STUDIES ON *Macrophomina phaseolina* (Tassi) Goid CAUSING CHARCOAL ROT OF SOYBEAN [*Glycine max* (L.) