery of four day old culture and was transferred aseptically into the liquid media flasks separately. Each treatment was replicated thrice. The flasks were incubated at 30 ± 1 °C. Since the growth of the fungus was found to be the maximum on the eleventh day (Satishchandra, 1977 and Ramamurthy, 1982) of incubation, all the cultures were harvested on the eleventh day. Cultures were filtered through Whatman No. 1 filter paper disc of nine cm. diameter, which were dried to a constant weight at 60 °C, prior to filtration. The filter papers, along with mycelial mat, were dried to a constant weight in an electric oven at 60 °C and weighed immediately on an electric balance and weight of dry mycelia was recorded. The data was analyzed statistically.

3.3.3 Morphological variability of isolates of *M. phaseolina*

All the isolates of *M. phaseolina* were characterised for colony colour and growth pattern on PDA plates. The mycelial discs of 5 mm diameter were cut from the edge of a four-day-old culture of different isolates and were transferred aseptically to a 90 mm petri dish containing 20 ml PDA and incubated for 8-9 days at 30 ± 1 °C. Each treatment was replicated thrice.

The colonies were characterised for phenotype and growth pattern according to Pearson *et al.* (1986); Atiq *et al.* (2001) and Kaur *et al.* (2013). Different morphotypes shape (irregular and regular); growth pattern (circular and feathery); texture (velvety and cottony) were observed *in vitro*. Similarly, colour was differentiated into black and greyish black; microsclerotia, sclerotia were classified as present or absent while mycelium was differentiated as immersed and aerial.

3.3.4 Molecular characterization of *Macrophomina phaseolina*

Molecular identities in fifty two isolates of *M. phaseolina* from pigeonpea were studied by ITS rDNA conserved region. Total genomic DNA from the fungal isolates was extracted by Cetyl trimethyl Ammonium Bromide (CTAB, Sigma Chemical CO., St. Louis, USA) method as follows.

3.3.4.1 Total genomic DNA extraction

Genomic DNA was extracted by using the CTAB method of Lee *et al.*, (1993), with some modifications. For DNA extraction, fungal mycelia were harvested from the isolates grown in PDB incubated for 15-20 days in orbital shaker in 2000 rpm at 30 ± 1 °C. After incubation, the fungal biomass was filtered through sterile cheese cloth and was ground to a fine powder in the presence of liquid nitrogen.

Approximately 200 g of lyophilized mycelium was taken into 1.5 ml eppendorf tube, crushed with micro-pestle, mixed with 300 µl of lysis solution (50 mM Tris-HCl, pH 7.8, 50 mM Na₂-EDTA, 3 % SDS) to which 1 per cent 2-mercaptoethanol was added freshly and incubated at 65 °C for 4 h. Proteinase K (600 µg ml⁻¹) was added and further incubated at 35 °C for 1 h. The extracted genomic DNA was purified by phenol-chloroform method and precipitated with ethanol. The pure quantified DNA samples were stored at 4 °C for further use.

3.3.4.2 Assessment of DNA quality and quantity

The extracted DNA was quantified by running 2 µl of each DNA sample on 1.5 per cent agarose gel with Lambda uncut DNA (30 and 60 ng) to get a final concentration of 15-20 ng/ µl. To assess the quality, the DNA was diluted with TE buffer and loaded on 1 per cent agarose gels in 0.5X TAE buffer (pH 8.0) in a gel electrophoresis apparatus (Biorad, USA) and electrophoresed for 30 min under constant voltage (70 V). The ethidium bromide (final concentration of 0.3 µg/ ml) was added to the molten agarose at the time of gel casting. The gel was visualized on a UV transilluminator, and photographs were taken using a Gel Documentation System (Alpha Innotech Corporation, USA).

3.3.4.3 Primers

The rDNA gene cluster, consisting of ITS-1, the 5.8 S rDNA and ITS-4, was amplified with primers homologous to conserved sequences within the

small subunit (SSU) rDNA gene. The ITS primers used were ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990). PCR was performed in a total volume of 50 µl containing 5 µl of 10 X 3 PCR buffer (100 mM, Tris-HCl, pH 8.3, 15 mM MgCl₂, 250 mM KCl), 1U Taq DNA polymerase (Bangalore Genei, India), 160 µM dNTP mixture, 50 pmol of each ITS-1 and ITS-4 primers, and 50 ng genomic DNA in sterile dH₂O. The PCR amplifications were performed by using thermal cycler (Mastercycler) programmed for initial DNA denaturation at 95 °C for 1 min, annealing at 50 °C for 30 sec and extension at 72 °C for 1 min 20 sec with a final extension step at 72 °C for 10 min. All the amplified DNA products were resolved by electrophoresis on agarose gel (1.8 %) in TAE (1 X) buffer, stained with ethidium bromide and photographs were taken by using gel documentation system.

3.3.4.4 Sequencing of ITS region

Twenty two isolates of *M. phaseolina* were selected out of fifty two isolates based on representation to geographic regions, morphological grouping for sequencing of ITS region. Sequencing was carried out using Sanger sequencing method (Amnion Biosciences Pvt. Ltd., Bangalore, India). The resulting ITS sequences were analyzed for homologies in NCBI BLAST database based on previously published database sequences, sequences were deposited in the GenBank and get accession numbers. Online software MEGA4 (Tamura *et al.*, 2007) was used to construct the phylogenic tree using maximum likelihood method (<http://magasoftware.net>).

3.4 Inter connectivity study between sclerotia and pycnidia

Cellophane tape inoculation method described by Mayee and Garud (1979); Besset *et al.* (1996) was followed in glass house condition. Stem detached method described by Subramaniam (1994) was used throughout in *in vitro* study with slight modification. Different parts of pigeonpea; stem, leaves and root were selected for complete experiment both under green house and *in-vitro*. Isolate MP-GB-25 was used on TS-3R variety for this experiment. Four days old culture of *M. phaseolina* grown on PDA from the periphery of the plate were placed on corresponding selected stem, roots and leaves of 90 days old crop.

Under *in vitro* condition healthy stems free from all infection were cut in to 5 cm length roots, stem and leaves were used for inoculation . All the stems, roots and leaves dipped in to 0.1 per cent sodium hypochlorite followed dipped in sterile distilled water then were air dried for 30 min. All the parts arranged in specific manner, then under laminar air flow stems, roots and leaves surfaces were superficially wounded to 1mm deep and 0.5 cm length with a razor blade. *M. phaseolina* grown at 30 ± 1 °C, pycnidia and sclerotia were inserted in to wounded area by sterilized needle in to stem, roots and leaves and then smeared by 2 % sugar solution and covered with cellophane tape and then incubated at 30 ± 1 °C under moist chamber and replicated thrice, maintained with moisture appropriately. Moisture stress was created after three days. Observations were recorded after 8, 12 and 15 days.

Set of experiment was followed under green house, plants raised and at 90 days/ flowering stage. Such plants were wounded and inoculated with *M. phaseolina*, pycnidia and sclerotia and inoculated portion covered with cellophane tape. The observation was recorded after the end of 21 days according to Besset *et al.* (1996).

3.5 Management studies on Macrophomina Stem Canker

3.5.1 Screening for disease resistance

The genotypes comprising of released and MLT lines were screened under natural field condition of ARS, Gulbarga. The details of genotypes are listed in Table 3 and disease scoring scale in Table 2. Further resistant identified under field condition was artificially inoculated under *in vitro* using blotter paper technique followed for chickpea (Nene *et al.*, 1981).

3.5.1.1 Protocol of the blotter paper technique

The fungus was cultured on PDB. Twenty ml of PDB was poured into 100 ml conical flasks and sterilized. The flasks were then inoculated and incubated for ten days. The mycelial mat from the flask was removed and macerated in a waring blender along with distilled water for a minute. The inoculum was later collected in a beaker. In the mean time, the pigeonpea seedlings were raised in polythene bags containing sterilized sand mixed soil. The six days old seedlings were uprooted and the roots were immersed in sterile water in order to remove the adhered soil particles. The 20 seedlings of each

test lines were immersed completely in the inoculum in a beaker for a minute. The seedlings particularly the root portion were then placed side by side on a blotter paper (45 cm x 25 cm with one fold); in such a way that only the cotyledons and roots are covered and the green tops of seedlings remained outside and then blotter paper was folded. The folded blotter paper were then placed in trays and kept in an incubator at 30 °C for eight days. The blotters were moistened with sterile water every day. At the end of the incubation period, the seedlings were examined for the extent of root damage and score for the disease based on disease rating scale (1-9). The fungus was then re-isolated on PDA slants, incubated for four days and compared with initially obtained culture.

3.5.2 *In-vitro* evaluation of non-systemic and systemic fungicides

The experiment was carried out in (CRD). The details of treatments for *in vitro* evaluation of fungicides are listed in Table 4. Twenty ml of PDA medium initially mixed with chemicals listed below were poured in to 90 mm diameter Petri dishes. Control was maintained without addition of fungicides. After solidification, 5 mm discs of *M. phaseolina* were placed at the centre of the plate. Each set of experiment was replicated thrice and plates were incubated at 30 ±1 °C for control when reached the periphery of plates. Observations were taken on parameters such as colony diameter and per cent inhibition of growth which was calculated using the formula (Vincent, 1927).

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

Where,

I = Per cent inhibition,

C = Radial growth of fungus in control

T = Radial growth of fungus in treatment.

Table 2. Disease rating scale for screening of Macrophomina Stem Canker of pigeonpea

(Modified from Agarwal, 2003)

Score	Description	Reaction
1	1-10% incidence with 1-5 lesions/plant	Resistant
2	10-25% incidence with 10-15 lesions/plant	Moderately Resistant
3	25-50% incidence with 15-20 lesions/plant	Moderately Susceptible
4	50-75% incidence with 20-25 lesions/plant	Susceptible
5	>75% incidence with >25 lesions/plant and drying of branches	Highly Susceptible

Table 3. List of pigeonpea genotypes used for the screening against the *M. phaseolina*

Sl. No.	Genotypes	Sl. No.	Genotypes
1	WRP-1	12	GRG- 822
2	Bennur local	13	BSMR- 736
3	TS- 3R	14	GC- 11- 39
4	Chaple	15	GRG- 811
5	Kari togari	16	GRG- 2009- 1
6	Katti Beeja	17	JKM-7
7	Gulyal red	18	JKM-189
8	GRG- 2009	19	GPHR-08-11
9	GRG- 333	20	PT-04-31
10	GRG- 2010	21	ICPL-8863 (Maruti)
11	GRG- 818	22	ICP-87119 (Asha)

Table 4. List of systemic and non systemic fungicides used for *in-vitro* evaluation against *M. phaseolina*

Sl. No.	Common name	Chemical name	Trade name
Non-systemic fungicides			
1.	Captan	N-Trichloromethyl-1-thio-4-cyclohexane-1, 2 dicorboximide	Merimain 50 % WP
2.	Mancozeb	Manganese ethylene bis dithiocarbamate	Dithane M-45 75% WP
3.	Ziram	Zinc dimethyl dithiocorbomate	Deviziram 27 % SC
4.	Copper hydroxide	Copper hydroxide	Kocide 77 % WP
5.	Carbendazim 12 % + Mancozeb 63 %	Methyl 1-1-2 benzimidazole carbonate + Manganese ethylene bis dithiocarbamate	Saaf 75 % WP*
Systemic fungicides			
1.	Carbendazim	2-methoxy-carbamoyl-benzimidazole	Bavistin 50 % WP
2.	Benomyl	Methyl-N-(1-butyl carbamyl) 2-benzimidazole carbomate	Benofit 50 % WP
3.	Hexaconazole	2-2,4-dichloro phenyl 1-(1H,1, 2, 4-triazol-1yl) hexan-2-01	Hexalife 5 % EC
4.	Thiophanate methyl	1,2, bis (3-methoxy caboryl-2-thioureido benzene)	Roko70 % WP
5.	Carbendazim 25 % + Mancozeb 50 %	Methyl 1-1-2 benzimidazole carbonate + Manganese ethylene bis dithiocarbamate	Sprint 75 % WP*
6.	Hexaconazole 4 % + Zineb 68 %	2-2,4-dichloro phenyl 1-(1H,1,2,4-triazol-1yl) hexan-2-01 + Zinc ethylene bis dithiocarbamate	Avtar 72 % WP*
7.	Tricyclazole 18 % + Mancozeb 62 %	5-methyl 1,2,4 triazole (3, 4, 6) beza thiozole +Manganese ethylene bis dithiocarbamate	Merger 80 % WP*

* Combi-products

3.5.3 *In-vitro* evaluation of bio-agents against *M. phaseolina*

Two isolates of *Trichoderma* spp. four *Pseudomonas fluorescens* isolates, one *Pseudomonas putida* and one *Bacillus subtilis* (Table 5) were evaluated for their efficacy through dual culture technique. The source of bio-agents is presented in Table 3. The fungal bio-agent and the test fungus were inoculated side by side on a single Petri plate containing solidified PDA medium. Whereas, the bacterial bioagents were streaked one day earlier to the test pathogen. Three replications were maintained for each isolate with one control by maintaining only pathogen and bio-agent. They were incubated for control reaches periphery of plates. The diameter of the colony of both bio-agent and the fungus was measured in both directions and average was recorded and the per cent inhibition on growth of the test pathogen was calculated by using the formula given below by (Vincent, 1927).

Table 5. List of fungal and bacterial bio-agents used for *in-vitro* evaluation against *M. phaseolina*

Sl. No.	Bio-agents	Source
1.	<i>Trichoderma viride</i> (Tv-R)	Department of Plant Pathology, Agriculture College, Raichur
2.	<i>T. harzianum</i> (Th-R)	Department of Plant Pathology, Agriculture College, Raichur
3.	<i>P. fluorescens</i> (RP-46)	Department of Plant Pathology, Agriculture College, Raichur
4.	<i>P. fluorescens</i> (RP-31)	Department of Plant Pathology, Agriculture College, Raichur
5.	<i>P. fluorescens</i> (RP-16)	Department of Plant Pathology, Agriculture College, Raichur
6.	<i>P. fluorescens</i> (RP-41)	Department of Plant Pathology, Agriculture College, Raichur
7.	<i>P. putida</i> (RP-56)	Department of Plant Pathology, Agriculture College, Raichur
8.	<i>Bacillus subtilis</i>	Department of Plant Pathology, Agriculture College, Dharwad

3.5.4 Management under field conditions

3.5.4.1 Integrated disease management

An integrated approach for the management of pigeonpea stem canker caused by *M. phaseolina* was taken through cultural, chemical and biological measures. Those chemicals and bio-agents showing superior performance under *in vitro* were imposed for seed treatment/ soil application/foliar spray under field conditions.

Carbendazim was used for foliar spray. Integrated disease management was carried out by various combinations of treatments by using RBD design with seven treatments, replicated thrice as shown in Table 7. Observations on per cent incidence were recorded at the end of cropping season. Yield will be recorded after the harvest of the crop.

Design : Randomized Block Design

Plot size : 4.5 x 4.8 m

Spacing : 90 x 60 cm

**Table 6. Details of integrated disease management under field conditions
(Var: TS-3R)**

Treatments	
T ₁	Seed treatment with captan @ 3.0 g/kg seed + Foliar spray of carbendazim @ 1.5 g/l
T ₂	Seed treatment with carbendazim @ 2.0 g/kg seed + Foliar spray of carbendazim @ 1.5 g/l
T ₃	Seed treatment with <i>Trichoderma harzianum</i> @ 4 g/kg + Foliar spray of carbendazim @ 1.5 g/l
T ₄	Seed treatment with <i>T. viride</i> @ 4 g/kg + Foliar spray of carbendazim @ 1.5 g/l
T ₅	Seed treatment with <i>T. harzianum</i> @ 4 g/kg + <i>T. harzianum</i> enrichment with FYM at 1 kg per tonne FYM + Foliar spray of carbendazim @ 1.5 g/l
T ₆	Seed treatment with <i>T. viride</i> @ 4 g/kg + <i>T. viride</i> enrichment with FYM at 1 kg per tonne FYM + Foliar spray of carbendazim @ 1.5 g/l
T ₇	Check

3.5.4.2 Statistical analysis

Statistical analysis was carried out as per the procedures given by Panse and Sukhatme (1985). Actual data in percentage were converted to angular values, before analysis according to the table given by Snedecor and Cochran (1967).

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The present investigation on various aspects of Macrophomina Stem Canker (MSC) of pigeonpea caused by *Macrophomina phaseolina* includes survey on incidence and collection of pathogenic isolates and study of diversity among isolates with respect to cultural, morphological and molecular variability. Inter connectivity studies between pycnidia and sclerotia were made between the fungal structures and different parts of pigeonpea. *In vitro* screening of bio agents, fungicides and pigeonpea genotypes against pathogen was undertaken and management of stem canker of pigeonpea was attempted under field condition at MARS Farm, UAS Raichur. Screening of genotypes against Macrophomina Stem Canker of pigeonpea was undertaken in ARS, Gulbarga. The results obtained on these aspects are presented here under.

4.1 Symptomatology and collection of pathogenic isolates of *Macrophomina phaseolina*

The symptoms of Macrophomina Stem Canker of pigeonpea was characterized by formation of spindle shaped lesion (Plate 1a and Plate 1b) surrounded by pycnidial bodies on stem, splitting of the lesions and also the lesions coalesced leading to drying of branches and finally wilting of plants (Plate 2). The lesions were restricted to phloem region of the plants with brown discoloration. Such infected plants were collected and isolation was done by using standard tissue isolation method and purified by hyphal tip culture method.

4.1.2 Isolation and identification of the fungus

The morphological characters of *M. phaseolina* such as mycelium and sclerotial formation were studied for identification. The mycelium varied from black to brown or gray and became dark in color with age and abundant aerial mycelium was produced in the culture plate with sclerotia imbedded within the hyphae. Hyphae are septate, initially hyaline turning to a honey or black color. Aerial mycelium aggregated to form sclerotia and numerous dark brown to black colored sclerotia were observed on the reverse side of the culture plate. The vegetative mycelium is characterized by the formation of monilid or barrel-shaped cells and the formation of septum near the branching of the mycelium. Branching occurred at right angle to parent hyphae, even branching at acute angles was also observed (Plate 3a). Pycnidia were not observed on cultural plate but observed in



Plate 1a. Field view of *Macrophomina* Stem Canker infected pigeonpea plants



Plate 1b. Closer view of *Macrophomina* Stem Canker infected pigeonpea plants



a) Single spindle shaped lesion on stem



b) Typically infected plant showing drying of branches



c) Perennial plant showing more number of spindle shaped lesions on plants



d) Closer view of pycnidial bodies and splitting of stem at point of infection

Plate 2. Different stages of *Macrophomina* Stem Canker symptoms of pigeonpea (a-d)



e) Drying of branches of the *Macrophomina* Stem Canker infected plant

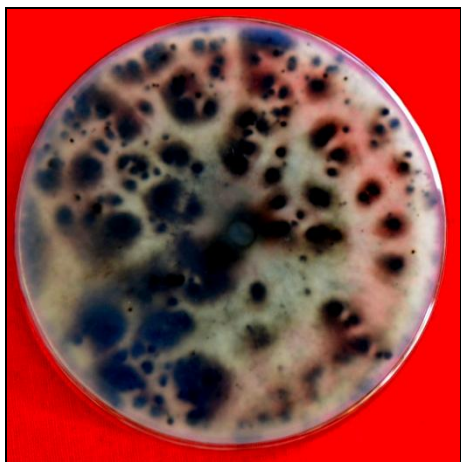


f) Closer view of dried branches of *Macrophomina* Stem Canker of pigeonpea

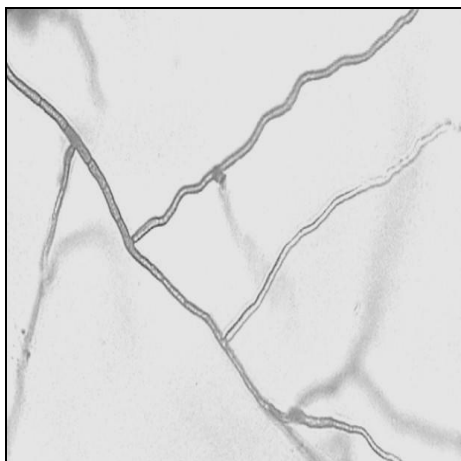
Plate 2. Different stages of *Macrophomina* Stem Canker symptoms of pigeonpea (e -f)



a) Aerial hyphae aggregated to form sclerotia and closer view of sclerotial bodies



b) Dark and numerous sclerotial bodies on the reverse side of the culture plate

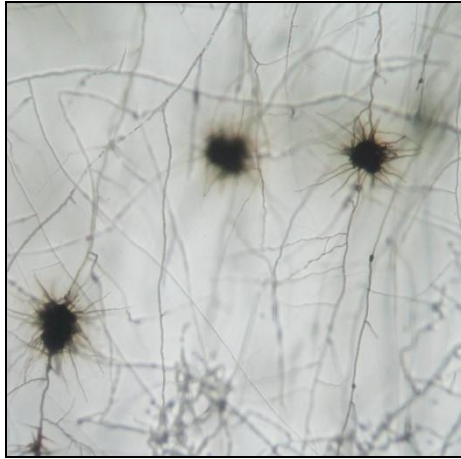


c) Right angle and acute angle branching of mycelium (10x)

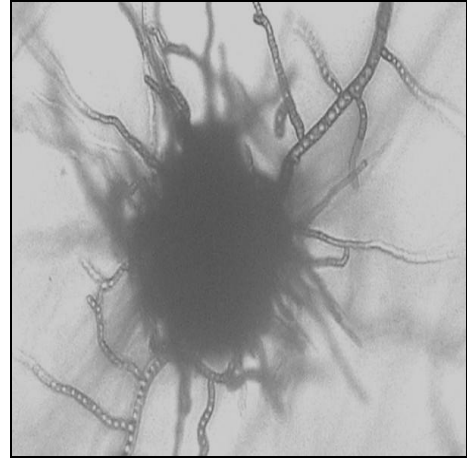


d) Initial stages of microsclerotia formation (10x)

Plate 3a. Morphological characteristics of *M. phaseolina* causing stem canker of pigeonpea (a-d)

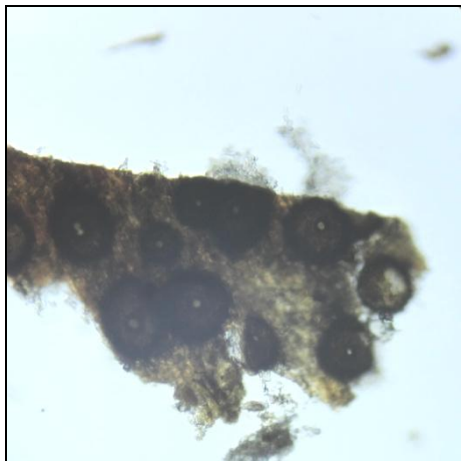


e) Microsclerotia (10x)



f) Microsclerotia (40x)

Plate 3a. Morphological characteristics of *M. phaseolina* causing stem canker of pigeonpea (e-f)



Pycnidial bodies in tissue section (10x) and pycnidiospores of *M. phaseolina* (40x)

Plate 3b. Intact tissue section of pycnidial bodies and pycnidial spores causing stem canker of pigeonpea

canker infected pigeonpea stem (Plate 3b). Based on the morphology and cultural characters of the mycelium and sclerotia, the fungus was identified as *M. phaseolina* and the isolates were designated as given in Table 7.

4.1.3 Pathogenicity test

Artificial inoculation of the fungus was carried out to prove the pathogenicity using cellophane tape method under glass house condition (Plate 4). Mycelium was inoculated in to 90 days old plant of pigeonpea variety TS-3R. After 21 days of inoculation, symptoms were expressed by formation of spindle shaped lesion producing numerous pycnidia on stem. *In vitro* detached stem technique was also carried out to prove pathogenicity (Plate 4), after six days of inoculation good number of pycnidia were noticed on stem incubated in moist chamber at 30 ± 1 °C. The fungus was re isolated and was found to resemble the original culture of *M. phaseolina*.

4.2. Survey on incidence of dry root rot and Macrophomina Stem Canker of pigeonpea

A roving survey was conducted during *Kharif*-2013 in different pigeonpea growing areas of North Eastern Karnataka regions which included Raichur, Gulbarga, Yadgir and Bidar districts to assess the status of Macrophomina Stem Canker (MSC) and dry root rot incidence under field condition. The data pertaining to survey are given in Table 8 and Fig. 1.

The mean maximum Macrophomina Stem Canker incidence was observed in Gulbarga district (25.02 %) followed by Bidar (18.11 %), Raichur (3.52 %) and the least (2.13 %) was in Yadgir. In Gulbarga, the highest incidence (77.33 %) was noticed in Agriculture Research Station (ARS), Gulbarga and the least (10.67 %) was in Handarki village. However, in Bidar district, the maximum incidence was noticed in Hudgi (48.00 %) and the least was (0.6 %) in Hankuni village. The maximum incidence in Raichur was in Yergera (10.30 %) and least was in Karigudda (0.33 %). However, in Yadgir district lowest incidence was recorded in Yargal (0.33 %) village and highest was found in Konapal with 5 per cent incidence. The overall incidence of MSC ranged from 0.33 to 77.33 per cent across the districts and taluks.

The highest average number of lesions and lesion length were recorded in ARS Gulbarga with 26.30 and 6.23 cm respectively. In ARS, Gulbarga two years old perennial

Table 7. Identity of *M. phaseolina* isolates of pigeonpea obtained from different regions of North Eastern Karnataka

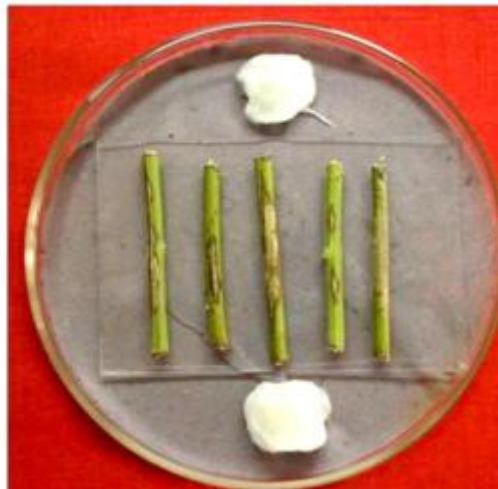
Sl. No.	Place of collection		Designation of the isolates
	Name of the District	Name of village	
1.	Raichur	UAS, Raichur	MP-RC-1
2.	Raichur	Yegera	MP-RC-2
3.	Raichur	Chickasugur	MP-RC-3
4.	Raichur	Jambaladinni	MP-RC-4
5.	Raichur	Kavithal	MP-RC-5
6.	Raichur	Mallat	MP-RC-6
7.	Raichur	Kallur	MP-RC-7
8.	Raichur	Kurdi	MP-RC-8
9.	Raichur	Jakkaldinni	MP-RC-9
10.	Raichur	Karigudda	MP-RC-10
11.	Raichur	Jalahalli	MP-RC-11
12.	Raichur	Alkod	MP-RC-12
13.	Yadgir	Yargal	MP-YD-13
14.	Yadgir	Gunjanaru	MP-YD-14
15.	Yadgir	Konapal	MP-YD-15
16.	Yadgir	Gajarkote	MP-YD-16
17.	Yadgir	B. Gudi	MP-YD-17
18.	Yadgir	Mudabala	MP-YD-18
19.	Yadgir	Gogi	MP-YD-19
20.	Yadgir	Madaraki	MP-YD-20
21.	Yadgir	Shorapur	MP-YD-21
22.	Yadgir	Mallaburjaka	MP-YD-22
23.	Yadgir	Yalagi	MP-YD-23
24.	Yadgir	Chamanahala	MP-YD-24
25.	Gulbarga	ARS,Gulbarga	MP-GB-25
26.	Gulbarga	Shirasigi	MP-GB-26
27.	Gulbarga	Bimalli	MP-GB-27
28.	Gulbarga	Hirapur	MP-GB-28

Contd....

Sl. No.	Place of collection		Designation of the isolates
	Name of the District	Name of village	
29.	Gulbarga	Kadaganchi	MP-GB-29
30.	Gulbarga	Telakarni	MP-GB-30
31.	Gulbarga	Honnahalli	MP-GB-31
32.	Gulbarga	Padavasavali	MP-GB-32
33.	Gulbarga	Halakatti	MP-GB-33
34.	Gulbarga	Diggao	MP-GB-34
35.	Gulbarga	Dandoti	MP-GB-35
36.	Gulbarga	Ramateerta	MP-GB-36
37.	Gulbarga	Kodla	MP-GB-37
38.	Gulbarga	Adaki	MP-GB-38
39.	Gulbarga	Handarki	MP-GB-39
40.	Gulbarga	Neelhalli	MP-GB-40
41.	Bidar	Janawada	MP-BD-41
42.	Bidar	Mirjapur	MP-BD-42
43.	Bidar	Honnadi	MP-BD-43
44.	Bidar	Bynaha	MP-BD-44
45.	Bidar	Hudgi	MP-BD-45
46.	Bidar	Nandagao	MP-BD-46
47.	Bidar	Kanakatta	MP-BD-47
48.	Bidar	Hankuni	MP-BD-48
49.	Bidar	Tadola	MP-BD-49
50.	Bidar	Hipparaga	MP-BD-50
51.	Bidar	Manavalli	MP-BD-51
52.	Bidar	Devanall	MP-BD-52



a) Control plant under glass house and plate under *in vitro* condition



b) Cellophane and Stem detached technique used for proving pathogenicity



c) Closer view of infected plant with spindle shaped lesion and pycnidial bodies

Plate 4. Pathogenicity test of *M. phaseolina* by inoculating to pigeonpea cultivar TS- 3R

Table 8. Incidence and PDI of Macrophomina Stem Canker (MSC) and dry root rot of pigeonpea in North Eastern Karnataka during Kharif-2013

Sl. No	District	Taluk	Villages	No. of fields	Variety	(Per cent incidence)		PDI of MSC	Average No. of lesions/ plant	Average lesion length/ plant (cm)
						Dry Root Rot	MSC			
1.	Raichur	Raichur	UAS, Raichur	3	TS-3R	13.67	6.30	12.88	3.30	2.3
			Yegera	3	Gulyal red	22.33	10.30	14.46	4.30	2.63
			Chickasugur	3	Local	16.33	3.30	12.88	3.30	2.63
			Jambaladinni	3	Kari togari	30.00	4.60	13.77	2.60	1.26
		Manvi	Kavithal	3	Kari togari	4.67	3.30	13.77	3.00	2.06
			Mallat	3	Kari togari	15.00	2.60	12.00	2.30	1.76
			Kallur	3	TS-3R	9.67	1.30	13.77	2.60	1.46
			Kurdi	3	TS-3R	20.67	3.00	12.00	1.30	1.76
		Deodurga	Jakkaldinni	3	TS-3R	22.33	4.60	13.77	2.00	2.13
			Karigudda	3	Local	15.33	0.33	12.00	1.30	0.93
			Jalahalli	3	Kari togari	12.67	1.30	13.77	2.30	1.8
			Alkoda	3	Local	0.00	1.00	12.00	1.00	1.46
		Mean					15.22	3.52	13.08	2.44
2.	Yadgir	Yadgir	Yargal	3	Local	21.67	0.33	12.00	0.60	0.63
			Gunjanaru	3	Local	13.33	3.00	12.00	1.30	1.63
			Konapal	3	Kari togari	15.00	5.00	13.77	2.00	1.86
			Gajarkote	3	Local	18.33	3.30	12.00	2.30	1.36
		Shahapur	B. Gudi	3	TS-3R	8.67	2.00	12.00	3.60	2.13
			Mudabala	3	Local	14.00	1.60	13.70	2.30	2.56
			Gogi	3	TS-3R	13.33	2.60	12.00	1.30	2.46
			Madaraki	3	Katti beeja	12.33	2.00	12.00	2.30	2.93
		Shorapur	Shorapur	3	TS-3R	5.33	0.60	13.77	1.30	2.73
			Mallaburjaka	3	Local	7.33	2.00	12.00	2.30	2.46
			Yalagi	3	Kari togari	8.33	1.60	12.00	1.30	3.30
			Chamanahala	3	Katti beeja	11.00	1.30	12.00	2.00	2.30
		Mean					12.39	2.13	12.43	1.88

Contd....

Sl. No.	District	Taluk	Villages	No. of fields	Variety	(Per cent incidence)		PDI of MSC	Average No. of lesions/ plant	Average lesion length /plant (cm)
						Dry Root Rot	MSC			
3.	Gulbarga	Gulbarga	ARS, Gulbarga	3	TS-3R	37.67	77.33	55.55	26.30	6.23
			Shirasigi	3	TS-3R	15.67	12.67	17.77	4.60	3.30
			Bimalli	3	TS-3R	23.67	28.67	27.55	6.30	3.7
			Hirapur	3	TS-3R	14.33	12.67	18.22	5.00	2.63
		Aland	Kadaganchi	3	TS-3R	30.67	13.33	20.00	7.60	2.10
			Telakarni	3	TS-3R	36.33	48.00	52.00	17.30	1.90
			Honnahalli	3	TS-3R	16.67	22.67	20.00	10.00	3.16
			Padavasavali	3	Gulyal red	25.67	18.00	13.77	2.00	2.60
		Chittapur	Ramateerta	3	TS-3R	32.00	46.00	52.00	5.00	3.30
			Diggao	3	TS-3R	17.33	28.00	27.12	6.00	3.43
			Dandoti	3	TS-3R	41.33	19.67	18.22	4.60	3.06
			Halakatti	3	local	9.00	20.67	27.55	5.30	3.86
		Sedam	Kodla	3	TS-3R	18.33	13.00	18.22	2.30	3.03
			Adaki	3	Kari togari	8.67	15.00	16.44	3.00	2.40
			Handarki	3	TS-3R	21.33	10.67	14.66	3.00	2.40
			Neelhalli	3	TS-3R	13.33	14.00	16.64	3.00	2.30
Mean						22.63	25.02	26.38	7.22	3.14
4.	Bidar	Bidar	Janawada	3	Maruti	8.67	6.30	13.77	1.60	1.26
			Mirjapur	3	BSMR 736	3.00	5.60	14.66	1.60	1.60
			Honnadi	3	Maruti	4.33	5.30	12.88	2.00	1.40
			Bynaha	3	Gulyal red	9.00	5.00	14.46	2.60	2.06
		Humnabad	Hudgi	3	TS-3R	0.00	48.00	52.00	4.00	2.86
			Nandagao	3	Asha	4.00	5.00	13.70	3.30	2.83
			Kanakatta	3	Asha	3.67	5.00	12.88	3.00	2.46
			Hankuni	3	Local	4.67	0.60	12.00	2.30	2.10
		Basavakalyana	Tadola	3	TS-3R	6.67	40.60	28.00	5.30	2.7.0
			Hipparaga	3	TS-3R	0.00	37.30	22.66	3.60	2.60
			Manavalli	3	TS-3R	7.67	32.60	20.76	3.60	2.16
			Devanall	3	TS-3R	5.00	26.00	24.00	2.00	2.26
		Mean						4.72	18.11	20.14

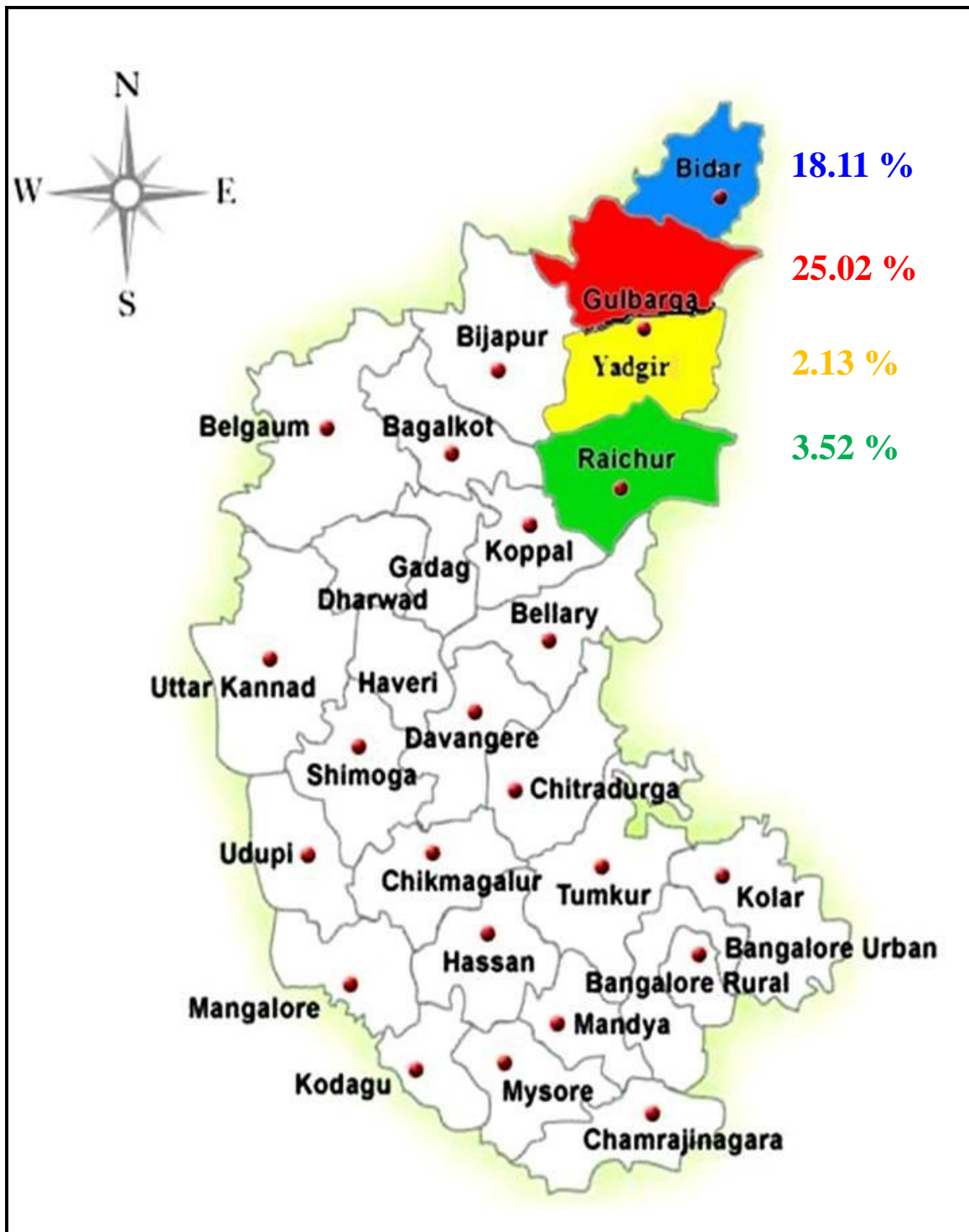


Fig. 1. Map showing incidence of Macrophomina Stem Canker of pigeonpea in different districts with special reference to North Eastern Karnataka during 2013

plant produced 89 lesions in a single plant with maximum lesion length of 13.60 cm with splitting of most of basal stem of lesions.

Maximum disease incidence of dry root of pigeonpea was recorded in Gulbarga district (22.63 %) followed by Raichur (15.22 %), Yadgir (12.39 %) and the least incidence (4.72 %) was in Bidar.

In Gulbarga, the highest incidence (41.33 %) was noticed in Dandoti village of Chittapur taluk and the least (8.67 %) was in Adaki village. However, in Raichur district, the maximum incidence was noticed in Jambaladinni (30.00 %) and the least was (0.00 %) in Alkoda village of Devodurga taluk. The maximum incidence was in Yargal (21.67 %) of Yadgir and least incidence was 5.33 per cent in Shorapur. However, in Bidar district lowest incidence was recorded in Hipparaga (0.00 %) and Hudgi village and highest of 9 per cent was found in Bynaha village of Bidar taluk. The overall incidence of dry root rot ranged from 0.00 to 41.33 per cent across the districts and taluks.

During survey higher disease incidence of MSC and dry root rot was noticed in TS-3R grown areas compared to other cultivars. In Gulbarga districts 80-90 per cent area is occupied by TS-3R variety and some villages like Hudgi (48.00 %) and Alkoda (1.00 %) belonged to Humnabad and Devodurga taluk respectively noticed only MSC with absence of dry root rot.

4.3 Cultural, morphological and molecular characterization of *M. phaseolina*

4.3.1 Growth and sclerotia production of *M. phaseolina* on different solid media

The results on growth and sclerotial production of isolate MP-GB-25 on different solid media are presented below.

Radial growth: The isolate MP-GB-25 showed difference in their growth in fourteen different synthetic and non synthetic solid media. Among different synthetic solid media, the maximum radial growth was recorded on Czapek's Dox agar with mean colony diameter of 50.67 mm followed by Sabouraud's dextrose agar (34.00 mm) and least mean colony diameter of 28.00 mm was observed in Richards' agar (Table 9a).

Among different non synthetic solid media, maximum radial growth was observed in Potato Dextrose Agar (PDA) with a diameter of 90.00 mm followed by host stem dextrose agar (80.33 mm) and least growth of 20.67 mm was observed in host leaf extract

Table 9a. Effect of different solid media on growth of *M. phaseolina* of pigeonpea

Sl. No.	Media	Average Radial growth (mm)*
1.	Richard's agar	28.00 (31.94)
2.	Czapek's Dox agar	50.67 (45.38)
3.	Asthana and Hawker's agar	30.00 (33.21)
4.	Sabouraud's dextrose agar	34.00 (35.66)
5.	Water agar	24.33 (29.55)
6.	Potato dextrose agar	90.00 (71.55)
7.	Carrot agar	46.33 (42.88)
8.	Potato carrot dextrose agar	80.00 (63.63)
9.	Malt extract agar	76.33 (60.88)
10.	Oat meal agar	62.33 (52.13)
11.	Host stem extract agar	55.33 (48.05)
12.	Host stem dextrose agar	80.33 (63.47)
13.	Host leaf extract agar	20.67 (27.04)
14.	Host leaf dextrose agar	26.00 (30.65)
	S. Em±	1.14
	C.D @ 1%	3.30

*Mean average of three replications

Observation taken after 72 hr incubation

Figures in parentheses are arc sine values

agar. Among the synthetic and non synthetic solid media, PDA, host stem dextrose agar and potato carrot dextrose agar showed maximum colony diameter and least was recorded in host leaf extract agar (Plate 5).

Production of sclerotia: Aerial mycelium aggregated to form sclerotia. The sclerotial production by isolate MP-GB-25 of *M. phaseolina* on different solid media is depicted in table 9b; and plate 6.

All the fourteen different synthetic and non synthetic media showed high variation in their sclerotial production. Among them, carrot agar recorded maximum 103 sclerotia per plate followed by PDA with 29 per plate. Least sclerotia were counted in malt extract agar (3/plate). The pycnidia were not produced in any of the tested media.

Observations on colony colour, type of margin, growth pattern and size of the sclerotia on different solid media of isolate MP-GB-25 of *M. phaseolina* at the end of ninth day of incubation are presented in table 9b. With reference to colony color and sclerotia productions, some non synthetic media such as carrot agar, PDA, potato carrot agar and malt extract agar produced the sclerotia with partial aerial mycelium and grayish black with a uniform margin. Rest of the media showed no sclerotia, immersed mycelium and light gray to dark gray colour, but oat meal agar showed fluffy growth and light gray colony colour and Richard's agar with a light pista colour. Among the synthetic media, Asthana and Hawker's agar produced sclerotia. Size of sclerotial bodies was recorded by using digital screw gauge.

Size of the sclerotia varied from 0.85 mm to 1.34 mm, highest sclerotial size (1.34 mm) was recorded in PDA, followed by carrot agar (1.12 mm) and least sclerotial size (0.85 mm) was recorded in malt extract agar.

4.3.2 Dry mycelial weight on different liquid media

Among the various liquid media tested, Richards' solution (465.33 mg) yielded significantly higher dry mycelial weight as compared to other liquid media followed by Malt extract solution (431.67 mg) (Table 10 and Plate 7). The dry weight of mycelium was moderately good in Czapek's Dox broth (360.67 mg), Sabouraud's dextrose broth (240.00 mg) followed by potato carrot dextrose broth (225.33 mg). Potato dextrose broth (315 mg), oat meal broth (324.33 mg) and host stem dextrose broth (326.33 mg) were

Table 9b. Cultural characteristics of *M. phaseolina* of pigeonpea on different solid media

Sl. No.	Media	Cultural characters of isolate				
		Colony colour	Type of growth	Type of margin	Sclerotial production per plate *	Sclerotial size (mm)
Synthetic Media						
1.	Richard's agar	Light pista	Immersed	Irregular	No	-
2.	Czapek's Dox agar	Grayish black	Immersed mycelium	Irregular	No	-
3.	Asthana and Hawker's agar	Light gray	Partially aerial mycelium	Irregular	5	0.96
4.	Sabouraud 's dextrose agar	Dark gray	Immersed mycelium	Irregular	No	-
5.	Water agar	Light black	Partially aerial mycelium	Uniform	No	-
Non Synthetic Media						
6.	Potato dextrose agar	Grayish black	Partially aerial mycelium	Uniform	29	1.34
7.	Carrot agar	Grayish black	Partially aerial mycelium	Uniform	103	1.12
8.	Potato carrot dextrose agar	Grayish black	Partially aerial mycelium	Uniform	17	1.02
9.	Malt extract agar	Grayish	Partially aerial mycelium	Uniform	3	0.85
10.	Oat meal agar	Light gray	Fluffy growth	Uniform	No	-
11.	Host stem extract agar	Dark gray	Partially aerial mycelium	Uniform	No	-
12.	Host stem dextrose agar	Grayish black	Immersed mycelium	Irregular	No	-
13.	Host leaf extract agar	Light gray	Immersed mycelium	Irregular	No	-
14.	Host leaf dextrose agar	Light gray with black centre	Partially aerial mycelium	Uniform	No	-

*Mean average of three replications

Observation taken 8-9 days after incubation

LEGEND

Plate. 5

Sl. No.	Solid media	Sl. No.	Solid media
1.	Potato carrot dextrose agar	8.	Richard's agar
2.	Potato dextrose agar	9.	Asthana and Hawker's agar
3.	Malt extract agar	10.	Host leaf dextrose agar
4.	Carrot agar	11.	Host leaf extract agar
5.	Host stem extract agar	12.	Czapek's Dox agar
6.	Host stem dextrose agar	13.	Water agar
7.	Oat meal agar	14.	Sabouraud's dextrose agar

Plate. 6

Sl. No.	Solid media	Sl. No.	Solid media
1.	Host leaf extract agar	8.	Host stem extract agar
2.	Sabouraud's dextrose agar	9.	Potato dextrose agar
3.	Richard's agar	10.	Malt extract agar
4.	Host stem dextrose agar	11.	Host leaf dextrose agar
5.	Czapek's Dox agar	12.	Oat meal agar
6.	Potato carrot dextrose agar	13.	Carrot agar
7.	Asthana and Hawker's agar	14.	Water agar

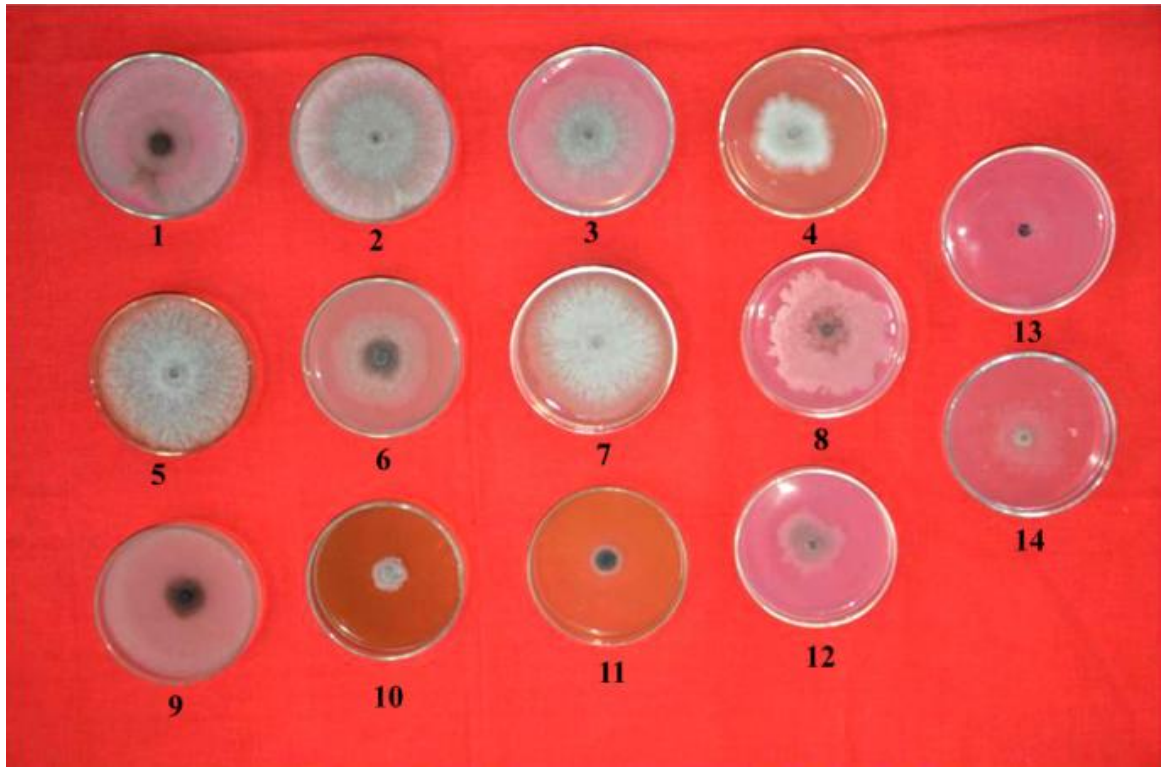


Plate 5. Effect of different solid media on radial growth of *M. phaseolina* at 3 days after inoculation



Plate 6. Effect of different solid media on radial growth and sclerotial production of *M. phaseolina* at 9 days after inoculation

Table 10. Effect of different liquid media on dry mycelial weight of *M. phaseolina* of pigeonpea

Sl. No.	Liquid media	Dry mycelial weight (mg)*
1.	Potato dextrose broth	315.00
2.	Potato carrot dextrose broth	225.33
3.	Carrot broth	399.33
4.	Oat meal broth	324.33
5.	Malt extract broth	431.67
6.	Richard's broth	465.33
7.	Asthana and Hawker's broth	183.00
8.	Czapek's Dox broth	360.67
9.	Sabouraud's dextrose broth	240.00
10.	Host stem extract broth	159.67
11.	Host stem dextrose broth	326.33
12.	Host leaf extract broth	120.67
13.	Host leaf dextrose broth	167.67
14.	Water	118.67
	S. Em±	9.39
	CD @ 1%	27.21

* Mean of three replications

LEGEND

Sl. No.	Liquid media
1.	Potato dextrose broth
2.	Potato carrot dextrose broth
3.	Carrot broth
4.	Oat meal broth
5.	Malt extract broth
6.	Richard's broth
7.	Asthana and Hawker's broth
8.	Czapek's Dox broth
9.	Sabouraud's dextrose broth
10.	Host stem extract broth
11.	Host stem dextrose broth
12.	Host leaf extract broth
13.	Host leaf dextrose broth
14.	Water

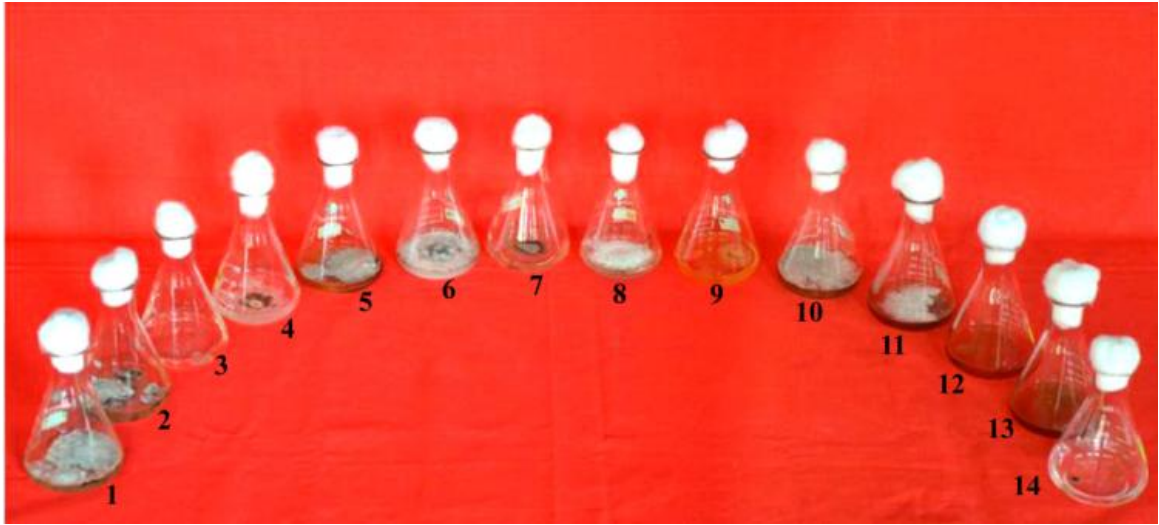


Plate 7. Effect of different liquid media on dry mycelial weight of *M. phaseolina* at 11 days after inoculation

Table 11a. Cultural and morphological diversity of different isolates of *M. phaseolina* of pigeonpea on PDA

Isolates	Mycelium characteristics						
	Radial growth (mm)*	Shape**	Growth pattern**	Texture**	Colour**	Mycelium**	Branching type**
MP-RC-1	90.00	Regular	Circular	Cottony	Grayish black	Partially aerial	Right & Acute angle
MP-RC-2	88.50	Regular	Circular	Velvety	Grayish with margin pista	Partially aerial	Right & Acute angle
MP-RC-3	84.30	Regular	Circular	Cottony	Grayish black	Aerial	Right & Acute angle
MP-RC-4	90.00	Irregular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-RC-5	90.00	Regular	Circular	Velvety	Light pista	Partially aerial	Right & Acute angle
MP-RC-6	90.00	Regular	Circular	Cottony	Light pista	Partially aerial	Right & Acute angle
MP-RC-7	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-RC-8	90.00	Regular	Circular	Velvety	Grayish black	Immersed	Right & Acute angle
MP-RC-9	90.00	Regular	Feathery	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-RC-10	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-RC-11	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-RC-12	90.00	Irregular	Circular	Cottony	Grayish black	Immersed	Right & Acute angle
MP-YD-13	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-YD-14	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-YD-15	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-YD-16	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-YD-17	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-YD-18	90.00	Regular	Feathery	Velvety	Grayish black	Aerial	Right & Acute angle

Contd....

Isolates	Mycelium characteristics						
	Radial growth (mm)*	Shape**	Growth pattern**	Texture**	Colour**	Mycelium**	Branching type**
MP-YD-19	90.00	Regular	Feathery	Cottony	Grayish with margin pista	Aerial	Right & Acute angle
MP-YD-20	90.00	Regular	Feathery	Cottony	Grayish black	Aerial	Right & Acute angle
MP-YD-21	90.00	Regular	Circular	Velvety	Light pista	Immersed	Right & Acute angle
MP-YD-22	90.00	Regular	Feathery	Velvety	Grayish black	Immersed	Right & Acute angle
MP-YD-23	85.20	Regular	Feathery	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-YD-24	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-GB-25	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-GB-26	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-GB-27	87.43	Regular	Circular	Cottony	Grayish black	Partially aerial	Right & Acute angle
MP-GB-28	90.00	Regular	Circular	Velvety	Grayish with margin pista	Immersed	Right & Acute angle
MP-GB-29	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-GB-30	90.00	Regular	Circular	Velvety	Light pista	Partially aerial	Right & Acute angle
MP-GB-31	87.02	Regular	Circular	Velvety	Grayish with margin pista	Partially aerial	Right & Acute angle
MP-GB-32	90.00	Regular	Circular	Cottony	Grayish black	Partially aerial	Right & Acute angle
MP-GB-33	90.00	Irregular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-GB-34	89.02	Irregular	Circular	Velvety	Grayish black	Immersed	Right & Acute angle
MP-GB-35	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-GB-36	90.00	Irregular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle

Contd....

Isolates	Mycelium characteristics						
	Radial growth (mm)*	Shape**	Growth pattern**	Texture**	Colour**	Mycelium**	Branching type**
MP-GB-37	85.68	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-GB-38	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-GB-39	90.00	Regular	Circular	Cottony	Light pista	Partially aerial	Right & Acute angle
MP-GB-40	90.00	Irregular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-BD-41	90.00	Regular	Circular	Cottony	Light pista	Partially aerial	Right & Acute angle
MP-BD-42	90.00	Irregular	Circular	Cottony	Grayish with margin pista	Partially aerial	Right & Acute angle
MP-BD-43	90.00	Regular	Feathery	Cottony	Grayish with margin pista	Partially aerial	Right & Acute angle
MP-BD-44	90.00	Irregular	Circular	Velvety	Light pista	Partially aerial	Right & Acute angle
MP-BD-45	81.63	Regular	Feathery	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-BD-46	90.00	Regular	Circular	Velvety	Light pista	Partially aerial	Right & Acute angle
MP-BD-47	80.30	Irregular	Feathery	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-BD-48	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-BD-49	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-BD-50	90.00	Irregular	Circular	Velvety	Light pista	Partially aerial	Right & Acute angle
MP-BD-51	75.17	Irregular	Feathery	Velvety	Grayish black	Partially aerial	Right & Acute angle
MP-BD-52	90.00	Regular	Circular	Velvety	Grayish black	Partially aerial	Right & Acute angle

* Mean of three replications, observation was taken 72hr DAI (Days after inoculation)

** Observation was taken 9 DAI

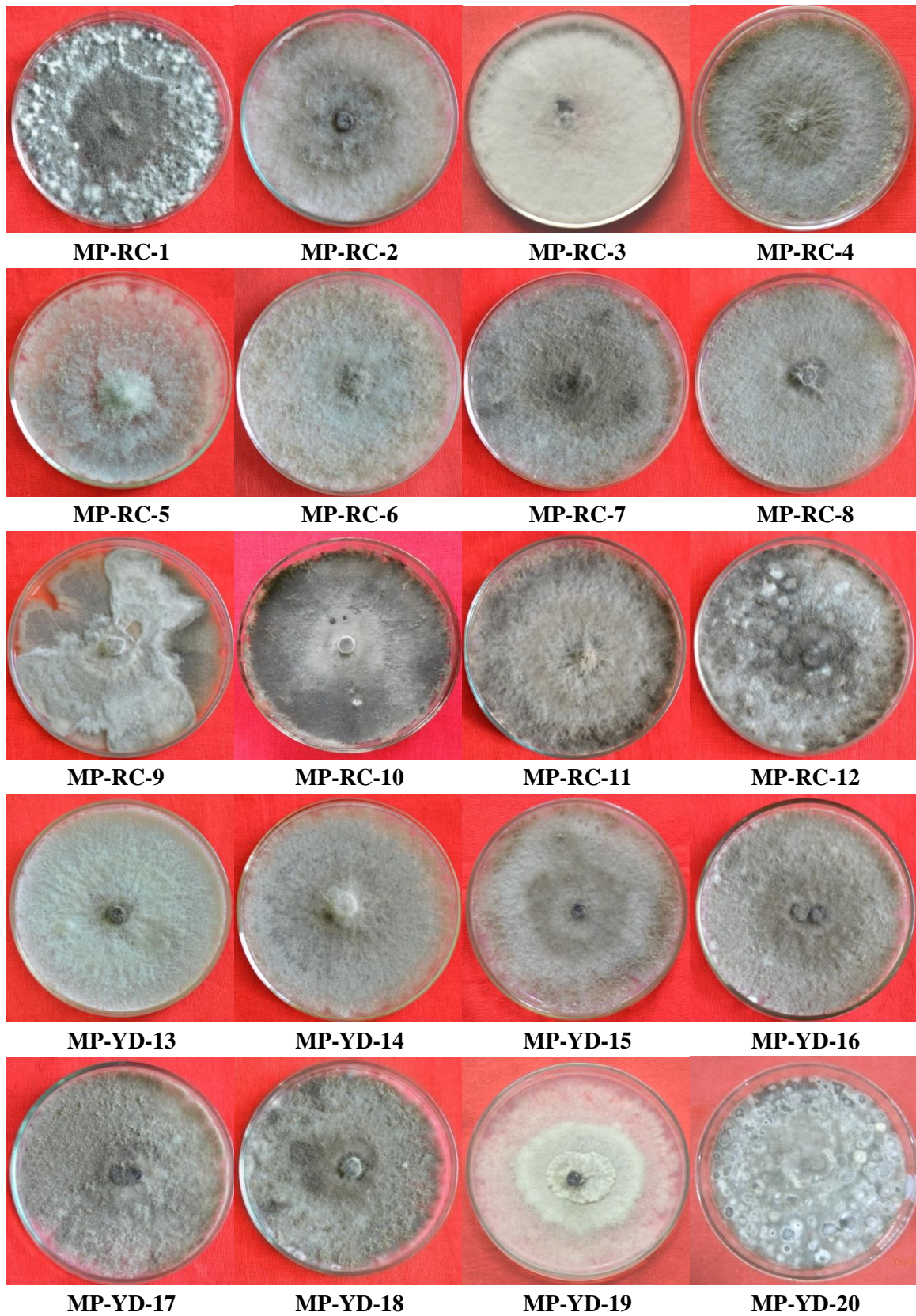


Plate 8. Diverse growth pattern of fifty two isolates of *M. phaseolina* on PDA (1-20)

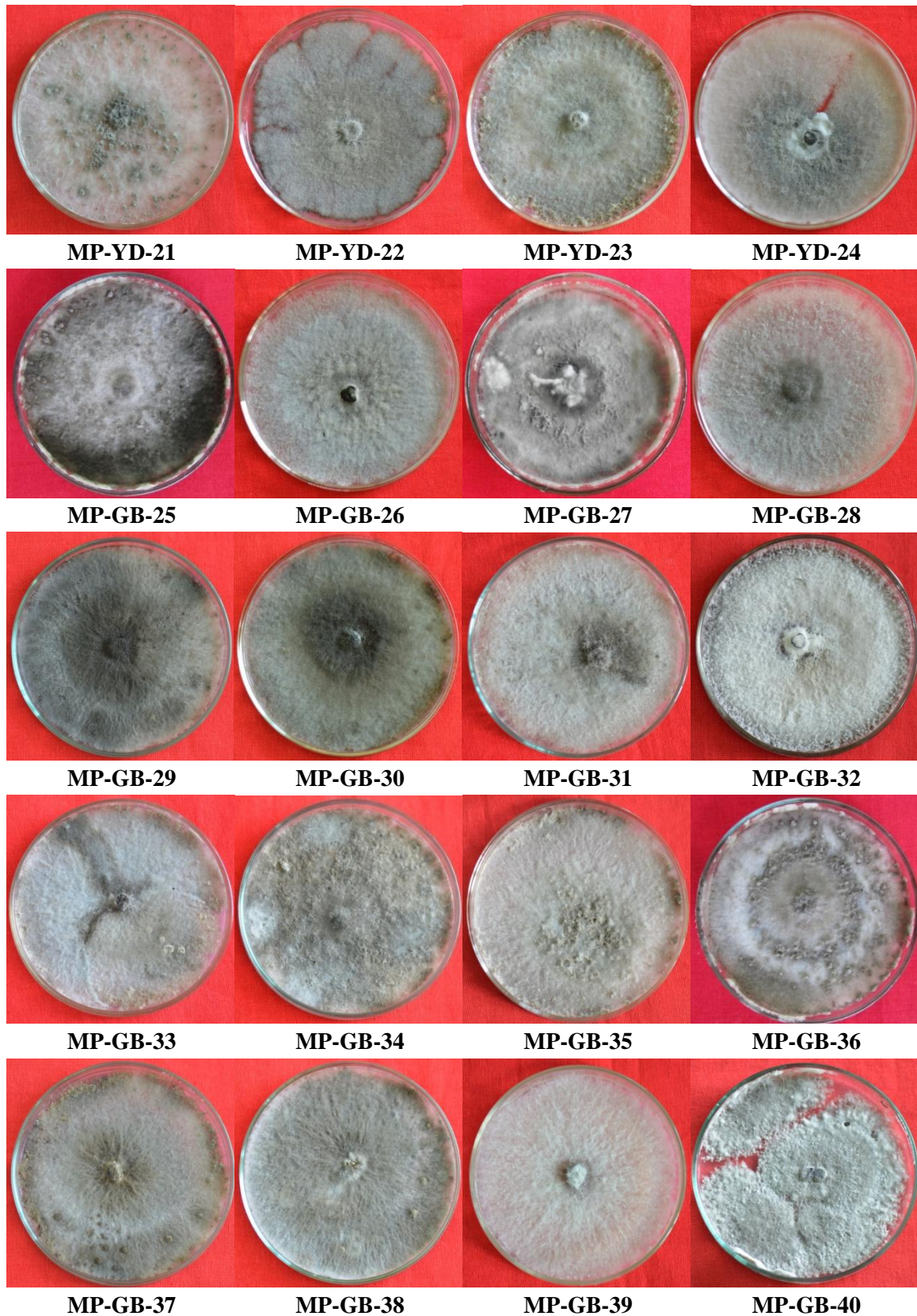


Plate 8. Diverse growth pattern of fifty two isolates of *M. phaseolina* on PDA (21-40)

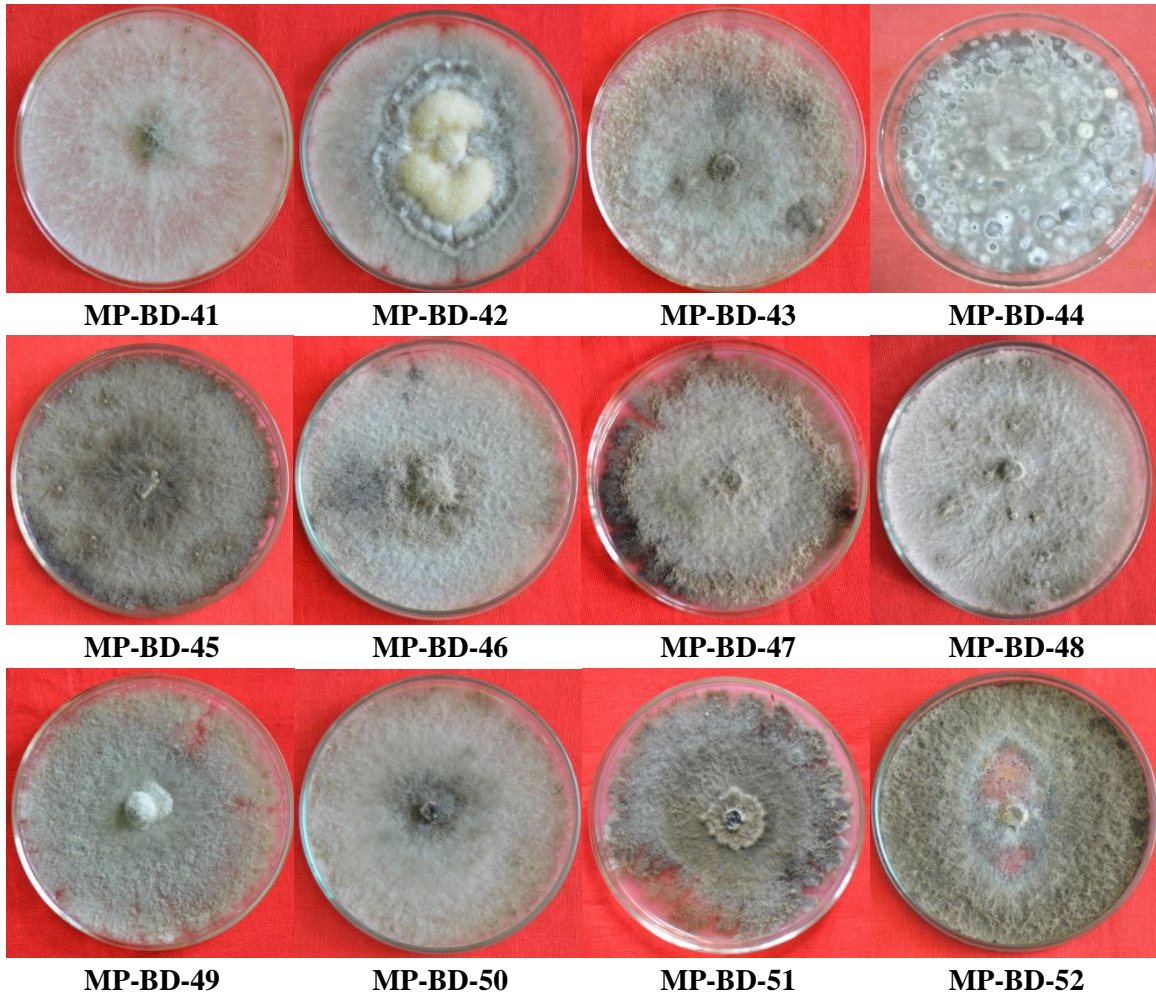


Plate 8. Diverse growth pattern of fifty two isolates of *M. phaseolina* on PDA (41-52)

found on par with each other. While in host leaf extract broth (120.67 mg) and water (118.67 mg) it was very poor. After 12 days of incubation mycelial mats were harvested.

4.3.3 Diversity in morphological and cultural characters of *M. phaseolina*

Morphological features of 52 isolates of *M. phaseolina* were described by growing the isolates on PDA medium and characterized with respect to different characters such as shape, growth pattern, colour, texture, mycelium growth, branching type and sclerotial/microsclerotia production. The results are summarized in table 11a with photograph depicted in Plate 8.

All the isolates were grown on both PDA and water agar (1 %) medium to study sclerotia, microsclerotia and their morphology and size. The data is presented in Table 12. Based on morphological characteristics of mycelium and sclerotia/microsclerotia the isolates were categorized in to three main groups and two subgroups described in Table 13.

Mycelium: Colony colour of *M. phaseolina* varied in culture from black to brown or gray and became dark in colour with advancement of age among the isolates. Hyphae are septate, branching at right angle to parent hyphae, but branching at acute angle was also common in all fifty two isolates. Morphological characteristics of all the isolates were studied by various parameters described in table 11a. Most of the isolates from Gulbarga and Bidar produced grayish black, velvety, partially aerial mycelium with right angle to acute angle and 11 isolates from Gulbarga, six isolates from Bidar produced sclerotia and microsclerotia. Few isolates of Raichur and Yadgir produced grayish to light pista, cottony to velvety aerial to immersed with right angle to acute angle and only three isolates each from Raichur and Yadgir produced sclerotia and microsclerotia. Based on mean colony diameter among isolates, forty two isolates were fast growing (90 mm), slow growing (75.17 mm) was found in MP-BD-50 and rest of nine isolates showed moderate growth ranging from 80 to 89.02 mm.

Among the phenotypic characters the right angle and acute angle branching was a common character with 100 per cent frequency and also circular pattern of growth was visible with 82.69 per cent, partial aerial of mycelium (80.77 %), regular pattern of shape (80.76 %) and velvety texture of fungus (76.93 %) with frequency ranking of I. However, phenotypic characters such as absence of microsclerotia (71.16 %), grayish black colour

(71.15 %) and absence of sclerotia (67.31 %) showed a frequency ranking of II. Presence of sclerotia and microsclerotia ranging from 25-50 per cent showed rank of III. The remaining isolates showed isolates with cottony texture (23.07 %), irregular shape (19.24 %), feathery growth pattern (17.31 %), light pista (17.30 %), grayish with margin pista (11.53%), immersed mycelium (11.54 %) and aerial mycelium with 7.69 per cent frequency and rank of IV. The data is presented in table 11b.

Sclerotia and microsclerotia: For production of sclerotia and microsclerotia all the isolates were grown in both PDA and water agar (1 %). Among fifty two isolates of *M. phaseolina* under PDA, 17 isolates produced sclerotia both on front side (1-68) and riverside side of the culture plates ranging from 3 to 136. Under water agar, microsclerotia were also observed under microscope. Among 52 isolates, 16 isolates produced microsclerotia ranging from 1 to 7.3 per microscopic field (10x).

Among 52 isolates, nine isolates produced with both sclerotia and microsclerotia under PDA and water agar respectively remaining twenty nine isolates did not produce any sclerotia and microsclerotia respectively.

Among 17 isolates, isolate MP-GB-36 produced highest number of sclerotia on both riverside side (136) and front side (68) of the PDA culture plate followed by MP-GB-27 (56 & 2) and MP-GB-25 (41 & 18) photograph depicted in plate 9. The isolate MP-GB-35 (8 & 1) and MP-BD-50 (7 & 1) recorded least production of sclerotia and also with respect to the size highest size was recorded in MP-BD-48 (1.94 mm) followed by MP-GB-37 (1.69 mm) and the least size was recorded in MP-GB-27 (0.61 mm). In water agar among 16 isolates, isolate MP-GB-25 produced highest microsclerotia (7.3/10x) under microscope followed by MP-GB-38 (5.4/10x) and highest size was recorded in MP-GB-25 (148.4 μ m) followed by MP-RC-11 (144.5 μ m) and least size was recorded in MP-RC-1 (84.5 μ m). Among 52 isolates, most (11) isolates from Gulbarga produced sclerotia and microsclerotia compared to Raichur, Yadgir and Bidar described in Table 12.

4.3.3.1 Grouping of isolates based on morphological and sclerotial characteristics

M. phaseolina

Diversity in morphological characters such as shape (regular/irregular), growth pattern (circular/feathery), texture (cottony/velvety), colour (grayish black/grayish with

Table 11b. Frequency distribution of isolates of *M. phaseolina* of pigeonpea based on morphological and cultural features obtained from different regions of North Eastern Karnataka

Sl. No.	Phenotypic characters		Frequency (%)	Ranking*
1.	Shape	Regular (RE)	80.76	I
		Irregular (IR)	19.24	IV
2.	Growth pattern	Circular (CIR)	82.69	I
		Feathery (FEA)	17.31	IV
3.	Texture	Velvety (VEL)	76.93	I
		Cottony (COT)	23.07	IV
4.	Colour	Grayish black (GB)	71.15	II
		Light pista (LP)	17.30	IV
		Grayish with margin pista (GMP)	11.53	IV
5.	Mycelium	Immersed (IM)	11.54	IV
		Partially aerial (PAL)	80.77	I
		Aerial (AL)	7.69	IV
6.	Branching type	Right angle(RA) & Acute angle (AA)	100.00	I
7.	Sclerotia	Presence (S+)	32.69	III
		Absence (S-)	67.31	II
8.	Microsclerotia	Presence (M+)	28.84	III
		Absence (M-)	71.16	II

* >75 % = I, 75-50 % = II, 50-25 % = III and < 25 % = IV

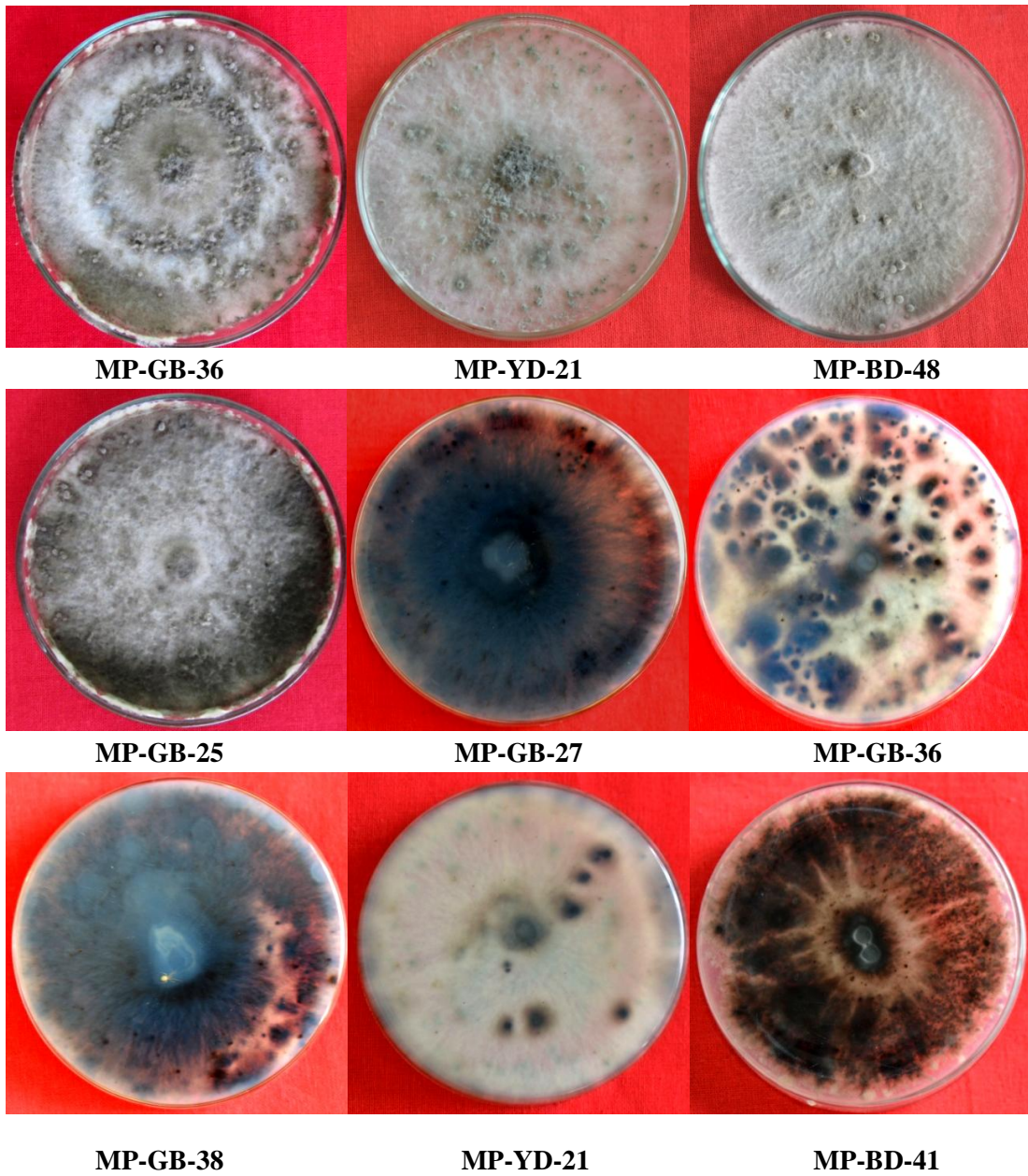


Plate 9. Diversity in sclerotia of *M. phaseolina* as varied from both front and reverse side of the culture plate

Contd....

Isolates	Potato dextrose Agar***					Water agar (1 %) ***			
	Presence or Absence	Presence of sclerotia				Absence	Presence of micro sclerotia		
		Reverse side of plate* (No./plate)	Front side* (No./plate)	Shape	Size* (mm)		(No./microscopic field, 10 X)**	Shape	Size* (µm)
MP-YD-18	-	-	-	-	-	-	-	-	-
MP-YD-19	-	-	-	-	-	-	-	-	-
MP-YD-20	-	-	-	-	-	-	-	-	-
MP-YD-21	+	8	7	Round	1.02	-	-	-	-
MP-YD-22	-	-	-	-	-	-	-	-	-
MP-YD-23	+	10	1	Round	1.21	+	4	Round	142.6
MP-YD-24	-	-	-	-	-	-	-	-	-
MP-GB-25	+	41	18	Round	1.38	+	7.3	Round	148.4
MP-GB-26	-	-	-	-	-	+	3	Round	94.2
MP-GB-27	+	56	2	Round	0.61	-	-	-	-
MP-GB-28	-	-	-	-	-	-	-	-	-
MP-GB-29	+	14	1	Round	1.31	+	4.2	Round	124
MP-GB-30	-	-	-	-	-	-	-	-	-
MP-GB-31	+	23	2	Round	1.39	-	-	-	-
MP-GB-32	-	-	-	-	-	-	-	-	-
MP-GB-33	+	9	6	Round	1.40	+	3.4	Round	124.4
MP-GB-34	-	-	-	-	-	-	-	-	-
MP-GB-35	+	8	1	Round	1.09	-	-	-	-
MP-GB-36	+	136	68	Round	1.31	-	-	-	-

Contd....

Isolates	Potato dextrose Agar***					Water agar (1 %) ***			
	Presence or Absence	Presence of sclerotia				Absence	Presence of micro sclerotia		
		Reverse side of plate* (No./plate)	Front side* (No./plate)	Shape	Size* (mm)		(No./microscopic field, 10 X)**	Shape	Size* (µm)
MP-GB-37	+	40	12	Round	1.69	+	4.6	Oblong	134.6
MP-GB-38	+	36	13	Round	1.57	+	5.4	Round	129.2
MP-GB-39	+	3	-	-	-	-	-	-	-
MP-GB-40	-	-	-	-	-	-	-	-	-
MP-BD-41	-	-	-	-	-	-	-	-	-
MP-BD-42	-	-	-	-	-	-	-	-	-
MP-BD-43	-	-	-	-	-	-	-	-	-
MP-BD-44	-	-	-	-	-	+	2.8	Oblong	103.4
MP-BD-45	-	-	-	-	-	+	2	Oblong	108.4
MP-BD-46	-	-	-	-	-	+	6	Oblong	111.3
MP-BD-47	+	12	-	-	-	-	-	-	-
MP-BD-48	+	17	8	Round	1.942	-	-	-	-
MP-BD-49	-	-	-	-	-	-	-	-	-
MP-BD-50	+	7	1	Round	1.30	+	3	Round	106.4
MP-BD-51	-	-	-	-	-	-	-	-	-
MP-BD-52	-	-	-	-	-	-	-	-	-

Size: *Mean average of three replications, Presence (+), Absence (-)

** Mean of 5 observations per microscopic field,

*** Observation was taken 9 DAI and

margin pista/light pista) and mycelium (aerial/partially aerial/immersed) and branching type (right angle to acute angle) were closely observed in fifty two isolates of *M. phaseolina*. Based on the striking difference of morphological characteristics of texture, shape and colour of the isolates were categorized in to three groups designated as G-I, G-II, and G-III and further based on the characteristics of sclerotia and microsclerotia presence/absence, numbers, size and shape again isolates were categorized in to sub groups in G-II (G-IIA and G-IIB), G-III (G-IIIA, G-IIIB) as described in Table 13.

G-I comprising of six isolates from Raichur, five from Gulbarga, four each from Yadgir and Bidar. G-II comprising of diverse isolates with respect to the striking phenotypic characters and sclerotia/microsclerotia produced and also absence of both sclerotia and microsclerotia isolates from all districts. It includes 19 isolates three each from Bidar and Raichur, six each from Gulbarga and Yadgir. In GII group out of 19 isolates, nine isolates belonged to G-II-A with production of both sclerotia and microsclerotia. In GII-B comprised of 10 isolates with absence of sclerotia and microsclerotia.

G-III comprised five isolates each from Gulbarga and Bidar, three isolates from Raichur and two isolates from Yadgir. In GIII out of 14 isolates, 7 isolates produced sclerotia without microsclerotia belonged to GIII-A and 7 from GIII-B produced microsclerotia without sclerotia.

Among fifty two isolates maximum frequency (36.53 %) was found in G-I and GII (GII-A (17.30 %), GII-B (19.23 %)). followed by G-III (GIIIA (13.46) and GIIIB (13.46) as described in Table 13.

4.3.4 Molecular characterization of *M. phaseolina* isolates

The extracted DNA was amplified with ITS primers and 52 *M. phaseolina* isolates were amplified and amplified product checked on 1.4 % agarose gel. The size of amplified DNA showed range of 550 to 600 bp length (Plate 10). Sequencing of them revealed that all the isolates belonged to the *M. phaseolina*. Twenty two isolates of *M. phaseolina* were selected out of fifty two isolates based on representation to geographic regions and morphological grouping. Such isolates were amplified and 5.8 S rDNA sequenced. The ITS rDNA were sequenced from Amnion Biosciences Pvt. Ltd., Bangalore, India. The sequences of representative isolates are given below.

Table 13. Grouping of 52 different *M. phaseolina* isolates of pigeonpea based on cultural, morphological and sclerotial characters

	Group I	Group II		Group III	
Mycelium					
Shape	Regular to irregular	Regular to irregular		Regular to irregular	
Growth pattern	Circular to Feathery	Circular to feathery		Circular	
Texture	Cottony to Velvety	Velvety		Cottony to Velvety	
Colour	Grayish black to margin pista	Grayish black to light pista		Light pista to grayish with margin pista	
Mycelium	Partially aerial to Aerial and Immersed	Partially aerial		Partially aerial to Aerial and Immersed	
Branching type	Right angle and Acute angle	Right angle and Acute angle		Right angle and Acute angle	
		II A	IIB	IIIA	IIIB
Sclerotia					
Sclerotia	Absence	Presence	Absence	Presence	Absence
Microsclerotia	Absence	Presence	Absence	Absence	Presence
Shape	-	Round	-	Round	Oblong
Isolates	19	9	10	7	7
	MP-RC-2, MP-RC-3, MP-RC-5, MP-RC-6, MP-RC-9, MP-RC-12, MP-YD-14, MP-YD-18, MP-YD-19, MP-YD-20, MP-YD-22, MP-GB-28, MP-GB-32, MP-GB-34, MP-GB-39, MP-BD-41, MP-BD-42, MP-BD-43, MP-BD-51	MP-RC-11, MP-YD-15, MP-YD-23, MP-GB-25, MP-GB-29, MP-GB-33, MP-GB-37, MP-GB-38 and MP-BD-50	MP-RC-8, MP-RC-10, MP-YD-13, MP-YD-16, MP-YD-17, MP-YD-24, MP-GB-30, MP-GB-40, MP-BD-49, and MP-BD-52	MP-YD-21, MP-GB-27, MP-GB-31, MP-GB-35, MP-GB-36, MP-BD-47 and MP-BD-48	MP-RC-1, MP-RC-4, MP-RC-7, MP-GB-26, MP-BD-44, MP-BD-45, and MP-BD-46
Frequency	36.53	17.30	19.23	13.46	13.46

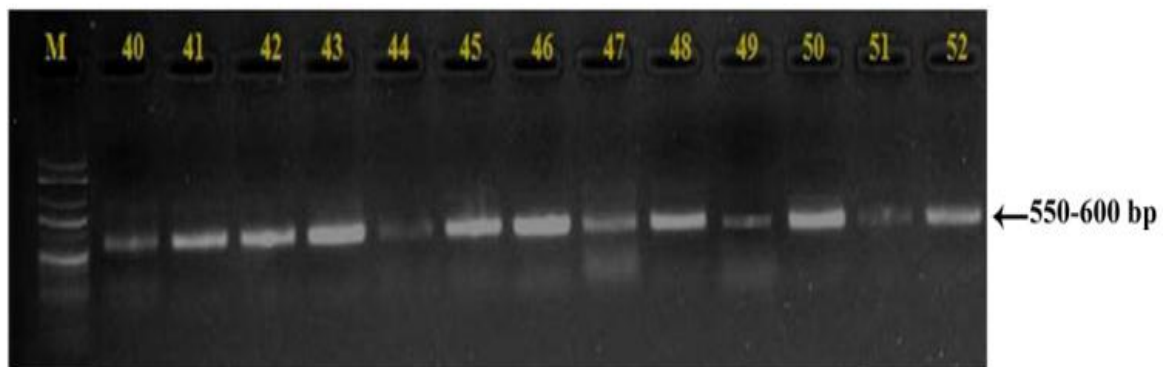
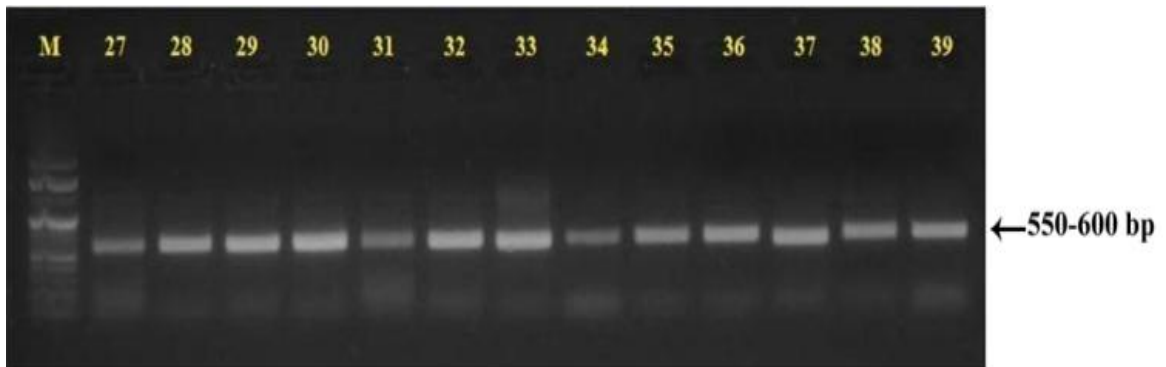
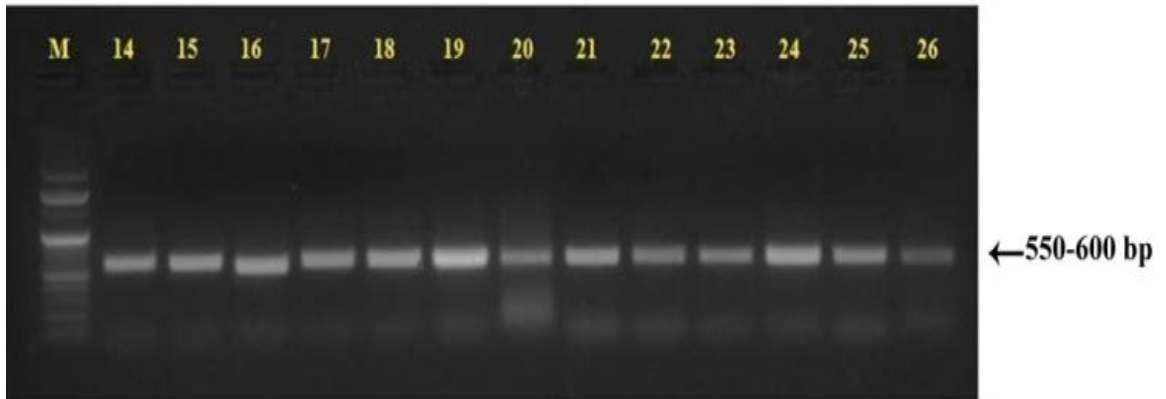
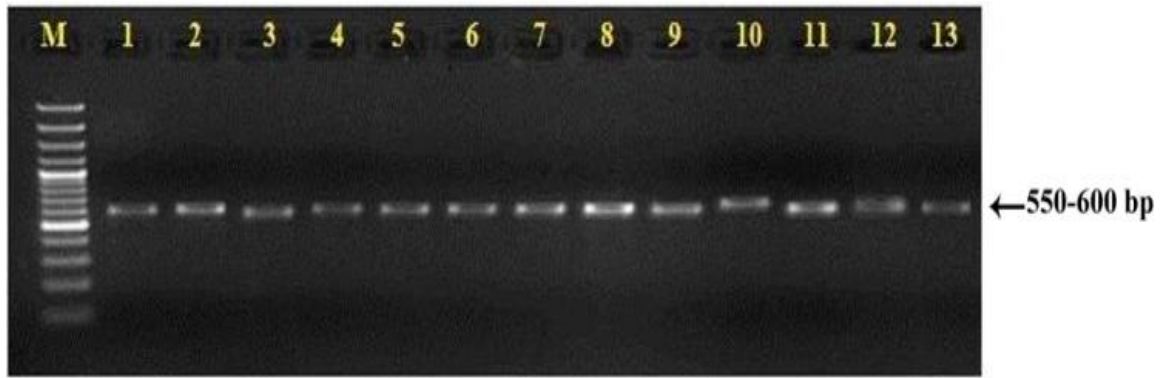


Plate 10. Amplification of ITS rDNA region of fifty two isolates of *M. phaseolina* ranging from 550 to 600bp

MP-RC-1

CAGGATGATCAGGCTCGGCCGATCCTCCCACCCTTTGTGTACCTACCTCTGTTGCTTTGGCGGGCCGCGGTCCCCCGCGG
CCGCCCCCTCCCCGGGGGTGGCTAGTGCCCGCCAGAGGACCATCAAACCTCCAGTCAGTGAACGTCGCAGCTGAAAA
AACATTTCAATAAACTAAAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTGGTATTCCGAGGGGCATGCCTG
TTCCGAGCGTCATTACAACCTCAAGCTCTGCTTGGTATTGGGCACCGCCCTTCTTCGGGCGCGCTCAAAGACCTCGGCG
GTGGCTGTCTTGCCCTCAAGCGTAGTAGAATACACCTCGCTTCGGAGCGCAGAGCGTCGCCCCCGGACGAACCTTCTGAA
CTTTTCTCAAGGTTGACCTCGGATCAGATAGGGATACCCGCTGAACTTAAGCATATCAATAAGCCGAAAAA

MP-RC-3

GTGAAATTTTCAAGGGCTTCGGCTCGACTCTCCCACCCTTTGTGAACGTACCTCTGTTGCTTTGGCGGCTCCGGCCGCCA
AAGGACCTCCAACTCCAGTCAGTAAACGCAGACGTCTGATAAAACAAGTAAATAAACTAAAACCTTTCAACAACGGATCTC
TTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCAAATCTT
TGAACGCACATTGCGCCCTTGGTATTCCGGGGGGCATGCCTGTTTCGAGCGTCATTACAACCTCAAGCTCTGCTTGGAA
TTGGGCACCGTCTCACTGCGGACGCGCTCAAAGACCTCGGCGGTGGCTGTTTCAGCCCTCAAGCGTAGTAGAATAACCC
TCGCTTTGGAGTGGTTGGCGTCGCCCCCGGACGAACCTTCTGAACTTTCTCAAGGTTGACCTCGGATCAGGTAGGGAT
ACCCGCTGAACTTAAGCATATCAATAAGCGGGAGGAA

MP-RC-6

GCCTGACAATTGCAGTGTCCGGTATCGGGCTCGGCCGATCCTCCCACCCTTTGTGTACCTACCTCTGTTGCTTTGGCGGG
CCGCGGTCCCCCGCGGCCGCCCCCCCTCCCCGGGGGGTGGCTAGTGCCCGCCAGAGGACCATCAAACCTCCAGTCAGTGA
CGTCGCAGTCTGAAAAACATTCAATAAACTAAAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTGGTATT
CCGAGGGGCATGCCTGTTTCGAGCGTCATTACAACCTCAAGCTCTGCTTGGTATTGGGCACCGCCCTTCTTCGGGCGCGC
CTCAAAGACCTCGGCGGTGGCTGTCTTGCCCTCAAGCGTAGTAGAATAACACCTCGCTTCGGAGCGCAGGGCGTCCGCCGCG
GGACGAACCTTCTGAACTTTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCATAACCC
GGAGGAAAATT

MP-RC-10

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CTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAACAAAATGCGATAAGTAATGTGAATTGCAGAATTC
AGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTCAACC
CTCAAGCTCAGCTTGGTGTGGGACTCGCGGTAACCCGCGTTCCCCAAATCGATTGGCGGTTCAGTCGAGCTTCCATAGC
GTAGTAATCATAACCTCGTTACTGGTAATCGTCGCGGCCACGCGGTAACCCCAACTTCTGAACTTTGACCTCGGATC
AGGTAGGAATACCCGCTGAACTTAGGCATATCAATAGCAGGAGG

MP-YD-14

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TGCCCTGTTTCGAGCGTCATTACAACCTCAAGCTCTGCTTGGTATTGGGCACCGCCCTTCTTCGGGCGCGCTCAAAGACC
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AT

MP-GB-25

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CCTGTTTCGAGCGTCATTACAACCTCAAGCTCTGCTTGGTATTGGGCACCGCCCTTCTTCGGGCGCGCTCAAAGACCTC
GGCGGTGGCTGTCTTGCCCTCAAGCGTAGTAGAATAACCTCGCTTCGGAGCGCAGGGCGTCCGCCCGCGGACGAACCTT
TGAACCTTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCCGAAAAA

MP-YD-18

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CGGCGGCCGCCCCCTCCCCGGGGGTGGCTAGTGCCCGCCAGAGGACCATCAAACCTCCAGTCAGTGAACGTCGCAGTCG
AAAAAACATTCAATAAACTAAAACCTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTGGTATTCCGAGGGGCAT
GCCTGTTTCGAGCGTCATTACAACCTCAAGCTCTGCTTGGTATTGGGCACCGCCCTTCTTCGGGCGCGCTCAAAGACCT

CGGCGGTGGCTGTCTTGCCTCAAGCGTAGTAGAATACACCTCGCTTCGGAGCGCAGGGCGTCGCCCGCCGGACGAACCTT
CTGAACTTTTCTCAAGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCATAGCCCCGAG

MP-YD-21

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GTAGTAGAATACACCTCGCTTCGGAGCGCAGGGCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGTTGACCTC
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MP-YD-22

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MP-GB-26

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MP-GB-28

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MP-GB-30

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MP-GB-31

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MP-GB-32

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MP-GB-33

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MP-GB-35

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MP-GB-36

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MP-GB-39

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MP-GB-40

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CGGCGGTGGCTGTCTTGCCCTCAAGCGTAGTAGAATACACCTCGCTTCGGAGCGCAGGGCGTCGCCCGCCGACGAACCT
CTGAACCTTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACCTAAGCATATCAATAAGCGGAGGAA

MP-BD-41

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GCGGTGGCTGTCTTGCCCTCAAGCGTAGTAGAATACACCTCGCTTCGGAGCGCAGGGCGTCGCCCCGGGACGAACCTTCT
GAACTTTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAAGGGGCACGAAGGGA

MP-BD-48

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AAGCGTAATAAAATACACCTCGCTTCGGAGCGCAGGGCGTCGCCCCGGGACGAACCTTCTGAACTTTTCTCAAGGTTGA
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MP-BD-50

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GCGGTGGCTGTCTTGCCCTCAAGCGTAGTAGAATACACCTCGCTTCGGAGCGCAGGGCGTCGCCCCGGGACGAACCTTCT
GAACTTTTCTCAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCATAAGCGCACGA

The NCBI, BLAST was carried out and the conformity of the isolates was obtained. Twenty two rDNA sequences were deposited in the GenBank, Mary land, USA database under the accession no. KJ629071-KJ629093. The list of representative isolates, accession number and per cent homology are given in a Table14.

Phylogenetic tree of *M. phaseolina* constructed using Mega.4.2 online software indicated variation among isolates from different district as well as within a district. The isolates MP-BD-41, MP-BD-50, MP-GB-33, MP-RC-6, MP-GB-25 and MP-YD-22 formed a separate cluster I with the 53 per cent dissimilarity to the clusters II. Isolates of cluster II, MP-GB31, MP-RC-1, MP-GB-36, MP-GB-28, MP-TD-14, MP-GB-30, MP-GB-40, MP-YD-18, MP- GB-35, MP-GB-32, MP-GB-26, MP-GB-39, MP-YD-21 and MP-BD-48 showed 97 per cent similarity but Isolates, MP-RC-3 and MP-RC-10 showed totally distinct variability from all isolate of same Species . The isolate used for *in vitro* study and interconnectivity studies belonged to cluster I having 53 per cent dissimilarity to other isolates. Majority of morphological and sclerotial/ microsclerotia varied isolates belonged to same cluster except MP-RC-3 and MP-RC-10. Most of Gulbarga isolates were in same cluster, no much molecular variation was found. MP-RC-3 and MP-RC-10 collected from Chickasugur and Karigudda village of Raichur district showed diverse variation.

Table 14. Comparison and identity of representative *M. phaseolina* isolates of pigeonpea sequenced and deposited in GenBank, Maryland, USA

Sl. No.	Isolates designation	Identified as	GenBank accession No.	Referenced strains, accession no. and host			% Homology
				Strain	Accession no.	Host	
1.	MP-BD-41	<i>M. phaseolina</i>	KJ629071	Ezh-1	KC822431	<i>Fragaria virginiana</i>	95
2.	MP-BD-50	<i>M. phaseolina</i>	KJ629072	NICS01	JX945162	<i>Sesamum indicum</i>	95
3.	MP-RC-3	<i>M. phaseolina</i>	KJ629073	Mp001NY12	KC800709	<i>Glycine max</i>	95
4.	MP-GB-30	<i>M. phaseolina</i>	KJ629074	NICS01	JX945162	<i>Sesamum indicum</i>	95
5.	MP-YD-18	<i>M. phaseolina</i>	KJ629075	Ezh-1	KC822431	<i>Fragaria virginiana</i>	95
6.	MP-BD-48	<i>M. phaseolina</i>	KJ629076	M1	KC20282	<i>Glycine max</i>	95
7.	MP-GB-39	<i>M. phaseolina</i>	KJ629077	M1	KC20283	<i>Glycine max</i>	95
8.	MP-GB-35	<i>M. phaseolina</i>	KJ629079	NICS01	JX945162	<i>Sesamum indicum</i>	95
9.	MP-GB-31	<i>M. phaseolina</i>	KJ629080	Hga-1-3	KC822432	<i>Fragaria virginiana</i>	95
10.	MP-RC-1	<i>M. phaseolina</i>	KJ629081	M1	KC20282	<i>Glycine max</i>	95
11.	MP-GB-26	<i>M. phaseolina</i>	KJ629082	NICS01	JX945162	<i>Sesamum indicum</i>	95
12.	MP-GB-40	<i>M. phaseolina</i>	KJ629083	CK-7	JX945170	<i>Ipomoea batatas</i>	95
13.	MP-GB-28	<i>M. phaseolina</i>	KJ629084	CK-7	JX945170	<i>Ipomoea batatas</i>	95
14.	MP-YD-21	<i>M. phaseolina</i>	KJ629085	NICS01	JX945162	<i>Sesamum indicum</i>	95
15.	MP-RC-6	<i>M. phaseolina</i>	KJ629086	Mp-1351	KF453968	<i>Cuminum cyminum</i>	95
16.	MP-GB-36	<i>M. phaseolina</i>	KJ629087	NICS01	JX945162	<i>Sesamum indicum</i>	95
17.	MP-GB-25	<i>M. phaseolina</i>	KJ629088	Ezh-1	KC822431	<i>Fragaria virginiana</i>	95
18.	MP-YD-22	<i>M. phaseolina</i>	KJ629089	NICS01	JX945162	<i>Sesamum indicum</i>	95
19.	MP-YD-14	<i>M. phaseolina</i>	KJ629090	NICS01	JX945162	<i>Sesamum indicum</i>	95
20.	MP-RC-10	<i>M. phaseolina</i>	KJ629091	MAC37	GU046864	<i>Helianthus annuus</i>	94
21.	MP-GB-33	<i>M. phaseolina</i>	KJ629092	CK-7	JX945170	<i>Ipomoea batatas</i>	95
22.	MP-GB-32	<i>M. phaseolina</i>	KJ629093	Ezh-1	KC822431	<i>Fragaria virginiana</i>	95

4.4 Inter connectivity study between sclerotia and pycnidia

Inter connectivity between sclerotia and pycnidia were studied using different parts of pigeonpea employing stem detached technique and cellophane tape method. Experiment was setup by using MP-GB-25 isolate inoculated in to healthy roots, stems and leaves of TS-3R variety of 90 days old plants both under *in vitro* (stem detached technique) and glass house (cellophane tape method). The extent of root infection, production of pycnidia on stem and infection on leaves were recorded.

Under *in vitro* condition, sclerotia from *R. bataticola* were inoculated in to roots, stems and leaves. Inoculated stems and roots showed infection where as leaves were not infected. Similarly, sclerotia produced mycelium of *M. phaseolina* (MP-GB-25) when inoculated on roots they did not produce any symptoms but stems were completely infected with more number of pycnidia and formed spindle shaped lesion but leaves were not infected. Pycnidia inoculated roots and leaves did not infect but stems were infected and produced less number of pycnidial bodies (Table 15a and Plate 11).

Under glass house condition also same experiment was conducted and sclerotia were inoculated in to roots. Such seedlings were dead, stem and leaves were not infected. Mycelium inoculated stems produced a spindle shaped lesions with numerous pycnidia but leaves were not infected (Table 15b and Plate 12). Similarly pycnidia inoculated stems produced pycnidia only but leaves were not infected. Comparing of these results no perfect connectivity was found between sclerotia and pycnidia but pycnidia had a major role for spreading of disease under field condition. The sclerotia produced on culture media, when inoculated did not establish infection on root system. This is the missing link between the fungal structures.

4.5 Management studies on Macrophomina Stem Canker

4.5.1 Varietal screening of pigeonpea genotypes against Macrophomina Stem Canker under natural condition

Totally, 22 genotypes were screened for their reaction to Macrophomina Stem Canker during the season *Kharif*-2013. Earlier there was no susceptible lines reported. Based on the performance of genotypes over the season, per cent incidence and average number of lesions was categorized by scoring as given below. Out of 22 genotypes

Table 15a. Interconnectivity study between sclerotia and pycnidia with different parts of the pigeonpea under *in vitro*

Sl. No.	Treatments		Symptoms development				
			3 DAI	6 DAI	9 DAI	12 DAI	Results
1.	Root	Sclerotia*	Infection started	Observed Few sclerotia	Area of root increased	Area of root increased	Infection: produce sclerotia, Slight drying of roots
		Only Mycelia**	No infection	No infection	No infection	No infection	No infection
		Pycnidia***	No infection	No infection	No infection	No infection	No infection
2.	Stem	Sclerotia*	Infection started	Observed Few sclerotia	25% stem covered with sclerotia	50% stem covered with sclerotia	Infection : produce numerous number of sclerotia on stem
		Only Mycelia**	Infection started	Observed Few pycnidia	50% stem covered with pycnidia	Half of stem covered with pycnidia	Infection: produce more number of pycnidia and spindle shaped lesion observed
		Pycnidia***	Infection started	Produced mycelium	Few pycnidia bodies	8-11 pycnidia on the stem	Infection : produce maximum 11 pycnidia on the stem
3.	Leaves	Sclerotia**	No infection	No infection	No infection	No infection	No infection
		Only Mycelia**	No infection	No infection	No infection	No infection	No infection
		Pycnidia***	No infection	No infection	No infection	No infection	No infection

* Sclerotia collected from infected root portion of pigeonpea (Dry Root rot caused by *R. bataticola*),

** Mycelium obtained from stem canker of pigeonpea caused by *M. phaseolina*,

*** Pycnidial bodies collected from infected stem canker of pigeonpea

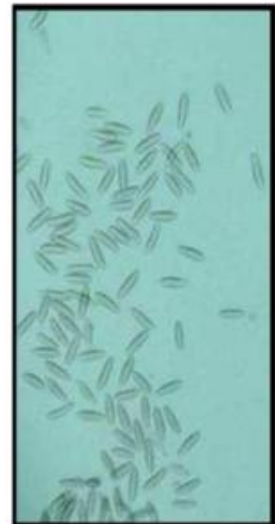
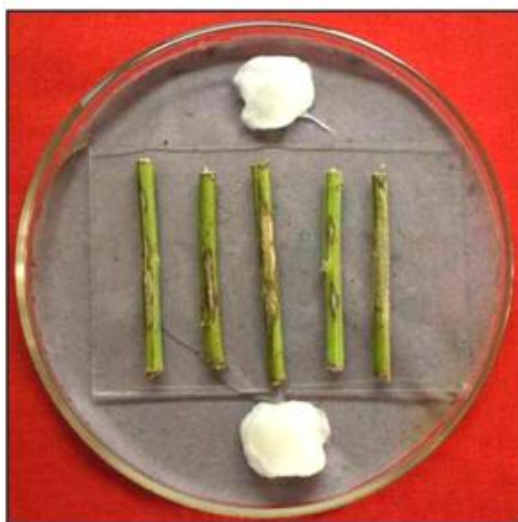
Roots, Stems and leaves collected from 90 days old plant for this study



a) Peeling and insertion of mycelium, pycnidia and sclerotia in to stem, leaf and root



b) Control plates of stem, leaf and root



c) Mycelium (*M. phaseolina*) inoculated to stem, spindle shaped lesion and pycnidiospores (10x)

Plate 11. Stem detached technique used for interconnectivity studies under *in vitro* (a-c)



d) Pycnidial (*M. phaseolina*) bodies inoculated to stem, spindle shaped lesion and pycnidiospores (10x)



e) Sclerotia (*R. bataticola*) inoculated to stem, formation of lesion and sclerotial bodies (10x)



f) Sclerotia (*R. bataticola*) inoculated to root and sclerotial bodies (10x)

Plate 11. Stem detached technique used for interconnectivity studies under *in vitro* (d-f)

Table 15b. Inter connectivity study between sclerotia and pycnidia with different parts of the pigeonpea under glass house

Sl. No.	Treatments		Results
1.	Root	Sclerotia*	Death of seedlings
		Only Mycelia**	No infection
		Pycnidia***	No infection
2.	Stem	Sclerotia*	No infection
		Only Mycelia**	Infection: produce more number of pycnidia and spindle shaped lesion observed
		Pycnidia***	Infection : pycnidia and spindle shaped lesion observed
3.	Leaves	Sclerotia**	No infection
		Only Mycelia**	No infection
		Pycnidia***	No infection
4	Free from pathogen		Healthy

* Sclerotia collected from infected root portion of pigeonpea (Dry Root rot caused by *R. bataticola*)

** Mycelium obtained from stem canker of pigeonpea caused by *M. phaseolina*

*** Pycnidial bodies collected from infected stem canker of pigeonpea

For leaves inoculum sprayed by hand sprayer

For stem inoculated by cellophane tape method

For root inoculum multiplied and dumped before sowing

Observation was taken 21 DAI



a) 90 days old TS-3R variety for studies pigeonpea



b) Peeling of epidermal layer of stem



c) Insertion of mycelium in to epidermal layer of the stem



d) Insertion of pycnidial bodies in to stem



e) Expression of symptoms on stem

Plate 12. Cellophane tape technique used for the sclerotia and pycnidial interconnectivity studies under glass house

screened (Table 16a), none of them showed resistance, While four genotypes viz., (GRG -2010, BSMR-736, ICP-8863 (Maruti) and ICP-87119 (Asha) showed moderately resistant. WRP-1, Bennur-local, Katti Beeja, GRG -2009, GRG -822, GC-11-39, GRG-2009-1, GPHR-08-11 and PT-04-31 showed moderately susceptible. Chaple, Kari togari, Gulyal local, GRG -333, GRG -818, GRG-811, JKM-7 and JKM-189 showed susceptible and TS-3R showed highly susceptible (Table 16b) reaction.

4.5.1.1 *In vitro* screening of pigeonpea genotypes against Macrophomina Stem Canker by using paper towel method

Under field condition none of them showed resistance. Four genotypes (GRG-2010, BSMR-736, ICP-8863 (Maruti) and ICP-87119 (Asha) showed moderately resistant such genotypes were selected for *in vitro* screening by paper towel method. Experiment was repeated five times but pathogen was not infected to the root so same genotypes were sown under glass house condition and artificially sprayed the inoculum and confirmed their resistant level.

4.5.2 Efficacy of non-systemic and systemic fungicides against *M. phaseolina*

4.5.2.1 Efficacy of non-systemic fungicides against *M. phaseolina* under *in vitro*

Efficacy of five contact fungicides was tested against *M. phaseolina* (MP-GB-25) by poisoned food technique. Among contact fungicides mancozeb, copper hydroxide and a combiproducts of carbendazim 12 % + mancozeb 63 % recorded maximum inhibition of (100 %) mycelial growth at all concentrations (0.10 %, 0.20 % and 0.30 %) and captan showed 92.20 % inhibition at 0.10 per cent concentration, 100 per cent inhibition at 0.2 and 0.3 per cent concentrations. Least inhibition of 86.00, 88.88 and 89.33 per cent was observed in case of ziram at 0.10, 0.20 and 0.30 per cent respectively (Table 17a and Plate 13a).

4.5.2.2 Efficacy of systemic fungicides against *M. phaseolina* under *in vitro*

Efficacy of seven systemic fungicides was tested against *M. phaseolina* (MP-GB-25) by poisoned food technique. Among systemic fungicides carbendazim, benomyl, thiophanate methyl, carbendazim 25 % + mancozeb 50 %, zineb 68 % + hexaconazole 14 % and tricyclazole 18 % + mancozeb 62 % showed 100 % inhibition at all concentrations (0.05, 0.10 and 0.20 %). Least inhibition was found in hexaconazole

Table 16a. Screening of pigeonpea genotypes against *Macrophomina* Stem Canker during *Kharif* 2013 at ARS Farm, Gulbarga

Sl. No.	Genotypes	Observation at 120 DAS				
		Average lesion/plant	Per cent Incidence	Per cent disease index	Average lesion length (cm)	Reaction
1.	WRP-1	12.80	38.00	27.30	3.71	MS
2.	Bennur-local	14.30	34.00	25.00	4.09	MS
3.	TS-3R	23.40	96.29	50.20	3.31	HS
4.	Chaple	17.6	66.66	34.15	3.37	S
5.	Kari togari	16.44	63.33	57.30	3.16	S
6.	Katti Beeja	13.53	49.73	38.40	3.00	MS
7.	Gulyal local	16.80	68.00	28.00	3.04	S
8.	GRG -2009	12.70	37.00	27.57	3.25	MS
9.	GRG -333	13.80	50.00	33.33	5.62	S
10.	GRG -2010	7.20	25.00	20.63	2.33	MR
11.	GRG -818	16.00	54.50	26.76	2.27	S
12.	GRG -822	11.49	33.33	25.00	2.06	MS
13.	BSMR-736	7.40	25.00	18.25	2.65	MR
14.	GC-11-39	13.20	32.00	23.80	5.87	MS
15.	GRG-811	15.80	54.50	23.23	2.84	S
16.	GRG-2009-1	14.00	45.00	28.28	4.02	MS
17.	JKM-7	17.80	57.00	34.92	3.42	S
18.	JKM-189	19.00	68.00	41.33	3.94	S
19.	GPHR-08-11	6.20	50.00	25.39	3.17	MS
20.	PT-04-31	6.00	43.33	22.96	2.58	MS
21.	ICP-8863 (Maruti)	5.80	23.00	20.51	2.23	MR
22.	ICP-87119 (Asha)	5.90	24.45	21.42	2.47	MR

Table 16b. Reaction of pigeonpea genotypes against Macrophomina Stem Canker based on per cent incidence and lesion per plant

Score	Reaction	List of entries
1.	1-10 % incidence with 1-5 lesions/plant (Resistant)	Nil
2.	10-25 % incidence with 10-15 lesions/plant (Moderately Resistant)	GRG -2010, BSMR-736, ICP-8863 (Maruti) and ICP-87119 (Asha)
3.	25-50 % incidence with 15-20 lesions/plant (Moderately Susceptible)	WRP-1, Bennur-local, Katti Beeja, GRG - 2009, GRG -822, GC-11-39, GRG-2009-1, GPHR-08-11 and PT-04-31
4.	50-75 % incidence with 20-25 lesions/plant (Susceptible)	Chaple, Kari togari, Gulyal local, GRG -333, GRG -818, GRG-811, JKM-7 and JKM-189
5.	>75 % incidence with >25 lesions/plant and drying of branches (Highly Susceptible)	TS-3R

Table 17a. Efficacy of non-systemic fungicides on the growth of *M. phaseolina* of pigeonpea under *in vitro*

Sl. No.	Fungicides	Per cent inhibition at different concentrations*			
		0.10 %	0.20 %	0.30 %	Mean
1.	Captan	92.20 (84.60)	100 (90.00)	100 (90.00)	97.40 (80.72)
2.	Ziram	86.00 (68.02)	88.88 (70.52)	89.33 (70.93)	88.07 (69.82)
3.	Mancozeb	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
4.	Copper hydroxide	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5.	Carbendazim 12 % + Mancozeb 63 %	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6.	Control	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)
	Mean	79.70 (63.22)	81.48 (64.51)	81.55 (64.56)	80.91 (64.09)

	S. Em±	CD @ 1 %
Fungicides (F)	0.36	1.40
Concentration (C)	0.25	0.99
F x C	0.63	2.44

*Mean average of three replications

Figures in parentheses are arc sine values

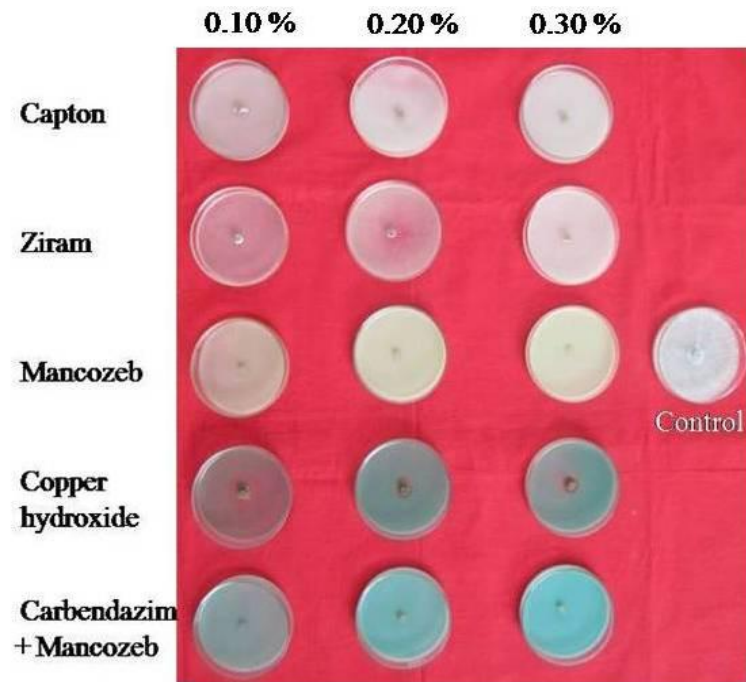


Plate 13a. *In vitro* evaluation of non-systemic fungicides on inhibition of mycelial growth of *M. phaseolina*

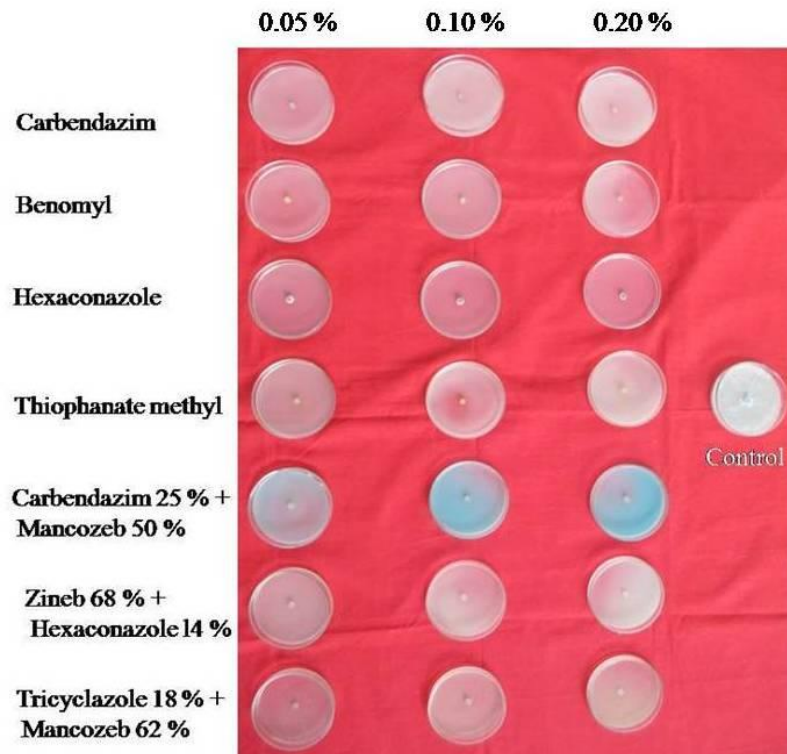


Plate 13b. *In vitro* evaluation of systemic fungicides on inhibition of mycelial growth of *M. phaseolina*

with 88.88, 90.44 and 91.88 per cent at 0.05, 0.10 and 0.20 per cent concentrations respectively (Table 17b and Plate 13b) with significant difference.

4.5.3 Efficacy of fungal and bacterial bio-agents against *M. phaseolina* under *in vitro*

Efficacy of bio agents was studied under *in vitro* and the results on inhibition of mycelial growth of *M. phaseolina* (MP-GB-25) was recorded and presented here under. Among the bio-agents tested (Table 18), *Trichoderma harzianum* (Th-R) was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (41.86 %) of *M. phaseolina* followed by *T. viride* (39.07 %). *Pseudomonas putida* (RP-56) was inhibited to the extent of 27.22 % compared to other strains of *Pseudomonas* spp. Among *P. fluorescens* strains, RP-41 showed least inhibition, RP-46 and RP-31 were on par with each other, RP-16 showed 23.10 per cent inhibition and *Bacillus subtilis* recorded 14.85 per cent of inhibition (Plate 14).

4.5.4 Integrated management against Macrophomina Stem Canker

The experiment was conducted under field conditions to manage stem canker in pigeonpea during *Kharif* 2013 by integration of best performing bio agents and chemicals. The results are presented here under Table 19 and general field view depicted in Plate 15.

4.5.4.1 Macrophomina Stem Canker incidence

The least stem canker incidence (9.87 %) was recorded in the combination with seed treatment and soil application with *T. harzianum* and foliar spray of carbendazim followed by *T. viride* seed treatment and soil application and foliar spray of carbendazim with mean incidence 12.50 per cent as against check with 34.55 per cent. While, the treatment of seeds with carbendazim followed by spray of carbendazim @ 1.5g/l recorded a mean incidence of 15.17 per cent (Table 19) followed by treatment of seeds with *T. Harzianum* + spray of carbendazim @ 1.5g/l (17.55 %) and *T. Viride* + spray of carbendazim @ 1.5g/l (18.96 %) which were on par each other.

4.5.4.2 Yield

The yield was higher (1509 kg/ha) in treatment with seeds treatment by *T. harzianum* + *T. harzianum* enrichment with FYM + foliar spray of carbendazim

Table 17b. Efficacy of systemic fungicides on the growth of *M. phaseolina* of pigeonpea under *in vitro*

Sl. No.	Fungicides	Per cent inhibition at different concentrations *			
		0.05 %	0.10%	0.20%	Mean
1.	Carbendazim	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
2.	Benomyl	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
3.	Hexaconazole	88.88 (70.52)	90.44 (71.98)	91.88 (73.44)	90.40 (71.98)
4.	Thiophanate methyl	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5.	Carbendazim 25 % + Mancozeb 50 %	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6.	Zineb 68 % + Hexaconazole 14 %	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
7.	Tricyclazole 18 % + Mancozeb 62 %	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
8.	Control	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)	0.00 (00.00)
	Mean	86.11 (68.11)	86.30 (68.27)	86.48 (68.42)	86.30 (68.27)

	S. Em±	CD @ 1 %
Fungicides (F)	0.10	0.39
Concentration (C)	0.06	0.24
F x C	0.17	0.68

*Mean average of three replications

Figures in parentheses are arc sine values

Table 18. Efficacy of bio-agents against *M. phaseolina* of pigeonpea under dual culture

Sl. No.	Bio-agents	Per cent of inhibition
1.	<i>Trichoderma viride</i> (Tv-R)	39.07 (38.66)
2.	<i>T. harzianum</i> (Th-R)	41.86 (40.31)
3.	<i>Pseudomonas fluorescens</i> (RP-46)	18.33 (25.34)
4.	<i>P. fluorescens</i> (RP-31)	18.77 (25.67)
5.	<i>P. fluorescens</i> (RP-16)	23.10 (28.72)
6.	<i>P. fluorescens</i> (RP-41)	12.66 (20.84)
7.	<i>P. putida</i> (RP-56)	27.22 (31.44)
8.	<i>Bacillus subtilis</i>	14.85 (22.66)
9.	Control	0.00 (0.00)
	S. Em±	0.55
	CD @ 1%	2.26

*Mean average of three replications

Figures in parentheses are arcsine value

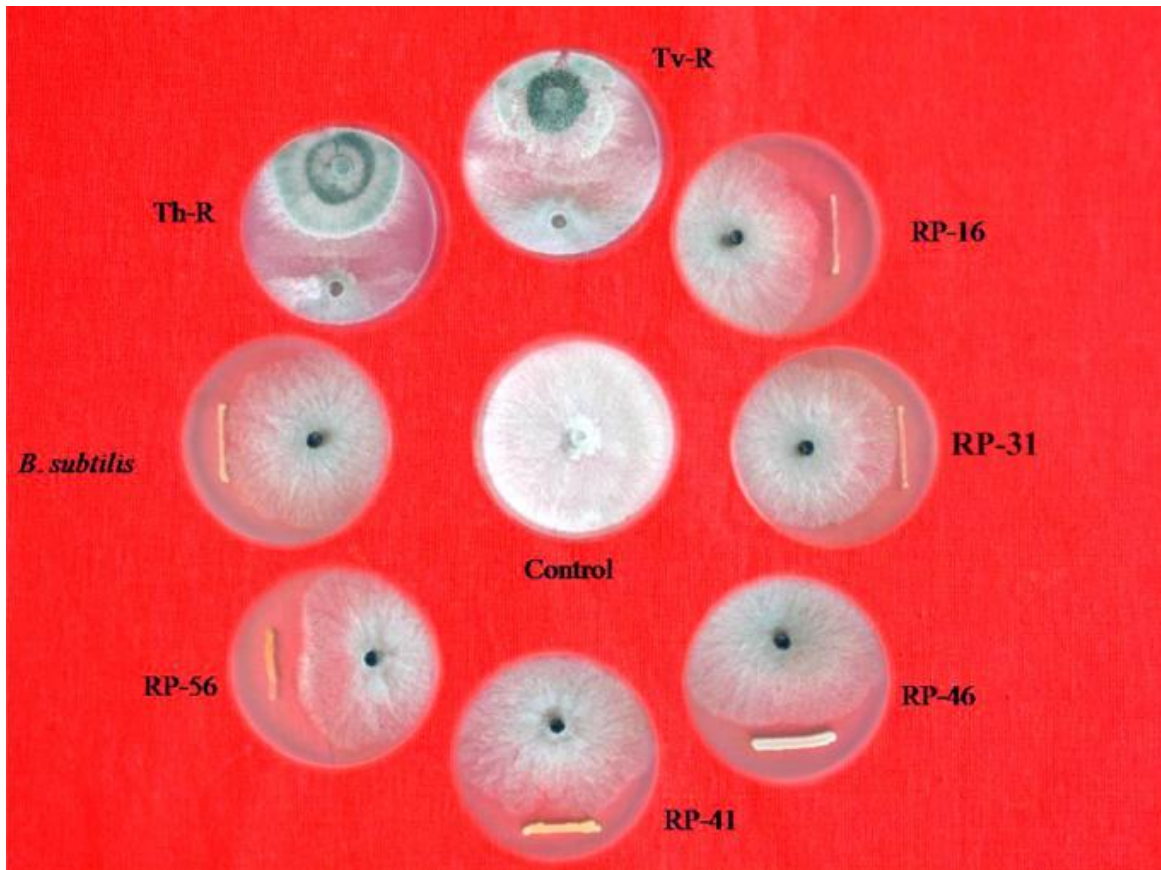


Plate 14. Efficacy of bio-agents in inhibition of mycelial growth of *M. Phaseolina* in dual culture

Table 19. Effects of application of bio-agents and fungicides on management of Macrophomina Stem Canker of pigeonpea

Sl. No.	Treatments	Per cent incidence		
		90 DAS	120 DAS	Terminal
1.	Seed treatment with captan @ 3.0 g/kg seed + Foliar spray of carbendazim @ 1.5 g/l	18.51 (25.48)	20.66 (27.03)	22.26 (28.14)
2.	Seed treatment with carbendazim @ 2.0 g/kg seed + Foliar spray of carbendazim @ 1.5 g/l	11.85 (19.47)	13.00 (21.14)	15.17 (22.92)
3.	Seed treatment with <i>Trichoderma harzianum</i> @ 4 g/kg + Foliar spray of carbendazim @ 1.5 g/l	14.07 (21.41)	15.78 (23.40)	17.55 (24.77)
4.	Seed treatment with <i>T. viride</i> @ 4 g/kg + Foliar spray of carbendazim @ 1.5 g/l	16.29 (23.22)	17.29 (24.57)	18.96 (25.81)
5.	Seed treatment with <i>T. harzianum</i> @ 4 g/kg + <i>T. harzianum</i> enrichment with FYM at 1 kg per tonne FYM + Foliar spray of carbendazim @ 1.5 g/l	8.88 (17.33)	9.01 (17.47)	9.87 (18.30)
6.	Seed treatment with <i>T. viride</i> @ 4 g/kg + <i>T. viride</i> enrichment with FYM at 1 kg per tonne FYM + Foliar spray of carbendazim @ 1.5 g/l	10.36 (18.78)	11.58 (19.89)	12.50 (20.79)
7.	Check	28.88 (32.50)	30.95 (33.80)	34.55 (36.00)
S. Em±		0.40	0.52	0.52
CD @ 5%		1.24	1.61	1.60

Figures in the parentheses are arc sine value



Plate 15. Field view of integrated disease management of *Macrophomina* stem canker at MARS Farm, UAS, Raichur

followed by *T. viride* (1485 kg/ha) seed treatment + soil application with FYM + spray of carbendazim @ 1.5g/l as compared to check recording 1262 kg/ha (Table 20).

Table 20. Effect of application of bio-agents and fungicides on yield of pigeonpea

Sl. No.	Treatments	Yield (kg/ha)	B:C ratio
1.	Seed treatment with captan @ 3.0 g/kg seed + Foliar spray of carbendazim @ 1.5 g/l	1413	2.60:1
2.	Seed treatment with carbendazim @ 2.0 g/kg seed + Foliar spray of carbendazim @ 1.5 g/l	1423	2.62:1
3.	Seed treatment with <i>T. harzianum</i> @ 4 g/kg + Foliar spray of carbendazim @ 1.5 g/l	1433	2.65:1
4.	Seed treatment with <i>T. viride</i> @ 4 g/kg +Foliar spray of carbendazim @ 1.5 g/l	1420	2.61:1
5.	Seed treatment with <i>T. harzianum</i> @ 4 g/kg + <i>T. harzianum</i> enrichment with FYM at 1 kg per tonne FYM + Foliar spray of carbendazim @ 1.5 g/l	1509	2.80:1
6.	Seed treatment with <i>T. viride</i> @ 4 g/kg + <i>T. viride</i> enrichment with FYM at 1 kg per tonne FYM + Foliar spray of carbendazim @ 1.5 g/l	1485	2.75:1
7.	Check	1262	
	S. Em±	46.87	
	CD @ 5%	144.44	

DISCUSSION

V. DISCUSSION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most important legume crops of the tropics and subtropics of Asia and Africa. Pigeonpea is also known by names such as redgram, *arhar* and *tur* in India. It is the main source of dietary protein to a large fraction of vegetarian population in developing countries. India is the world's largest producer and consumer of pulses including pigeonpea. About 90 per cent of the global pigeonpea area (4.9 m ha.) is in India contributing to 93 per cent of the global production. Maharashtra, Uttar Pradesh, Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh, Tamil Nadu and Bihar are the other major growing States of our country (Anon., 2011).

Pigeonpea is affected by several abiotic (water-logging, drought, temperature, photoperiodism, mineral deficiency) and biotic stresses (fungal pathogens, insects and storage pests) that limit the realization of true potential of pigeonpea. The crop is affected by more than 60 pathogens including fungi, bacteria, viruses, mycoplasma and nematodes but fortunately, only few diseases cause economic losses. The most widespread and destructive of which is Fusarium wilt (*Fusarium udum* Butler), sterility mosaic and Phytophthora blight (*Phytophthora drechsleri* f.sp. *cajani*) which are important in India and Cercospora leaf spot can cause serious losses under humid conditions in Asia and Africa (Hillocks *et al.*, 2000 and Reddy *et al.*, 2012).

Gulbarga is a very potential district in the country for extensive cultivation of pigeonpea. It is also grown in Bidar, Bijapur, Dharwad, Bellary and Belgaum districts of Northern Karnataka. *Kharif* is the growing season of pigeonpea in India. Moisture stress and sudden drop in temperature coupled with frost and foggy weather during the pod development stage, and terminal drought cause yield reduction leading to instability in production (Anonymous, 2012). Pigeonpea is cultivated largely in Northern parts of Karnataka although the black cotton soils in these areas are very ideal for cultivation of pigeonpea. Among a wide range of list of diseases on pigeonpea, the minor one which is now emerging as a threat to pigeonpea production recently is Macrophomina Stem Canker caused by *Macrophomina phaseolina*.

Macrophomina is primarily a soil and seed-borne fungal pathogen that incites the disease by producing microsclerotia/pycnidia (Farr *et al.* 1995 and Pun *et al.*, 1998). It

has a wide host range more than 500 species from 75 families. In India, the first report of stem canker occurrence was reported by Kannaiyan *et al.* (1979) in part of Eastern Uttar Pradesh (Varanasi and Mirzapur districts). Hot (>30 °C) and dry weather encourage disease development which is more prevalent on vertisols than alfisols. Rain after a prolonged dry spell predisposes plants to the disease. The disease poses a greater problem in pigeonpea cultivation and causes considerable loss (Bajpal *et al.*, 1999). Recently, the stem canker incited by *M. phaseolina* has emerged as one of the important pathogen of different agricultural crops, including pigeonpea (Kaur *et al.*, 2012a).

The present investigations on Macrophomina Stem Canker of pigeonpea due to *M. phaseolina* is focused on the survey, prevalence and assessment on the incidence of stem canker of pigeonpea in North Eastern Karnataka, collection of pathogenic isolates from different geographic regions to explore the cultural, morphological and molecular characterization, interconnectivity studies between sclerotia and pycnidial structures and between different parts of pigeonpea, screening of spectrum of chemicals and bio agents against *M. phaseolina* under *in vitro* and identification of source of resistance in management of Macrophomina Stem Canker under field condition. The results of the investigation are discussed in the pages to follow.

The symptoms of Macrophomina Stem Canker of pigeonpea was characterized by formation of spindle shaped lesion in the initial stages on stems with numerous pycnidial bodies. Then splitting of stems in point of lesion followed by coalescing of lesions and drying of branches, finally wilting of plants. Similar symptomatic observations were made by several workers (Reddy *et al.*, 1989; Agarwal and Gupta, 1991; Bajpal *et al.*, 1999; Kaur *et al.*, 2009; Kaur *et al.*, 2012a; Kaur *et al.*, 2012b; Kaur *et al.*, 2013 and Maurya *et al.*, 2013).

The survey on the incidence of Macrophomina Stem Canker and dry root rot of pigeonpea was carried out to know the prevalence and distribution of the disease in four districts of North Eastern Karnataka viz., Raichur, Yadgir, Gulbarga and Bidar representing rain fed conditions. Survey of the disease over a period of time provides information about the intensity with which it affects the yield. In addition, it will be a source of information about severity and indicates hot spot of disease in relation to soil environmental and edaphic factors.

5.1 Isolation and identification of the fungus

The samples of infected stems with typical spindle shaped lesions having pycnidial bodies were collected from pigeonpea fields during the survey. The fungus from the infected stems was confirmed as *Macrophomina phaseolina* based on their morphological, cultural, mycelia and sclerotia characters as described by Dhingra and Sinclair (1978); Short *et al.* (1978) and Kaur *et al.* (2013). The pathogenicity was proved by cellophane tape method and stem detached technique (modified from Mayee and Garud, 1979; Besset *et al.*, 1996). Fifty two isolates of *M. phaseolina* were collected from pigeonpea fields of North Eastern Karnataka during *Kharif* -2013 and they were isolated by standard tissue isolation technique and purified by hyphal tip culture technique. The identity was also confirmed by amplification and sequencing of ITS rDNA by NCBI, BLAST.

5.2 Survey on incidence of Macrophomina Stem Canker and dry root rot of pigeonpea

In recent years, *Macrophomina* Stem Canker is an nearly emerging disease in North Eastern Karnataka. Stem canker was more severe (25.02 %) in Gulbarga district compared to Bidar (18.11 %), Raichur (3.49 %) and Yadgir (2.10 %). The incidence of *Macrophomina* Stem Canker was maximum (77.33 %) in Agriculture Research Station (ARS), Gulbarga district and least was noticed in Karigudda (0.33 %) and Yargal (0.33 %) district of Raichur and Yadgir respectively. Basavakalyana taluk showed highest incidence of MSC with 34.13 per cent showed in Fig 2a and 2b. The highest number of lesions (89/plant) and maximum lesion length (13.60 cm) were recorded in ARS, Gulbarga. This is mainly attributed to variation in weather parameters which affected growth and development of disease. The average temperature of 30.04 °C and prolonged dry spell (19.20 mm rainfall) from flowering to harvesting stage in Gulbarga during November 2013 to February 2014 was recorded that led to enhanced disease during maturity stage. Kaur *et al.* (2012b) revealed that mean temperature during the months of October to March varied from 28.54 °C-19.55 °C and 27.35 °C - 14.12 °C and the rainfall recorded during October to March in 2008-09 and 2009-10 was 1.0 mm and 62.1 mm which was most favorable conditions for enhancing the disease development. These observations corroborate the results of Nobel and Richardson (1968) and Bajpal *et al.*

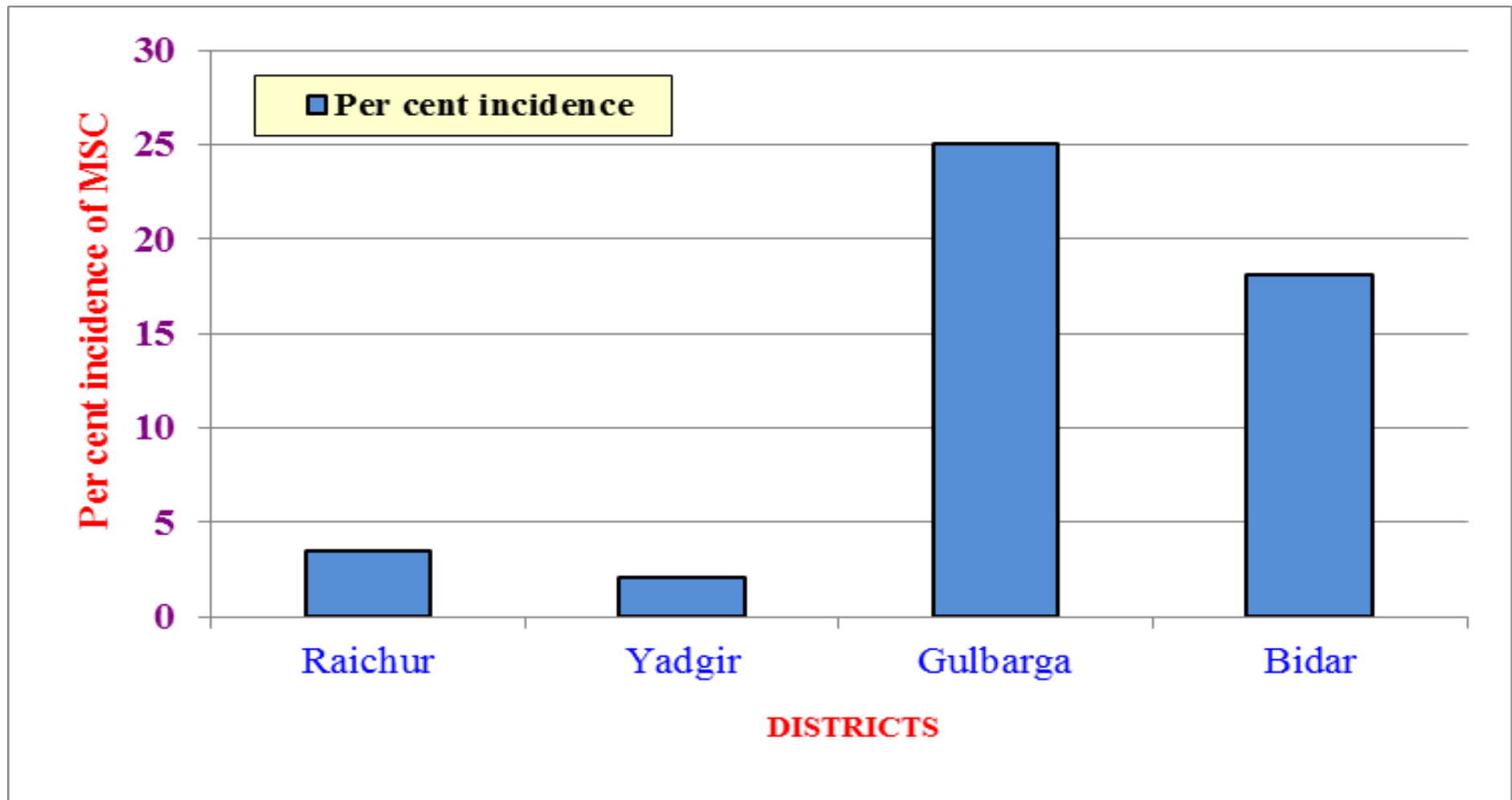


Fig. 2a. Incidence of *Macrophomina* Stem Canker of pigeonpea in different districts of North Eastern Karnataka during *Kharif* 2013

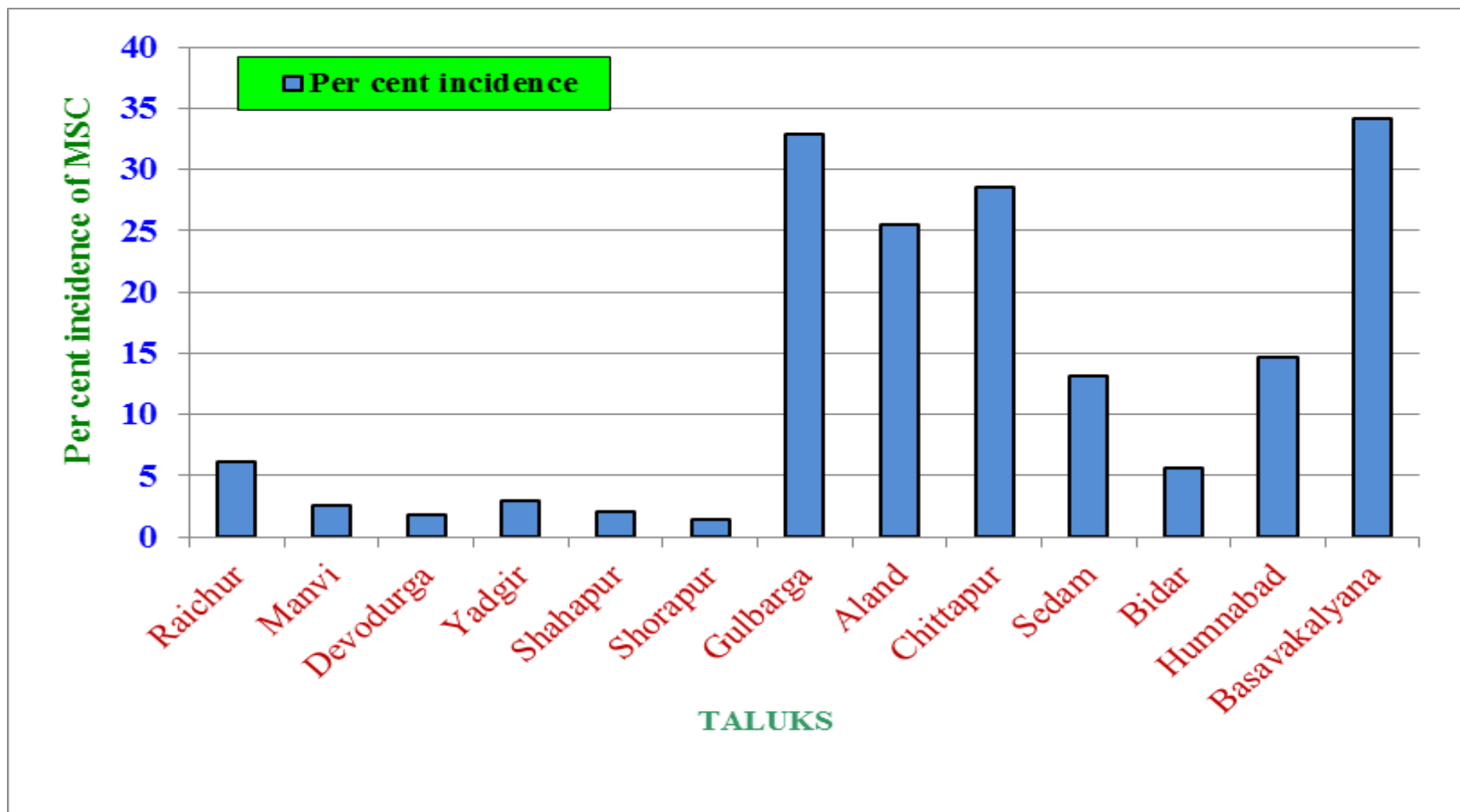


Fig. 2b. Incidence of *Macrophomina* Stem Canker of pigeonpea in different taluks of North Eastern Karnataka during *Kharif* 2013

(1999). Mono-cropping with closer spacing and also drought and high temperature increased the vulnerability of the crop and enhanced the aggressiveness of the pathogen. The findings are supported by Garrett *et al.* (2006) and Kaur *et al.* (2012b). Interestingly, more number of lesions were observed in a closer cropping system with higher incidence of drying of branches but few lesions with increased of lesion length was common in wider spaced crop.

The incidence of dry root rot pigeonpea was highest in Gulbarga district (22.63 %) compared to other districts (Fig 3a) and lowest was recorded in Bidar (4.72 %). The maximum (41.33 %) incidence was observed in Dandoti village of Chittapur taluk and least was (0.00 %) recorded in Alkoda village of Devodurga taluk. Among the taluks, Aland showed highest incidence with 27.33 per cent of dry root rot (Fig 3b) which could be due to highest area covered by TS-3R variety in Aland and hence TS-3R variety showed susceptibility. The survey conducted during *Kharif* season of 2013 revealed that *Macrophomina* Stem Canker and dry root rot of pigeonpea varied from locality to locality, obviously due to different soil conditions, cultivars used, cultivation practices and environmental conditions prevailing over these tracts.

During roving survey of dry root rot and MSC, some of fields infected only with MSC without any dry root rot and some fields infected only with dry root rot with less incidence of MSC but majority of fields were found infected with both dry root rot and MSC with different levels of incidence. Majority of TS-3R cultivar grown area was found with MSC incidence than dry root rot. It showed TS-3R cultivar more vulnerable to *M. phaseolina*. Hudgi village of Humnabad taluk and Alkoda village of Devodurga taluk was found to have only stem canker infection of 48.00 per cent and one per cent without any dry root rot disease respectively.

Stem canker and dry root rots are mainly enhanced by moisture stress and prolonged dry spell during the maturity stage (November to December). The pigeonpea crop entirely depends upon the rainfall. Since 2011, there has been prolonged dry spell during the maturity. In Gulbarga, particularly in ARS farm recycling of pigeonpea infected stem is a common practice for maintenance of *Fusarium* wilt sick plot. Apart from that heaping of uprooted stems on around the bunds of farmers field during the summer is another reason for activating the pycnidia

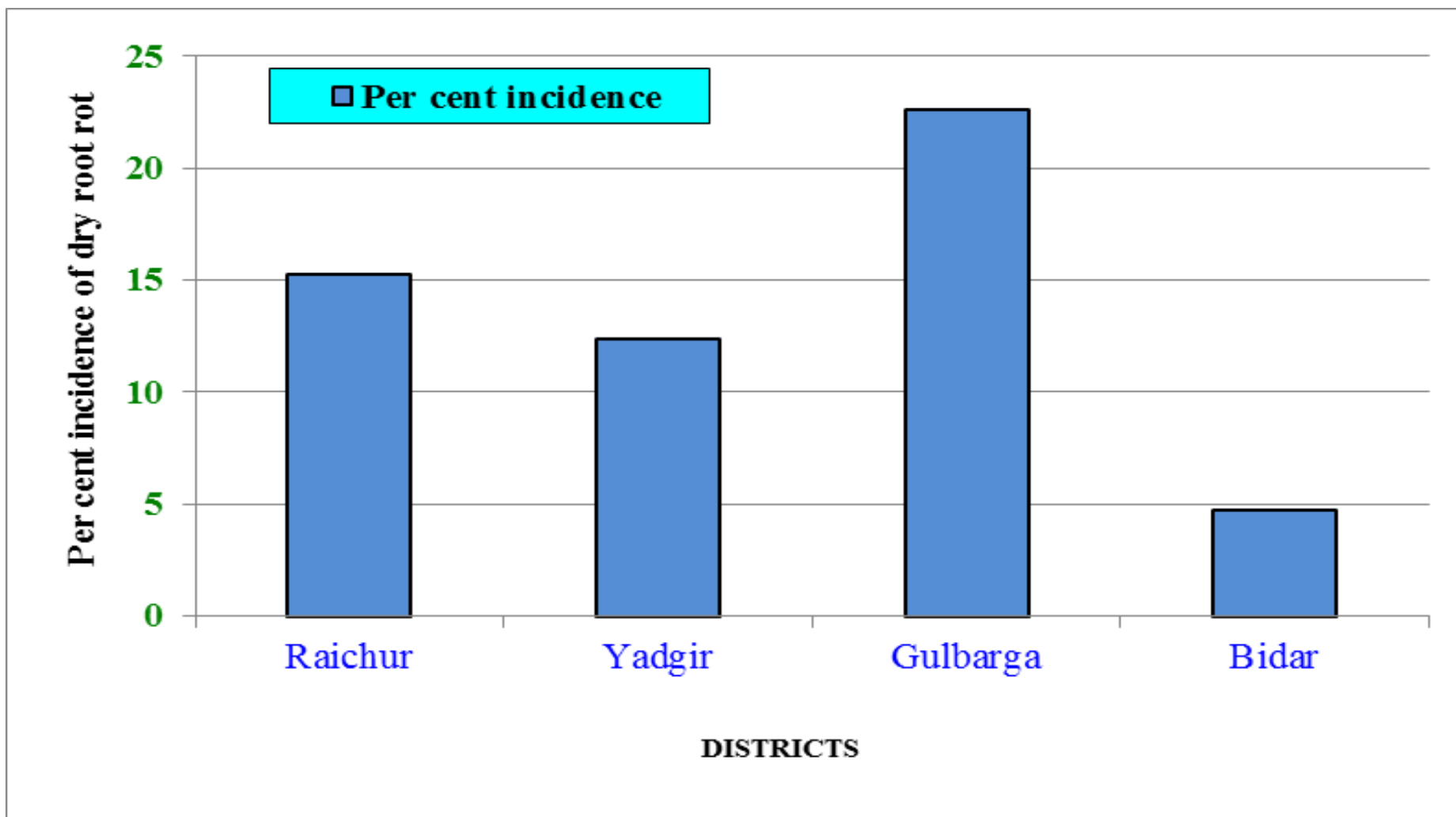


Fig. 3a. Incidence of dry root rot of pigeonpea in different districts of North Eastern Karnataka during *Kharif* 2013

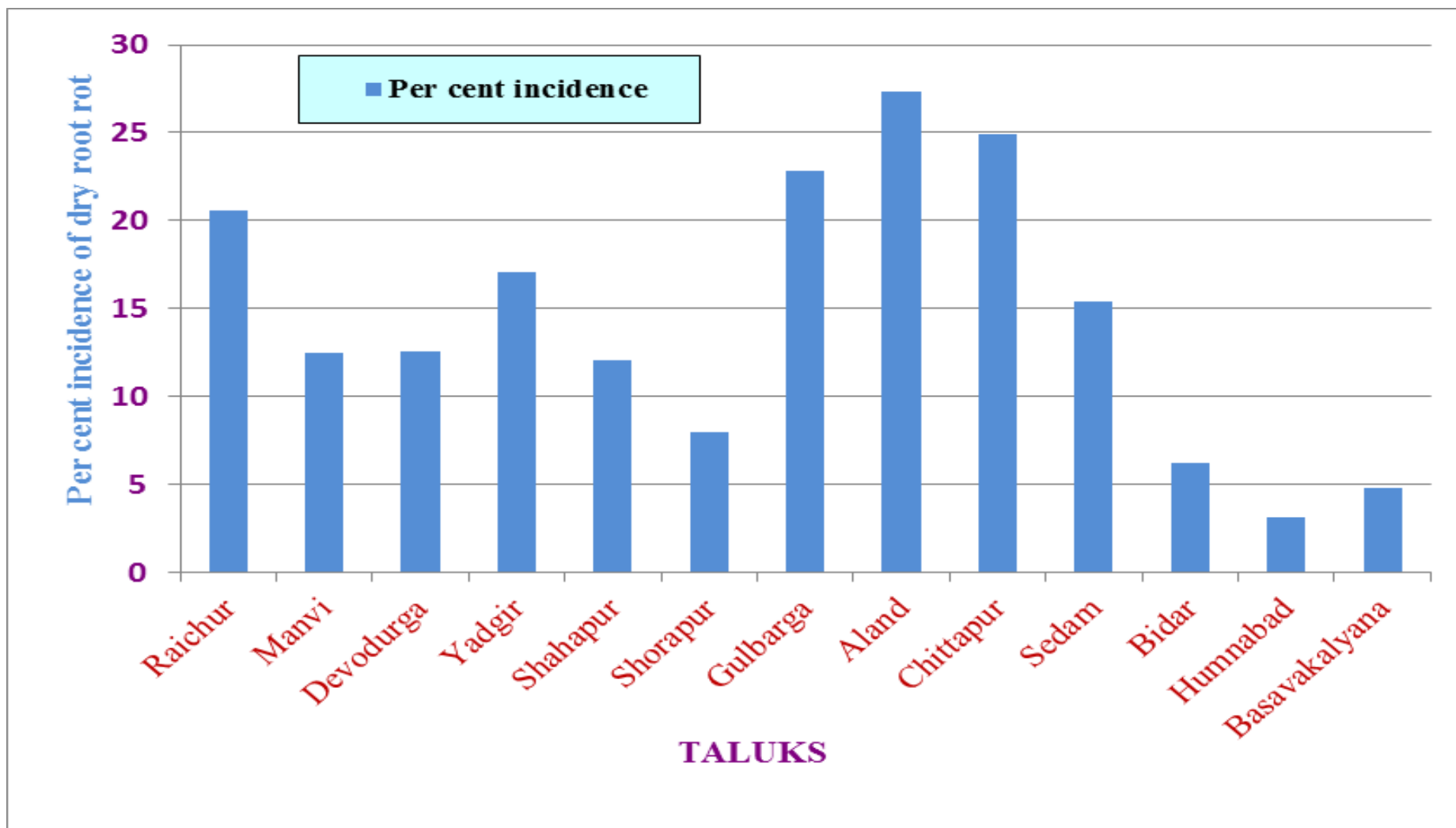


Fig. 3b. Incidence of dry root rot of pigeonpea in different taluks of North Eastern Karnataka during *Kharif* 2013

during rainy season. Thus the infection progressed during early planted crop during favorable condition. Due to this reason, higher incidence of MSC was noticed in Gulbarga and also ARS, Gulbarga is one of the “hot spots” for recurrence of Macrophomina Stem Canker.

5.3 Cultural, morphological and molecular characterization of *M. phaseolina*

The cultural and *in vitro* studies were carried on MP-GB-25 isolate. The isolate produced sclerotia on reverse side of plate with aerial mycelium aggregated to form sclerotia on front side also. The isolate produced microsclerotia in water agar and the isolate was obtained from ARS, Gulbarga with highest incidence of 77.33 per cent of stem canker. Cultural studies of the fungus such as growth of the pathogen on different solid media, liquid media, mycelium colour, sclerotia production, size and shape of sclerotia presence or absence of pycnidia were recorded.

M. phaseolina isolates were cultured on different solid media. It's necessary to provide essential nutritional components in the medium for better growth. Among the various solid media significantly more growth was observed on PDA (90 mm) followed by host stem dextrose agar (80.33 mm) and potato carrot dextrose agar (80.00 mm) which were on par with each other (Fig. 4a). Good growth on PDA may be attributed to rich carbohydrates (17.47 g per 100 g) present which might stimulate the vegetative growth and provision of some additional nutrients as reported by several workers (Wasser *et al.*, 1991; Sahi *et al.*, 1992; Monga and Sheo Raj, 1994; Singh and Kasir, 1994; Jha and Dubey, 2000; Meena *et al.* 2001; Veerendra Kumar, 2004 and Tandel *et al.*, 2012). Suriachandraselvan and Seetharaman (2003) reported that the mycelial growth of *M. phaseolina* was highest in potato dextrose agar medium. The pycnidial stage was never produced in culture (Grover and Sakhuja, 1981). Host leaf extract agar appeared to be of poor quality growth due to poor quality sugars in the media and the work has been supported by Anil Kumar and Sastry (1980).

The sclerotial production also varied among different solid media tested. The highest number of sclerotia was recorded in carrot agar which recorded maximum of 103 sclerotia per plate (Fig 4b) followed by PDA with 29 per plate and least sclerotia were counted in malt extract agar (3/plate). In carrot agar, the presence of lower amount (9.6 g per 100 g) carbohydrates compared to potato agar (17.47 g per 100 g) helps to sporulate

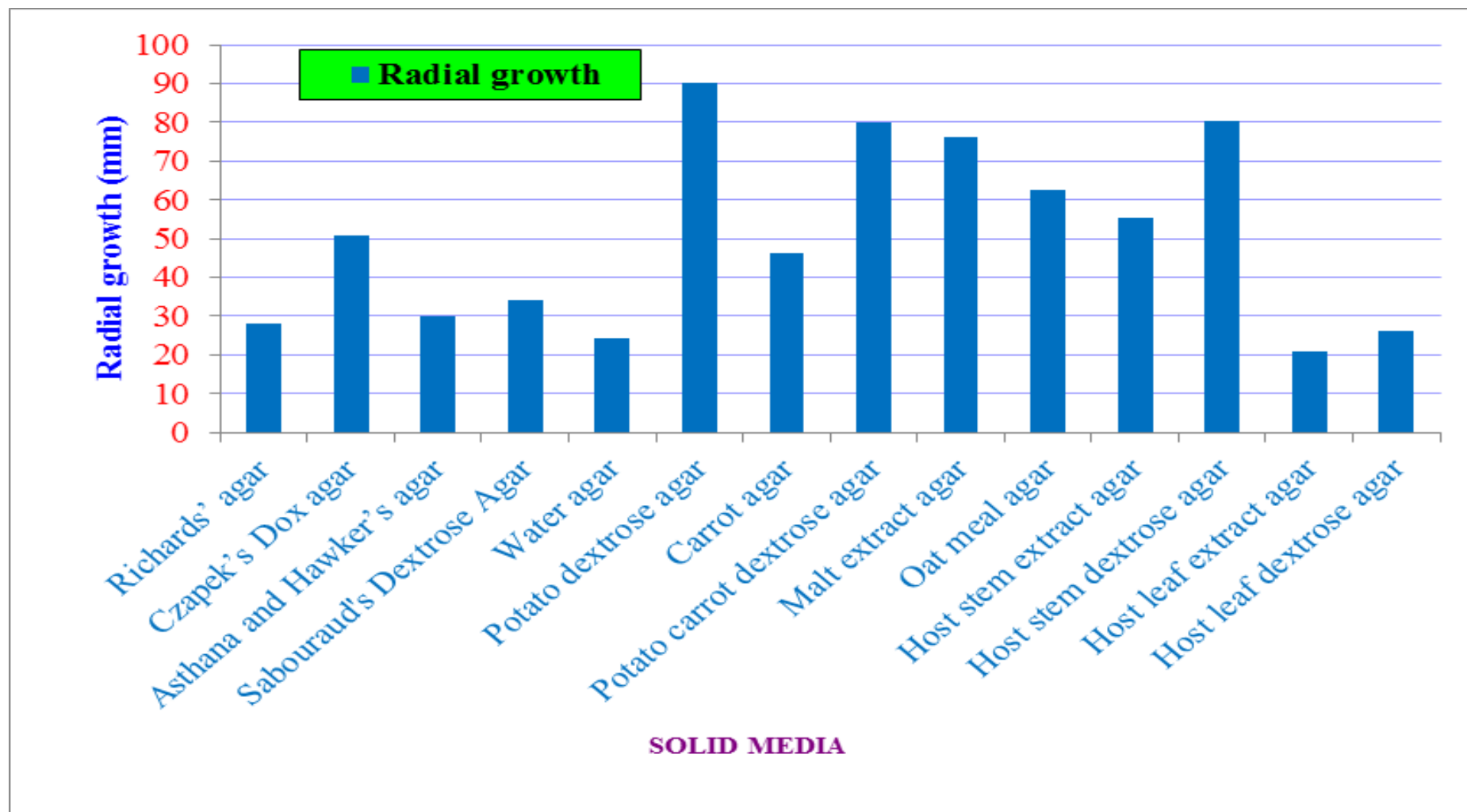


Fig. 4a. Effect of different solid media on growth of *M. phaseolina* of pigeonpea

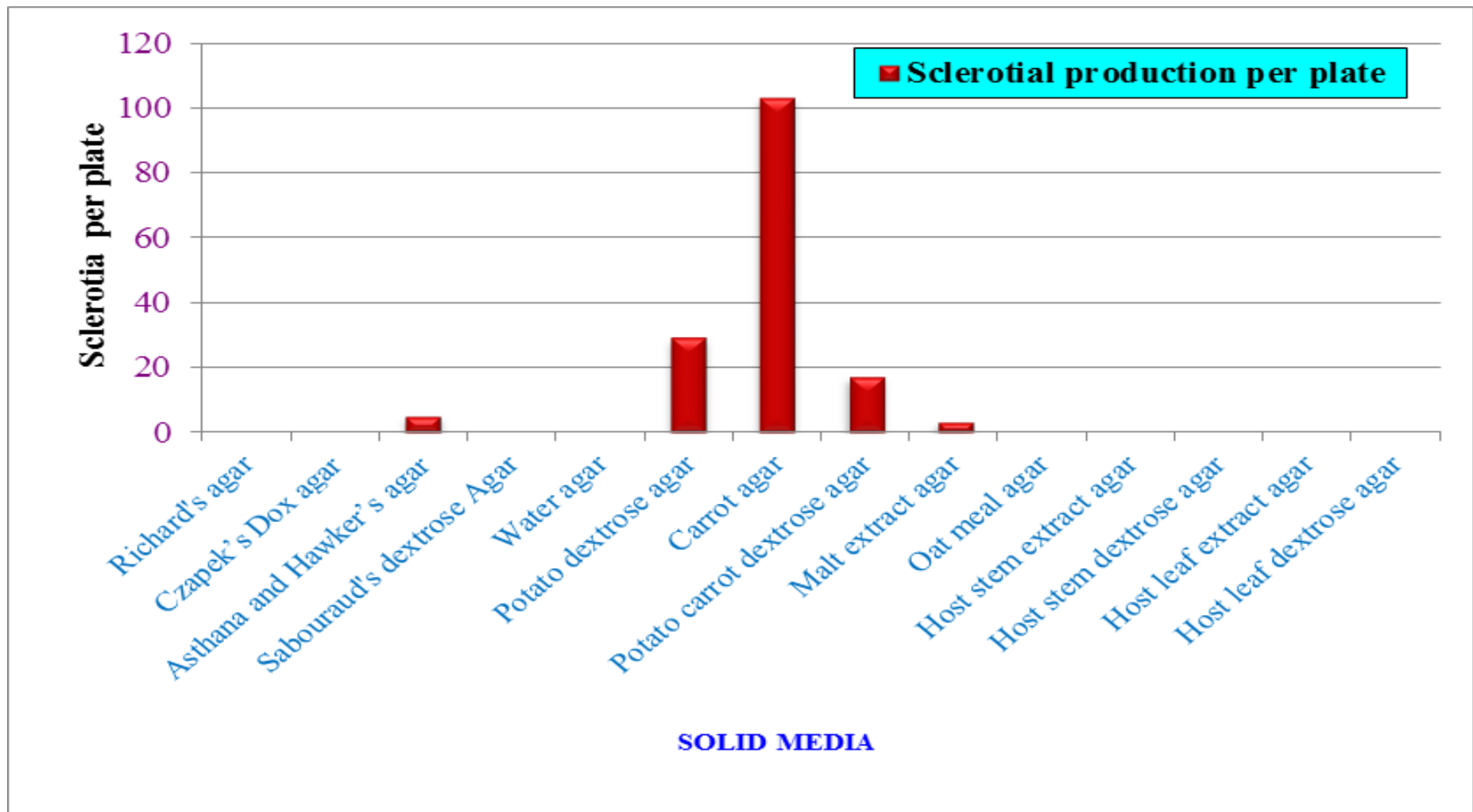


Fig. 4b. Effect of different solid media on sclerotial production of *M. phaseolina* of pigeonpea

in many imperfect fungi (Kirk *et al.*, 2008). Size of the sclerotia varied from 0.85 mm to 1.34 mm, highest sclerotial size (1.34 mm) was recorded in potato dextrose agar, followed by carrot agar (1.12 mm) and least sclerotial size (0.85 mm) was recorded in malt extract agar. These findings are confirmatory with the observations made by Chowdary and Govindaiah (2007) and Tandel *et al.* (2012). The pycnidia were not produced in any of the tested media (Grover and Sakhuja, 1981).

With reference to colony color and sclerotia productions, observations were taken nine days after incubation (Kaur *et al.*, 2013). Carrot agar, PDA, potato carrot agar and malt extract agar produced sclerotia with partial aerial mycelium and grayish black colour with a uniform margin. These results are supported by Suriachandraselvan and Seetharaman (2003) and Tandel *et al.* (2012). Oat meal agar showed fluffy growth and light gray coloured colony and Richard's agar with a light pista colour. Among the various liquid media, Richard's solution (465.33 mg) yielded significantly higher dry mycelial weight as compared to other liquid media (Fig 5) followed by malt extract solution (431.67 mg). In Richard's solution presence of high sucrose content helps in assimilation of carbon source for more biomass of fungus rather than faster growth. Similar results were found by Jha and Dubey (2000); Suriachandraselveran and Seetharaman (2000) and Tandel *et al.* (2012). Least mycelial growth was recorded in host leaf extract agar and water agar.

Diversity in morphological characters of fifty two isolates of *M. phaseolina* was studied. Cultural and morphological characters on potato dextrose agar (PDA) and water agar medium showed variation among 52 isolates of *M. phaseolina*. The isolates of *M. phaseolina* exhibited several morphological variations in culture. The isolates were characterised for branching type (right angle to acute angle), microsclerotia and sclerotia (present/ absent), type of mycelium (aerial/ partial aerial/submerged), mycelium colour (greyish black/ light pista/black greyish with margin pista) and shape of the colony (regular/irregular). Among fifty two isolates, 42 isolates showed fast growth (90 mm) and MP-BD-50 isolate showed least growth (75.17 mm). Rests of the nine isolates showed moderate growth ranging from 80 to 89.02 mm.

M. phaseolina isolates with right angle to acute angle branching type was most common in all the isolates. The colonies were characterised for phenotypic features and growth pattern according to Pearson *et al.* (1986) and Atiq *et al.* (2001). Most of the isolates from

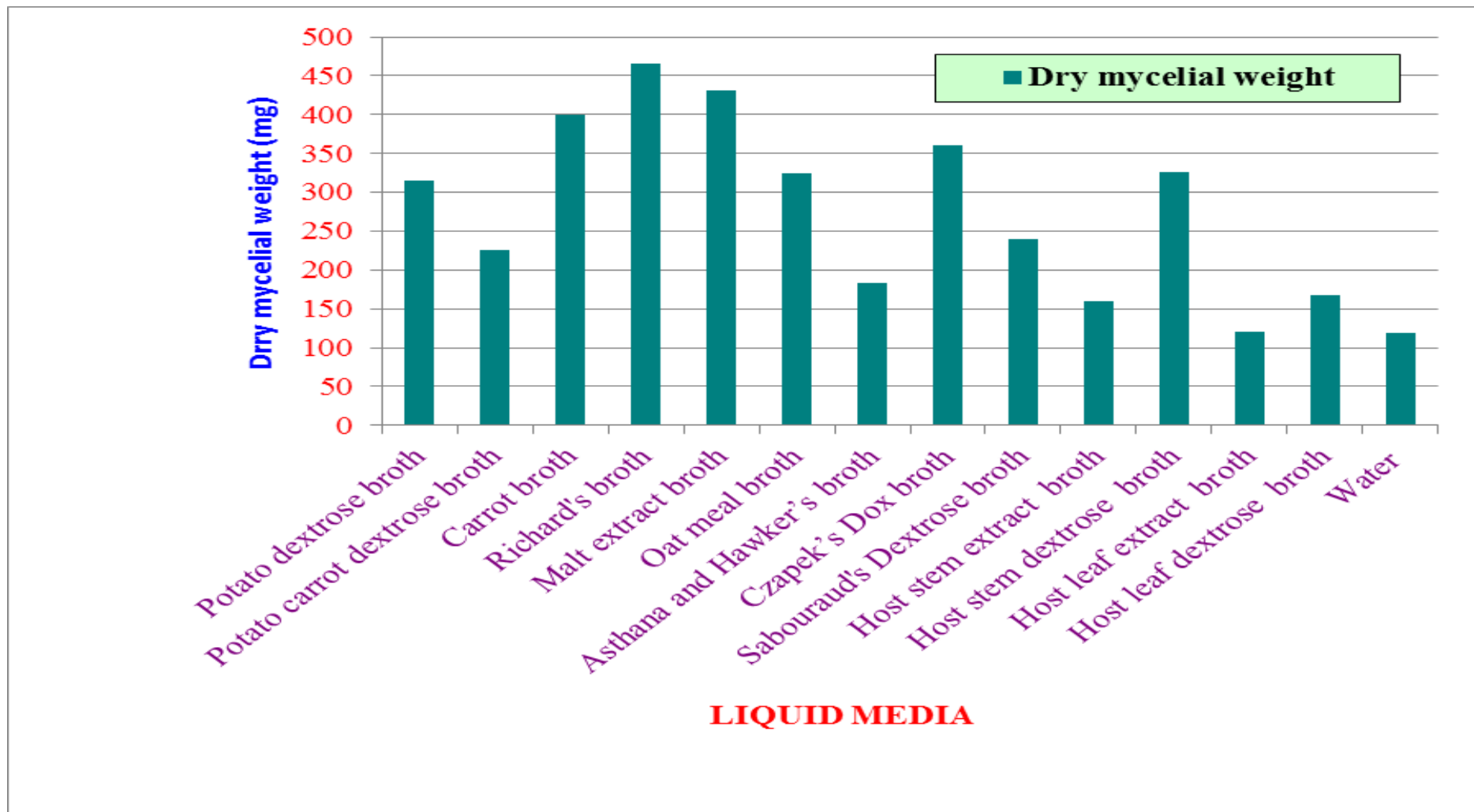


Fig. 5. Effect of different liquid media on dry mycelial weight of *M. phaseolina* of pigeonpea

Gulbarga and Bidar produced grayish black, velvety, partial aerial mycelium with right angle to acute angle and 11 isolates from Gulbarga, six isolates from Bidar produced sclerotia and microsclerotia. Few isolates of Raichur and Yadgir produced grayish to light pista, cottony to velvety aerial to immersed with right angle to acute angle. Three isolates from Yadgir produced sclerotia and microsclerotia. *M. phaseolina* isolates with circular shape, velvety texture, greyish black colour, partial aerial mycelium and without any production of sclerotia and microsclerotia were observed with maximum frequency. Similar findings were reported by Kaur *et al.* (2013).

Among the phenotypic characters the right angle and acute angle branching was a common character with 100 per cent frequency and also circular pattern of growth was visible with 82.69 per cent, partial aerial mycelium (80.77 %), regular pattern of shape (80.76 %) and velvety texture of fungus (76.93 %) with frequency ranking of I. However, phenotypic characters such as absence of microsclerotia (71.16 %), grayish black colour (69.24 %) and absence of sclerotia (67.31 %) showed a frequency ranking of II. Presence of sclerotia and microsclerotia ranging from 25-50 per cent showed rank of III (Fig 6). Similar findings were reported by Kaur *et al.* (2013).

M. phaseolina isolates collected from the different regions of North Eastern Karnataka exhibited diverse variation with respect to the sclerotia and microsclerotia production on both PDA and water agar. Dark brown to black coloured sclerotia were produced on reverse side of the culture plate and aerial mycelium aggregated to form sclerotia on front side of the plate which varied in number and size (Kaur *et al.*, 2012a). Out of fifty two isolates seventeen isolates produced the sclerotia under PDA and sixteen isolates produced microsclerotia under water agar (1 %). Out of 16 isolates collected from Gulbarga (MP-GB-27, MP-GB-31, MP-GB-35, MP-GB-36 and MP-GB-39) five isolates produced only sclerotia without microsclerotia, one (MP-GB-26) isolate produced only microsclerotia without sclerotia and five isolates (MP-GB-25, MP-GB-29, MP-GB-33, MP-GB-37 and MP-GB-38) isolate produced both sclerotia and microsclerotia. Rest of the 36 isolates one from Yadgir, two from Bidar produced only sclerotia but none of the isolates from Raichur produced sclerotia, three each from Raichur and Bidar produced only micro sclerotia but none of the isolates from Yadgir produced microsclerotia and one each from Raichur and Bidar, two from Yadgir and produced both sclerotia and microsclerotia. Sclerotia and microsclerotia producing isolates possessed more virulence

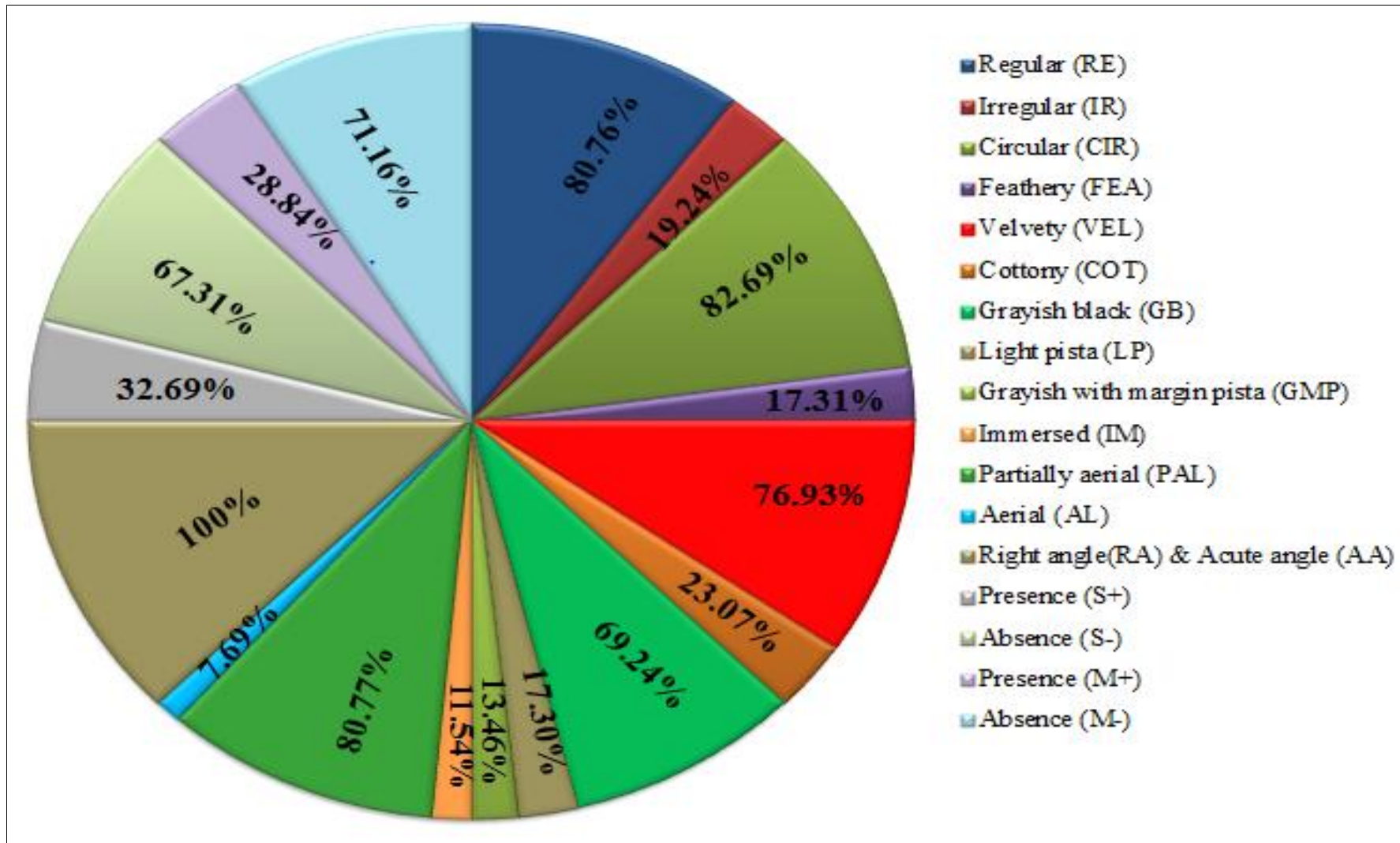


Fig. 6. Frequency distribution of isolates of *M. phaseolina* of pigeonpea obtained from different regions of North Eastern Karnataka

and aggressiveness compared to the isolates without sclerotia and microsclerotia with respect to the higher lesion length and AUPDC. Isolates of *M. phaseolina* producing microsclerotia were also reported to produce higher amount of different hydrolytic enzymes, such as cellulases, hemicellulase and amylase as compared to isolate with no microsclerotia production (Kaur *et al.*, 2012c). These findings are supported with Kaur *et al.* (2013).

Among fifty two isolates, seventeen of them produced sclerotia under PDA and sixteen isolates produced microsclerotia under water agar (1 %). Out of seventeen, eleven of them produced sclerotia and out of sixteen, six isolates produced microsclerotia which belonged to Gulbarga regions. It helps to perpetuate and survival of *M. phaseolina*. So this is one of the factors Gulbarga district attributed highest incidence of MSC. Highest sclerotia were (136/plate) produced in MP-GB-36 isolate and highest microsclerotia were found in MP-GB-25 (7.3/10x). MP-GB-36 isolated from Ramateerta village of Chittapur Taluk with the recorded incidence of 46 per cent and MP-GB-25 isolated from ARS, Gulbarga with the highest recorded incidence of 77.33 per cent. These isolates are more virulent compared to other isolates. Isolates with fast mycelial growth and abundant sclerotial production were more pathogenic than isolates growing more slowly (Purkayastha *et al.* 2004) and those isolates produced more number of sclerotia and microsclerotia are virulent compared to isolates without sclerotia (Kaur *et al.*, 2012c).

The differences were striking particularly in mycelial colour and sclerotia and microsclerotia. Based on the morphological and cultural characteristics, the isolates of *M. phaseolina* were grouped into three and designated as G-I, G-II and G-III and two each sub groups in G-II (GII-A and GII-B) and G-III (GIIIA and GIIIB). G-I comprising of six isolates from Raichur, five isolates from Gulbarga, four each from Yadgir and Bidar. G-II comprising of diverse isolates with respect to the striking phenotyping characters and sclerotia/microsclerotia produced and also absence of both sclerotia and microsclerotia isolates from all districts. It includes 19 isolates three each from Bidar and Raichur, six each from Gulbarga and Yadgir. G-III comprised of five isolates each from Gulbarga and Bidar, three isolates from Raichur and two isolates from Yadgir. Among fifty two isolates, maximum frequency 36.53 per cent (Fig 7) was found in G-I and GII (GII-A (17.30 %), GII-B (19.23 %)). Similar grouping of isolates was supported by Kaur *et al.* (2013) and Almomani *et al.* (2013).

Grouping of isolates

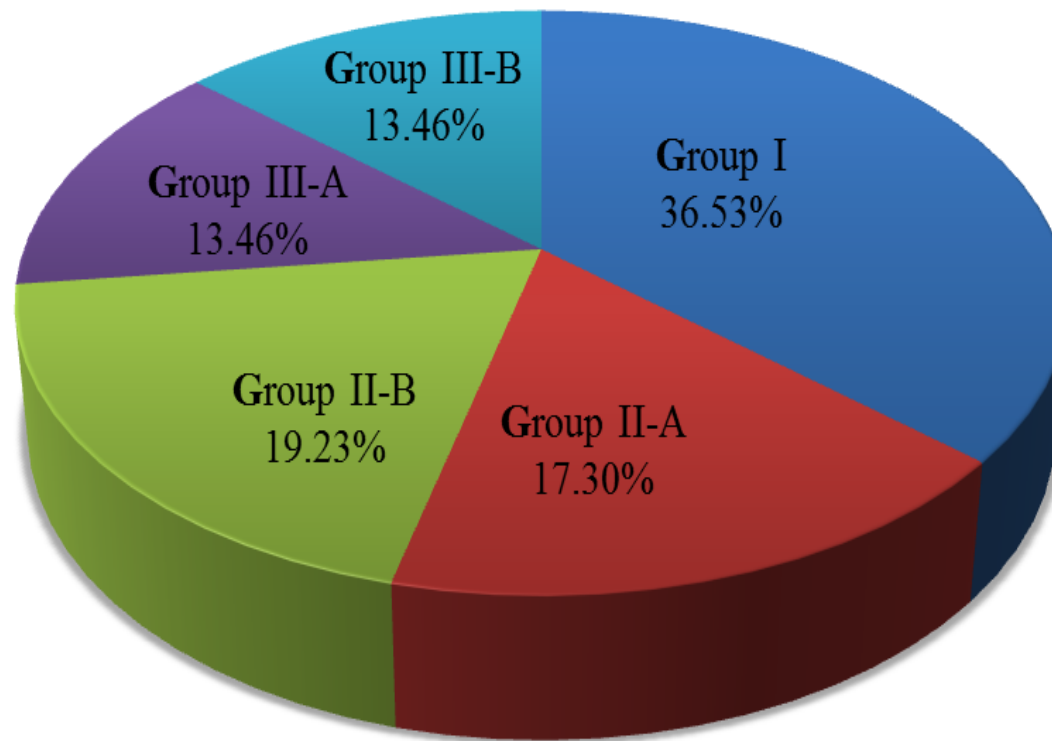


Fig. 7. Frequency distribution of grouping of isolates of *M. phaseolina* of pigeonpea based on morphological and sclerotial features

The amplification of isolated DNA from the 52 pathogenic cultures using ITS primers (ITS-1& ITS-4) showed 550 to 600 bp which is comparable with the markers supported by Babu *et al.* (2007) and Almomani *et al.* (2013). This indicates that all 52 isolates belong to the same species and represented as *M. phaseolina*. Among 52, twenty two isolates were selected based on representation to geographic regions and morphological grouping. Such isolates were amplified and 5.8 S rDNA was sequenced. The NCBI, BLAST was carried out and the conformity of the isolates was obtained. The twenty two sequences of rDNA were deposited in the GenBank, Mary land, USA database under the accession no. KJ629071-KJ629093. The isolates showed level of genetic similarity within a range of 47 to 97 per cent (Fig 8).

Isolate MP-GB-25 was used for *in vitro* and interconnectivity studies and it was 53 per cent dissimilar to other isolates and most of morphological and sclerotia/microsclerotia varied among isolates (MP-RC-1, MP-YD-14, MP-YD-18, MP-GB-26, MP-GB-28, MP-GB-30, MP-GB-31, MP-GB-32, MP-GB-35, MP-GB-36, MP-GB-39 and MP-GB-40) were in the same polymorphism with 97 per cent similarity but MP-RC-3 and MP-RC-10 showed diverse variability among the isolates. Infact, MP-RC-10 isolate is less virulence due to absence of both sclerotia and microsclerotia collected from Karigudda village of Raichur with the very lowest (0.33 %) incidence in local cultivar. Isolates within a state showed similarity suggesting that these isolates may not evolve independently from each other and therefore, they may be considered as a part of the same ancestral population (Almeida *et al.*, 2003). Very little variability was observed among the isolates it might be due to same climatic condition, same type of soil, in North Eastern Karnataka. Gulbarga isolates were morphologically different but not much variation was observed at molecular level which might be due to mono-variety (TS-3R) grown across the region and cultivation practices leading to interaction of similar type of host-pathogen interaction.

5.4 Inter connectivity studies between pycnidia and sclerotia

Interconnectivity between sclerotia and pycnidia was studied by stem detached technique and cellophane method. Fungus (*M. phaseolina*) was isolated from infected stem canker (presence of pycnidia on stem) under culture plate and it produced sclerotia (aerial mycelium aggregated form sclerotia on front side and reverse side of plate) and microsclerotia. Such fungus (only aerial mycelium) was inoculated in to root, stem and

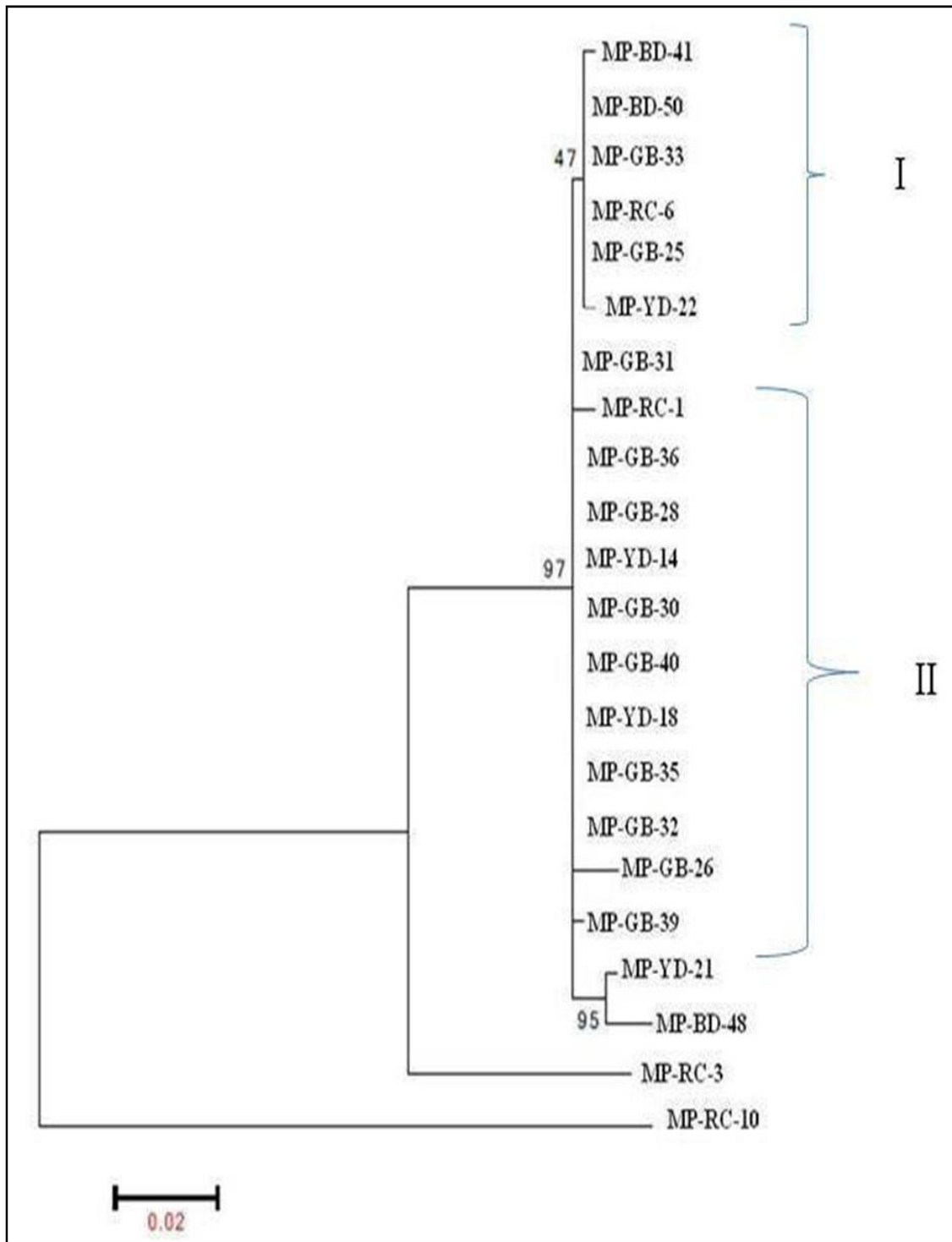


Fig. 8. Phylogeny tree showing the similarity among the *M. phaseolina* representative isolates based on their sequence

leaves, again stem was infected with pycnidial bodies but leaf and root were not infected. Similarly, fungus (*R. bataticola*) isolated from dry root rot infected plant (presence of sclerotia) under culture plate it produced sclerotia (microscopic view), such fungus was inoculated in to root, stem and leaves. Root and stem were infected with sclerotia but leaf did not show infection. Sclerotia and microsclerotia produced from *M. phaseolina* did not cause dry root rot. Similarly, sclerotia produced from *R. bataticola* did not cause stem canker. This is the missing link between sclerotia and pycnidia. Pycnidia from *M. phaseolina* was rarely produced on cultural plate only when exposed to UV light or highly specific media like peanut butter extract agar and soya butter extract agar as reported by Kaur *et al.* (2012a). Similarly, there is no report on pycnidia production from *R. bataticola*. This is also a missing link between *R. bataticola* of sclerotia and pycnidia of *M. phaseolina*.

5.5 Management studies on Macrophomina Stem Canker

Effective method of managing soil borne disease of crop plants is by using resistant varieties which is most economical, inexpensive and eco-friendly for resource poor farmers as compared to chemicals. None of them showed resistance against *M. phaseolina*. GRG -2010, BSMR-736, ICP-8863 (Maruti) and ICP-87119 (Asha) showed moderately resistant response. Chaple, Kari togari, Gulyal local, GRG -333, GRG -818, GRG-811, JKM-7 and JKM-189 showed susceptible and TS-3R showed highly susceptible reaction. ICP-8863 which was regarded as resistant (Anonymous, 1983) but present investigation showed moderately resistant reaction. Susceptibility of pigeonpea genotypes to *M. phaseolina* by the higher activity of pectin and polygalactouronate trans-eliminase and reduced the activity of these enzymes in resistant genotypes which has been observed by Srivastava (1987).

In vitro evaluation of fungicides provides useful preliminary information regarding its efficacy against a pathogen with in a shortest period of time and therefore serve as guide for further field testing. In the present study, among five non- systemic fungicides (Fig 9a), mancozeb, copper hydroxide and a combiproducs of carbendazim 12 % + mancozeb 63 % recorded maximum inhibition of (100 %) mycelial growth at all concentrations (0.10 %, 0.20 % and 0.30 %). Bavistin was most effective fungicide in reducing the mycelial growth of *M. phaseolina* and gave complete inhibition of mycelial

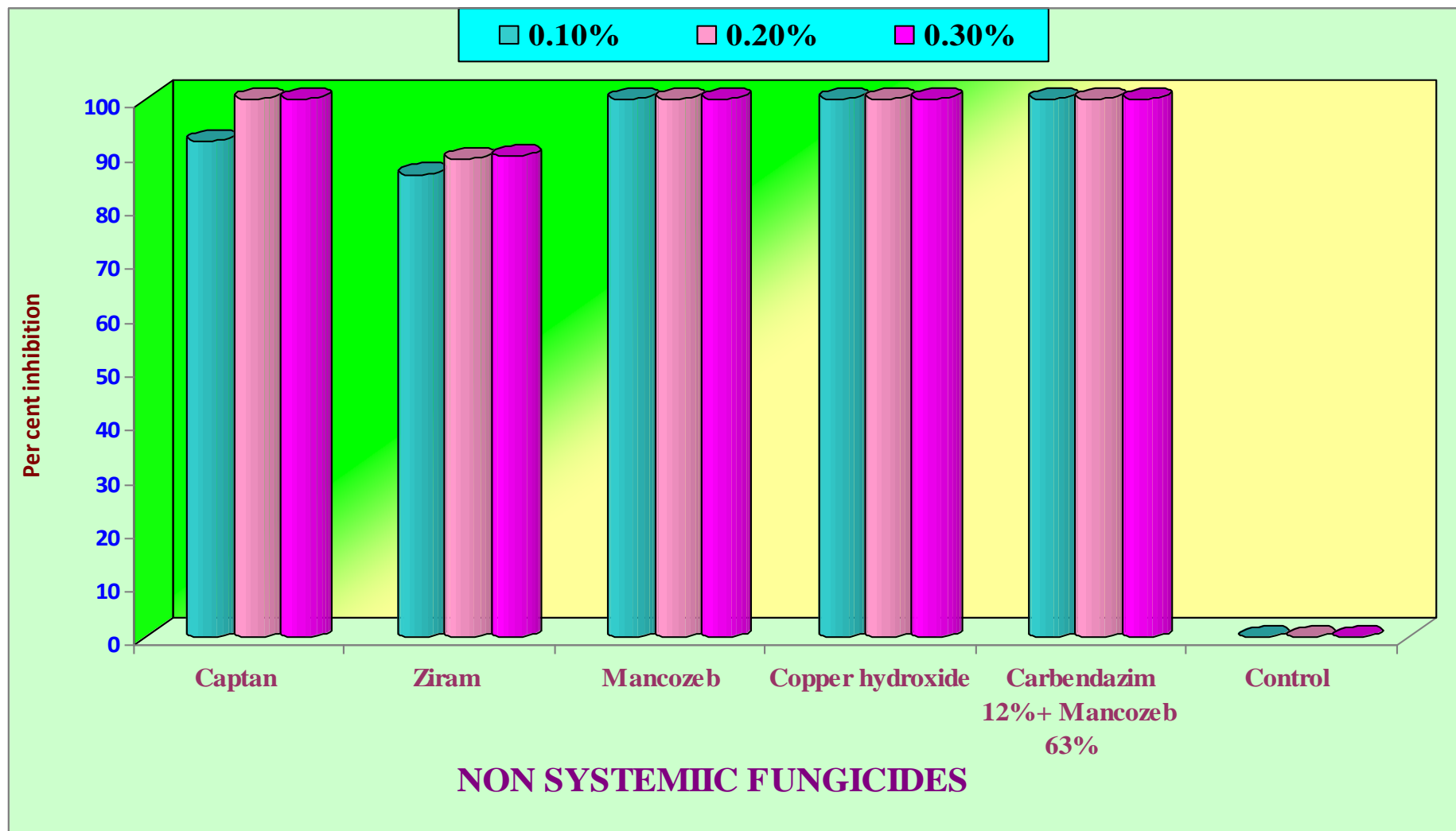


Fig. 9a. Efficacy of non-systemic fungicides on the growth of *M. phaseolina* of pigeonpea under *in vitro*

growth and action of these chemicals inhibit the germination, growth and multiplication of the fungus or are directly toxic (Kumari *et al.*, 2012). Ramadoss and Sivapraskasam (1994) reported that sclerotial production of *M. phaseolina* was completely inhibited by carbendazim. Similar results were obtained by several workers (Prashanthi *et al.*, 2000; Prajapati *et al.*, 2002; Lokesha, 2003; Konde *et al.*, 2008; Khan *et al.*, 2012 and Nagamma *et al.*, 2012).

Among the systemic fungicides (Fig 9b) carbendazim, benomyl, thiophanate methyl, carbendazim 25 % + mancozeb 50 %, zineb 68 % + hexaconazole 14 % and tricyclazole 18 % + mancozeb 62 % showed 100 per cent inhibition at all concentrations (0.05, 0.10 and 0.20 %). The efficacy of the triazoles fungicides in a combiform such as hexaconazole and tricyclazole may be attributed to their interference with the biosynthesis of fungal sterols and inhibition of ergosterol biosynthesis. In many fungi, ergosterol is essential to the structure of cell wall and its absence causes irreparable damage to cell wall leading to death of fungal cell. A similar study was reported for the effectiveness of triazoles, which inhibit the biosynthesis pathway in fungi (Nene and Thapliyal, 1973). Complete 100 per cent inhibition of carbendazim, thiophanate methyl, carbendazim 25 % + mancozeb 50 % and tricyclazole 18 % + mancozeb 62 % were reported earlier. These findings are supported by earlier workers (Prajapati *et al.*, 2002; Lokesha, 2003; Konde *et al.*, 2008; and Nagamma *et al.*, 2012).

Biological control is a potential non-chemical and eco friendly means for plant disease control by reducing harmful effects of a pathogen through the use of other living entities. It is now widely recognized that biological control of plant pathogens using antagonistic fungi and bacteria is a distinct possibility for future and can be successfully utilized especially within the frame work of integrated disease management system (Muthamilan and Jeyarajan, 1996). In the present study, investigations were carried out on the *in vitro* evaluation of bio-agents on inhibition of growth of *M. phaseolina*.

Among the bio-agents tested, *T. harzianum* was more effective as compared to other bio-control agents and inhibited maximum fungal growth (41.86 %) of *M. phaseolina* followed by *T. viride* 39.07 per cent (Fig 10). These findings are similar to that supported by several other workers (Elad *et al.*, 1986 Ghaffar *et al.*, 1988; Indra *et al.*, 2003; Suriachandraselvan *et al.*, 2004b; Surendra Singh *et al.*, 2007; Manjunatha,

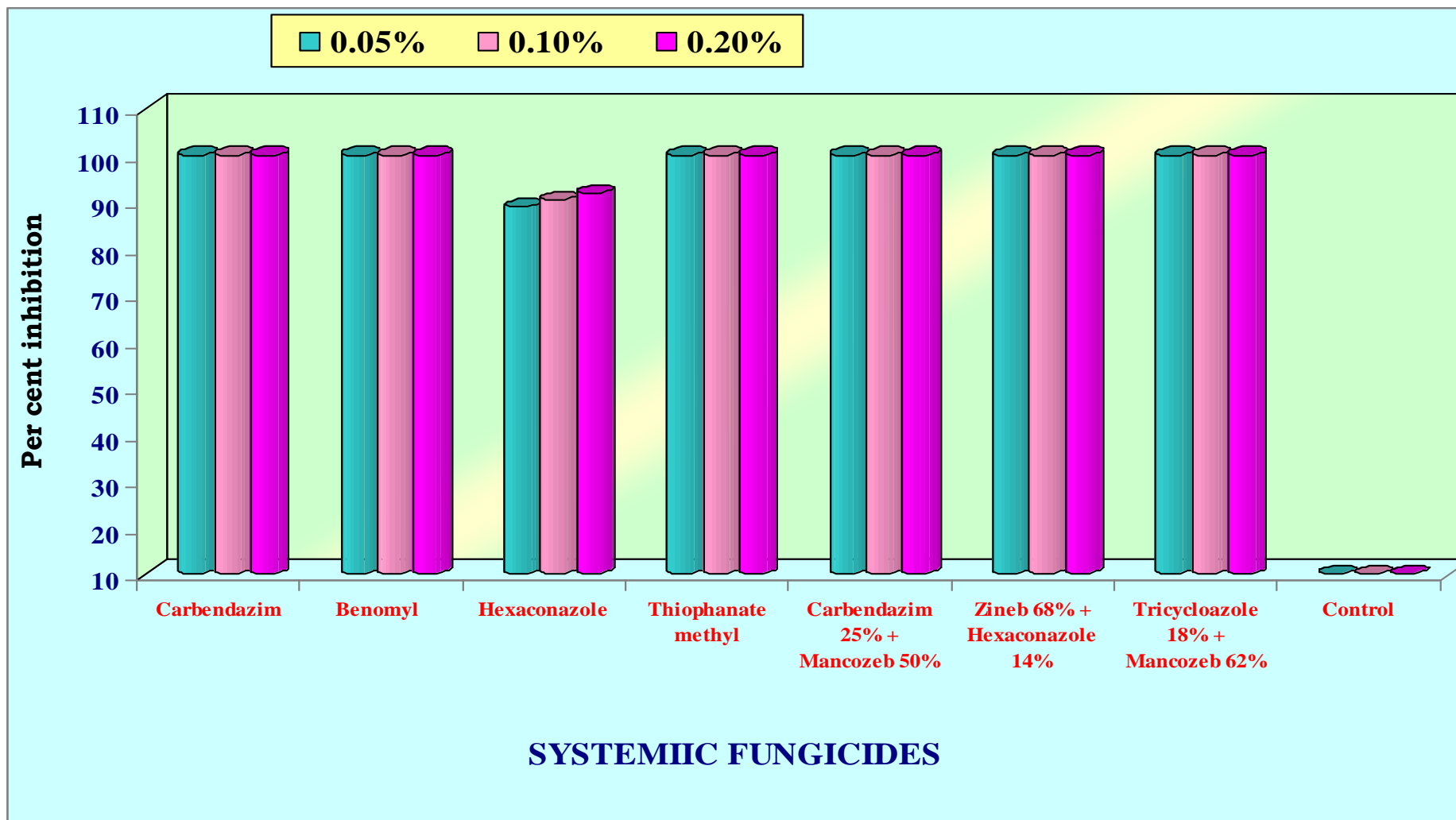


Fig. 9b. Efficacy of systemic fungicides on the growth of *M. phaseolina* of pigeonpea under *in vitro*

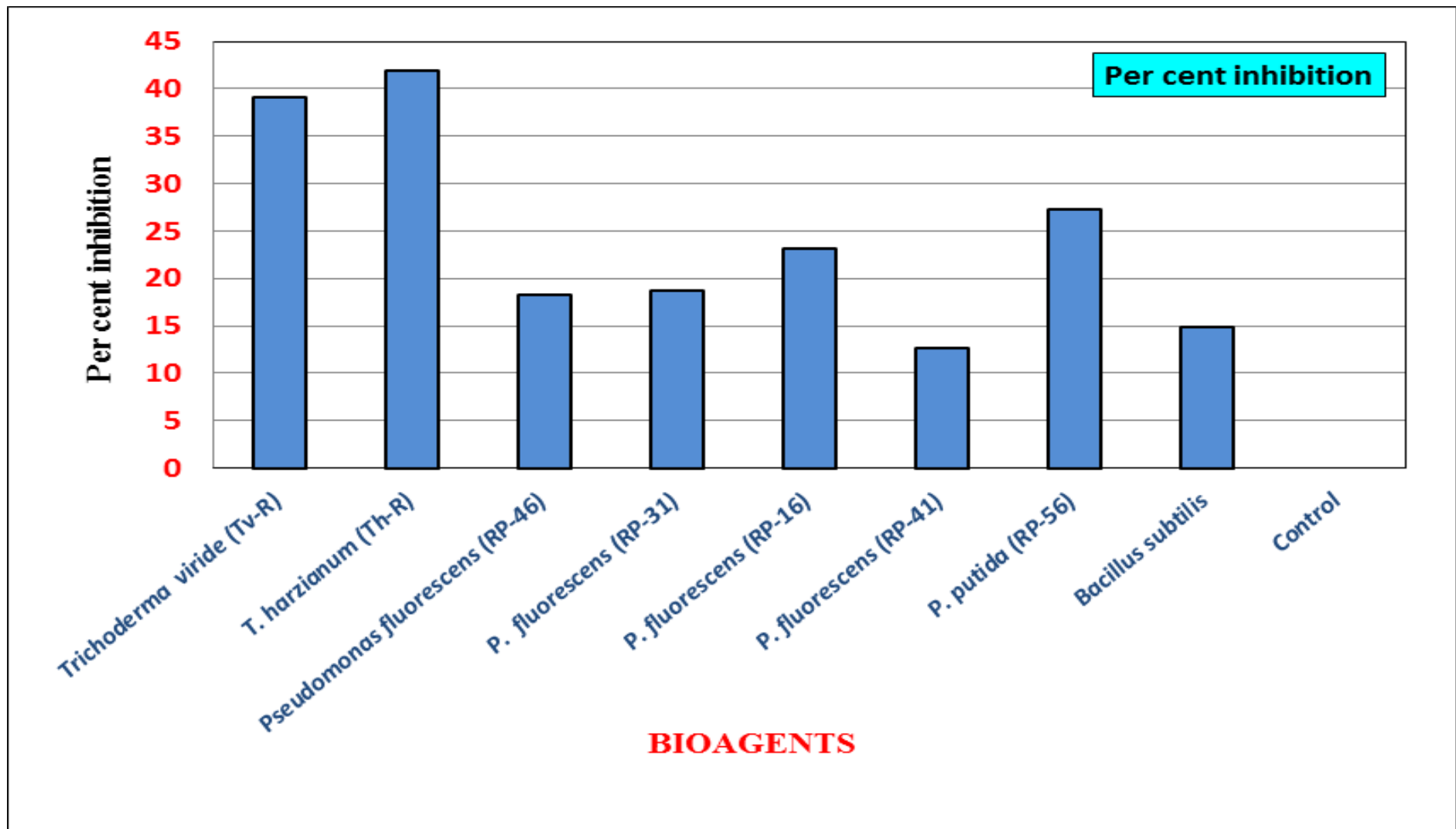


Fig. 10. Efficacy of bio-agents against *M. phaseolina* of pigeonpea under dual culture

2009; Ammajamma *et al.*, 2009; Shekhar and Kumar, 2010; Nagamma *et al.*, 2012; Kumari *et al.*, 2012 and Manjunatha *et al.*, 2013). Mechanisms for bio-control of plant pathogens by *Trichoderma* are antibiosis, lysis competition and mycoparasitism. *T. harzianum* and *T. viride* both suppressed the growth of *M. phaseolina* and this may be due to coiling and disintegration of hyphae of the test fungus resulting in loss of competitive saprophytic ability (Naik and Sen, 1995 and Naik *et al.*, 2009). Manczinger *et al.* (2002) reported that *T. harzianum*, *T. viride* and *T. polysporum* have a strong antagonistic against soil borne pathogens.

In vitro screening of fungicides and antagonists provides preliminary information regarding their efficacy against *M. phaseolina* and with a hope to utilize the promising bio-agents and fungicides for management of stem canker of pigeonpea under field conditions by integrating the methods to apply bio-agents, fungicides and with other components of integrated disease management. The least stem canker incidence (9.87 %) was recorded in combination with seed treatment and soil application with *T. harzianum* and foliar spray of carbendazim followed by *T. viride* seed treatment and soil application and foliar spray of carbendazim with mean incidence 12.60 per cent as against check with 34.55 per cent. Application of bio-agents significantly reduced the disease by enhancing the induced systemic resistance and also enhances the growth component (Durai *et al.*, 2006). The combination of seed treatment with *T. harzianum*, *T. viride* and enrichment with FYM application along with chemical spray reduced the maximum incidence of root rots as reported by Kumar *et al.* (2011); Kumari *et al.* (2012) and Manjunatha *et al.* (2013).

Macrophomina Stem Canker incidence was reduced to a greater extent (24.68 %) as against check (34.55 %). Seed treatment and soil application with bio-agent along with fungicidal spray was followed for managing pigeonpea stem canker.

Future line of work

1. Studies are needed to determine the temporal and spatial distribution of the pathogen.
2. Toxin characterization for sclerotia and microsclerotia produced isolates and studies for understanding the role of toxin in pathogenicity and disease development shall be taken up.

3. Survival studies are needed on isolates which don't produce sclerotia and microsclerotia.
4. More studies are needed to find out interconnectivity between sclerotia and pycnidia.
5. Evaluation of the genetic diversity of *M. phaseolina* isolates across the India will be an initial step towards understanding the population structure of *M. phaseolina*.
6. The identification of traits depicting disease resistance to stem canker would enhance the resistance breeding program.
7. Need to develop IDM package.

SUMMARY AND CONCLUSION

VI. SUMMARY AND CONCLUSIONS

Macrophomina phaseolina, a global devastating fungal pathogen, infects more than 500 plant hosts, inciting a stem canker disease in many crops that is often referred to as charcoal rot, due to the charcoal type discoloration imparted to the colonized plant tissues. *M. phaseolina* is primarily soil borne in nature, causes both pre and post emergence plant mortality. Characteristic post-emergence symptoms include the development of spindle shaped lesions with dark border and light gray centre covered with small pinhead-sized microsclerotia and sometimes pycnidia. Macrophomina stem canker of pigeonpea caused by *M. phaseolina* is an emerging disease in North Eastern Karnataka. The present study comprised of survey on incidence of stem canker of pigeonpea, collection of pathogenic isolates for exploring cultural, morphological and molecular characterization, interconnectivity studies between pycnidia and sclerotia, *in vitro* screening of bio agents, fungicides and screening of pigeonpea genotypes against MSC and the management of stem canker of pigeonpea under field condition using seed treatment and soil application. Results of the investigations are summarized here under.

Roving survey was carried out during *Kharif* season of 2013 in four districts of North Eastern Karnataka consisting of Raichur, Yadgir, Gulbarga and Bidar. Severity of the Macrophomina Stem Canker incidence was more in Gulbarga district (25.02 %) followed by Bidar (18.11). However, high (77.33 %) incidence of Macrophomina Stem Canker was noticed in ARS, Gulbarga and the least was in Karigudda (0.33 %) of Raichur district. The highest average number of lesions (26.30/plant) and lesion length (6.23 cm) were recorded in ARS, Gulbarga.

Maximum disease incidence of dry root of pigeonpea was recorded in Gulbarga district (22.63 %) and the least incidence was (4.72 %) in Bidar. However, high (41.33 %) incidence of dry root rot was noticed in Dadoti village of Chittapur taluk and no incidence in Alkoda village of Devodurga taluk and Hudgi village of Bidar district.

Symptoms appeared as spindle shaped lesions on stem with increased number of lesions coalesced on drying branches and finally plants wilted. The fungus was isolated from infected stem having pycnidial bodies by standard tissue isolation technique. As many as fifty two isolates of *M. phaseolina* were obtained from pigeonpea growing North

Eastern districts of Karnataka. The fungus produced rapid growth on PDA with mycelium black to brown or grayish of pista.

The formation of mycelial branching was at right angle to acute angle. Sclerotia and microsclerotia were round to oblong with dark brown to grayish colour. Further, the isolates were identified as *M. phaseolina* based on morphological features and they were subjected to pathogenicity test. The identity of *M. phaseolina* isolates was confirmed amplification and sequencing of ITS r DNA by NCBI, BLAST and deposited in GenBank (Mary land, USA).

Among solid media, PDA showed fast growth (90 mm) and least growth of 20.67 mm was observed in host leaf extract agar. More number (103/plate) of sclerotial bodies were produced in carrot agar followed by PDA (29/plate) and was absent in oat meal agar, host stem extract agar, host stem dextrose agar, host leaf extract agar, host leaf dextrose agar, Richards' agar, Czapek's Dox agar, Sabouraud dextrose agar and water agar. Highest sclerotial size (1.34 mm) was recorded in PDA and least sclerotial size (0.85 mm) was recorded in malt extract agar with grayish to light gray colour common in tested solid media. Pycnidial bodies were not found in any tested media. Maximum dry mycelial weight was noticed in Richard's solution (465.33 mg), while it was very poor growth in host leaf extract broth (120.67 mg) and water solution (118.67 mg).

Diversity in morphological characters of fifty two isolates of *M. phaseolina* was studied. Morphological characterization of different isolates indicated the existence of variation among isolates in shape, growth pattern, colour, texture, mycelium growth, branching type and sclerotial/microsclerotia production. Most of the isolates from Gulbarga and Bidar produced grayish black, velvety, partial aerial mycelium with right angle to acute angle. Eleven isolates from Gulbarga, 6 isolates from Bidar produced sclerotia and microsclerotia. Forty two isolates were fast growing (90 mm) and only one isolate (MP-GB-50) showed slow growth (75.17 mm).

Among the phenotypic characters, the right angle and acute angle branching was a common character with 100 per cent frequency. Partial aerial mycelium, regular pattern of shape and velvety texture of fungus were frequency under rank I. Presence of sclerotia and microsclerotia belonged to rank III. Among 52 isolates, most (11) isolates from Gulbarga produced sclerotia and microsclerotia compared to Raichur, Yadgir and Bidar. In PDA among the 17 isolates, isolate MP-GB-36 produced highest number of sclerotia

on both riverside side (136) and front side (68) of the culture plate. In water agar among 16 isolates, isolate MP-GB-25 recorded highest microsclerotia (7.3/10x).

Based on the striking differences in morphological characteristics *viz.*, texture, shape and colour the isolates were categorized in to three groups designated as G-I, G-II, and G-III. G-I includes striking morphological features such as circular to feathery growth, cottony to velvety texture, grayish black to margin pista colour, partial aerial to aerial mycelium immersed and absence of both sclerotia and microsclerotia. Again on the basis of characteristics of sclerotia and microsclerotia presence/absence, numbers, size and shape G-II and G-III are subdivided in to sub groups in G-II (G-IIA and G-IIB), G-III (G-IIIA, G-IIIB).

G-II comprising of diverse isolates with phenotyping characters such as regular to irregular, circular to feathery growth, velvety texture, grayish black to light pista colour, partial aerial mycelium and right angle and acute angle branching and presence of both sclerotia/microsclerotia and also absence of both sclerotia and microsclerotia isolates from all districts. It includes 19 isolates three each from Bidar and Raichur, seven from Gulbarga and six from Yadagiri. In GII group, out of 19 isolates nine isolates belong to G-II-A which produced both sclerotia and microsclerotia and in GII-B comprising 10 isolates without sclerotia and microsclerotia. Among fifty two isolates maximum frequency (36.53 %) was found in G-I and GII (GII-B (19.23 %), GII-A (17.30 %), followed by G-IIIA (presence of sclerotia with absence of microsclerotia) and GIIIB (absence of sclerotia with presence of microsclerotia) which were found in equal frequency of 13.46 per cent.

Identity of *M. phaseolina* isolates, the 5.8 S rDNA-ITS (ITS-1 and ITS-4) region of all the 52 isolates was amplified with a range of 550 to 600 bp length. Twenty two isolates of *M. phaseolina* were selected out of fifty two isolates based on representation to geographic regions and morphological grouping. Such isolates were amplified and 5.8 S rDNA was sequenced. The NCBI, BLAST was carried out and the conformity of the isolates was obtained. Twenty two rDNA sequences were deposited in the NCBI, USA GenBank database under the accession no. KJ629071-KJ629093. The isolates MP-GB-25 and MP-GB-36 belonged to cluster I and cluster II respectively. The isolates MP-RC-3 and MP-RC-10 showed diverse variability which were collected from Raichur district but most of Gulbarga isolates belonged to same clusters.

Interconnectivity between sclerotia and pycnidia was studied. Fungus (*M. phaseolina*) was isolated from infected stem canker (presence of pycnidia on stem). From pycnidia, sclerotia and microsclerotia were formed under culture plate. The pycnidia were inoculated in to stem, root and leaves. The roots were not infected but stem was infected and caused canker disease. Similarly, fungus (*R. bataticola*) isolated from dry root rot infected plant (presence of sclerotia) was inoculated in to root, stem and leaves of pigeonpea. Root was infected by sclerotia, stem was also infected by sclerotia only but leaf was not infected by sclerotia. Sclerotia and microsclerotia produced from *M. phaseolina* did not cause dry root rot. Similarly, sclerotia produced from *R. bataticola* did not cause stem canker. This is the missing link between sclerotia and pycnidia.

Out of 22 genotypes screened, none of them showed resistance, while four genotypes (GRG -2010, BSMR-736, ICP-8863 (Maruti) and ICP-87119 (Asha) showed moderately resistant reaction. Chaple, Kari togari, Gulyal local, GRG -333, GRG -818, GRG-811, JKM-7 and JKM-189 showed susceptible and TS-3R showed highly susceptible reaction.

Among contact fungicides mancozeb, copper hydroxide and a combi-products carbendazim 12 % + mancozeb 63 % recorded maximum inhibition of (100 per cent) mycelial growth at all concentrations (0.10 %, 0.20 % and 0.30 %). Among systemic fungicides carbendazim, benomyl, thiophanate methyl, carbendazim 25 % + mancozeb 50 %, zineb 68 % + hexaconazole 14 % and tricyclazole 18 % + mancozeb 62 % showed 100 per cent inhibition at all concentrations (0.05, 0.10 and 0.20 %). Among the bio-agents tested, *Trichoderma harzianum* (Th-R) was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (41.86 %) of *M. phaseolina* followed by *T. viride* (39.07 %). Pf-16 showed 23.10 per cent inhibition and *Bacillus subtilis* recorded 14.85 per cent of inhibition.

The promising bio-agents identified *in vitro* were imposed under field condition for management of Macrophomina Stem Canker of pigeonpea using *var* TS-3R. The least stem canker incidence (9.87 %) was recorded in combination with seed treatment and soil application with *T. harzianum* and foliar spray of carbendazim followed by *T. viride* seed treatment and soil application and foliar spray of carbendazim with mean incidence of 12.60 per cent as against check with 34.55 per cent, subsequently the yield (1509 kg/ha) was also higher in these treatments.

Conclusions

- The maximum severity of stem canker of pigeonpea was 25.02 per cent in Gulbarga and the least was in Yadgir (2.13 %) during *Kharif* -2013.
- The maximum severity of dry root rot of pigeonpea was in Gulbarga district (22.63 %) and lowest was recorded in Bidar (4.72 %) during *Kharif* 2013.
- Fifty two fungal isolates were collected from four districts of pigeonpea growing areas in North Eastern Karnataka and were identified as *M. phaseolina* based on morphological and cultural features. The identity was also confirmed by sequencing the ITS rDNA and NCBI, BLAST.
- 52 isolates of *M. phaseolina* was amplified with range of 550 to 600 bp length but 22 isolates were sequenced and MP-RC-10 which showed diverse variability among the isolates.
- The sequences were deposited with accession no. KJ629071-KJ629093 at NCBI, GenBank, Mary land, USA.
- PDA (90 mm), host stem agar and potato carrot agar supported good growth. Carrot agar supported 103 sclerotia per plate and PDA supported for sclerotia production with highest size (1.34 mm). Richards' solution (465.33 mg) showed maximum dry mycelial weight.
- Based on cultural and morphological characters, fifty two were grouped into three groups (G-I (36.53 %), G-II and G-III) and based on sclerotia and microsclerotia again divided in to two subgroups GII (GII-A (17.30 %), GII-B (19.23 %) and G-III comprising of G-IIIA (13.46 %) and G-IIIB (13.46 %).
- Interconnectivity between pycnidia and sclerotia was not clearly established and needs much more study.
- GRG -2010, BSMR-736, ICP-8863 (Maruti) and ICP-87119 (Asha) showed moderately resistant reaction. Chapple, Kari togari, Gulyal local, GRG -333, GRG - 818, GRG-811, JKM-7 and JKM-189 showed susceptible and TS-3R showed highly susceptible reaction.

- Mancozeb, copper hydroxide and a combi-products of carbendazim 12 % + mancozeb 63 % recorded maximum inhibition of (100 %) mycelial growth at all concentrations (0.10 %, 0.20 % and 0.30 %) among contact fungicides.
- Carbendazim, benomyl, thiophanate methyl, carbendazim 25 % + mancozeb 50 %, zineb 68 % + hexaconazole 14 % and tricyclazole 18 % + mancozeb 62 % showed 100 per cent inhibition at all concentrations (0.05, 0.10 and 0.20 %) among systemic fungicide.
- *Trichoderma harzianum* was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (41.86 %).
- Combination of seed treatment and soil application with *T. harzianum* and foliar spray of carbendazim resulted in incidence of 9.87 per cent against check with 34.55 per cent. Which subsequently gave higher (1509 kg/ha) yields against check (1262 kg/ha).

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VII. REFERENCES

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*- Originals are not seen

APPENDICES

APPENDIX – I

The composition and preparation of the synthetic and non synthetic media

1. Potato Dextrose agar (PDA)

In most of the experimental studies PDA medium was used. The composition of PDA is as follows

Peeled potato	200.00 g
Dextrose	20.00 g
Agar-agar	20.00 g
Distilled water	1000 ml (volume to make up)

Two hundred g of peeled potato was cut into small bits and boiled in distilled water and extract was collected by filtering through muslin cloth. Dextrose 20 g and Agar- Agar powder 20 g each were dissolved in potato extract and the final volume was made up to 1000 ml with distilled water. Later, it was sterilized at 1.1 kg/cm^2 pressure for 15 min and preserved for future use.

2. Potato carrot agar

Grated potato	20 g
Grated carrot	20 g
Agar-agar	20 g
Distilled water (to makeup)	1000 ml

Grated vegetables were boiled in 400 ml in distilled water. This was strained through fine sieve. Agar-agar was melted separately in 400 ml distilled water. Both the solutions were mixed thoroughly. Volume was made up to 1000 ml with distilled water and sterilized at 1.1 kg/cm^2 pressure for 15 min.

3. Carrot Dextrose agar

Peeled carrot	200.00g
Dextrose	20.00g
Agar-agar	20.00g
Distilled water	1000 ml (volume to make up)

Two hundred g of peeled carrot was cut into small bits and boiled in distilled water and extract was collected by filtering through muslin cloth. Dextrose 20 g and Agar- Agar powder 20 g each were dissolved in potato extract and the final volume was made up to 1000 ml with distilled water. Later, it was sterilized at 1.1 kg/cm^2 pressure for 15 min and preserved for future use.

4. Richard's agar

Sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$)	: 50 g
Potassium dihydrogen phosphate (KH_2PO_4)	: 5 g
Potassium nitrate (KNO_3)	: 10 g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	: 2.5 g
Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$)	: 0.02 g
Agar-agar	: 20 g]
Distilled water (to makeup)	: 1000 ml

All the above ingredients except potassium dihydrogen phosphate and agar-agar dissolved in 450 ml of distilled water. Agar-agar was melted separately in 500 ml of distilled water and was mixed with the above solution. The volume was made up to 950 ml. Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were sterilized at 1.1 kg/cm^2 pressure for 15 min and subsequently mixed together.

5. Malt extract agar

Malt Extract	: 20.00 g
Agar - agar	: 20.00 g
Distilled water	: 1000 ml (volume to make up)

All the ingredients were dissolved in 400 ml distilled water and agar was dissolved separately in 500 ml of distilled water and mixed with the above solution and the volume was made up to one L. The medium was sterilized at 1.1-kg/cm^2 pressure for 15 min.

6. Oat meal agar

Oat meal powder	: 40.00 g
Agar - agar	: 20.00 g
Distilled water	: 1000 ml (volume to make up)

The oat meal powder was dissolved in 500 ml of distilled water and Agar Agar was melted in 500 ml of distilled water separately. Both the solutions were mixed thoroughly. Then volume was made up to one liter and sterilized.

7. **Asthana and Hawker's medium**

Glucose (C ₆ H ₁₂ O ₆)	: 5.0 g
Potassium nitrate (KNO ₃)	: 3.5 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	: 1.75 g
Magnesium sulphate (MgSO ₄ . 7H ₂ O)	: 0.75 g
Agar-agar	: 15.0 g
Distilled water (to makeup)	: 1000 ml

Agar-agar was melted in 400 ml of distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to 1000 ml by adding distilled water. Sterilized at 1.1 kg/cm² pressure for 15 min.

8. **Czapek's agar**

Sucrose	: 30.00 g
Sodium nitrate (NaNO ₃)	: 2.00 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	: 1.00 g
Magnesium sulphate (MgSo ₄ 7H ₂ O)	: 0.50 g
Ferric chloride (FeCl ₃ 6H ₂ O)	: 0.01 g
Potassium chloride (Kcl)	: 0.50 g
Agar - agar	: 20.00 g
Distilled water	: 1000 ml (volume to make up)

Agar - agar was melted in 500 ml distilled water. The other ingredients were dissolved in remaining 500 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one liter. The medium was sterilized at 1.1 kg/cm² pressure for 15 min.

9. Sabouraud's agar

Dextrose	: 40.0 g
Neo peptone	: 10.0 g
Agar agar	: 20.0 g
Distilled water	: 1000 ml.

All the ingredients were dissolved one by one in 400ml distilled water and agar was dissolved separately in 500ml distilled water and mixed with the above solution and the volume was made to one liter before sterilization.

10. Host stem extract agar

Stem	: 200.0 g
Agar-agar	: 20.00 g
Distilled water	: 1000 ml

Pigeonpea stem were boiled for 10-15 min in 400 ml distilled water, the extract was squeezed and filtered through muslin cloth and collected in a beaker and remaining ingredients were added to same and volume was made to one liter before sterilization.

11. Host stem Dextrose extract agar

Stem	: 200.0 g
Dextrose	: 20.00 g
Agar-agar	: 20.00 g
Distilled water	: 1000 ml

Pigeonpea stem were boiled for 10-15 min in 400 ml distilled water, the extract was squeezed and filtered through muslin cloth and collected in a beaker and remaining ingredients were added to same and volume was made to one liter before sterilization.

12. Host leaf extract agar

Leaves	: 200.0g
Agar-agar	: 20.00 g
Distilled water	: 1000 ml

Pigeonpea fresh leaves were boiled for 10-15 min in 400 ml distilled water, the extract was squeezed and filtered through muslin cloth and collected in a beaker and remaining ingredients were added to same and volume was made to one liter before sterilization.

13. Host leaf Dextrose extract agar

Leaves	:	200.0 g
Dextrose	:	20.00 g
Agar-agar	:	20.00 g
Distilled water	:	1000 ml

Pigeonpea fresh leaves were boiled for 10-15 min in 400 ml distilled water, the extract was squeezed and filtered through muslin cloth and collected in a beaker and remaining ingredients were added to same and volume was made to one liter before sterilization.

14. Water agar

10 g of Agar Agar dissolved in distill water and volume was made up to 1000 ml with distilled water and sterilized at 1.1 kg/cm pressure for 15 min.

APPENDIX – II

Monthly meteorological data during the research period (2013) recorded at Agricultural Research Station, Gulbarga

Month	Temperature (°C)		Relative humidity (%)		Rainfall (mm)
	Max	Min	Morning	Afternoon	
January	31.39	22.90	65.75	52.08	0.00
February	32.38	24.02	64.68	56.73	31.40
March	36.58	27.58	49.95	42.65	0.00
April	39.71	27.38	50.06	39.19	44.40
May	40.85	24.31	54.30	33.40	59.40
June	32.69	20.72	78.91	61.76	108.60
July	28.34	19.84	86.06	76.84	174.40
August	30.31	19.65	83.98	67.15	61.00
September	30.38	19.47	85.12	73.27	174.40
October	30.98	19.13	80.86	70.44	27.40
November	30.18	13.68	66.41	57.07	16.60
December	29.61	9.87	65.42	46.70	0.00
Total	32.78	20.71	69.29	56.44	697.60

**BIOLOGY AND MANAGEMENT OF MACROPHOMINA STEM CANKER OF
PIGEONPEA CAUSED BY *Macrophomina phaseolina* (Tassi) Goid.**

CHIKKANNA SWAMY

2014

M. K. NAIK
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ABSTRACT

The incidence of *Macrophomina* Stem canker (MSC) of pigeonpea was high during maturity stage of the crop. The stem canker incidence ranged from 0.33 to 77.33 per cent and dry root rot incidence ranged from 0.00 to 41.33 per cent in four districts of North Eastern Karnataka. The typical symptoms first appeared as spindle shaped lesion surrounded by pycnidial bodies on stem, splitting of the lesions and also the lesions coalesced leading to drying of branches and finally wilting of plants. The fungus was identified as *Macrophomina phaseolina* based morphological and cultural characteristics and was confirmed by molecular amplification of ITS rDNA region and its subsequent sequencing blasting in NCBI BLAST programmed bioinformatic tool, Maryland, USA. Maximum growth of the fungus was obtained on 9th day incubation and PDA was best among the solid media and maximum dry mycelial weight was observed in Richard's solution. Most of isolates showed fast growth, few with moderate and one was in slow growth. Majority of the fifty two isolates showed distinct variability with regard to mycelial characters and morphological features of sclerotia and microsclerotia. The isolate MP-GB-25 produced highest microsclerotia (7.3/10x) where as MP-GB-36 produced highest sclerotia of 136/plate. Twenty two isolates were selected for sequencing based on geographical representation and cultural and morphological features. Phylogenetic analysis of 22 isolates framed two clusters (I and II) without much diversity but MP-RC-10 showed wide divergence. Interestingly, the isolate was obtained from Karigudda village of Raichur district with lower incidence (0.33 %) of stem canker. The interconnectivity studies revealed no perfect connectivity between sclerotia and pycnidia but pycnidia had a major role for spreading disease on stems under field condition. None of the variety showed resistance, TS-3R was highly susceptible. Captan among non-systemic fungicides, carbendazim among systemic fungicides and *Trichoderma harzianum* among the bio-agent were effective under *in-vitro* selected for integrated management under field condition. Seed treatment with *T. harzianum*+ *T. harzianum* enrichment with FYM + foliar spray of carbendazim showed least incidence (9.87 %) and highest yield (1509 kg/ha).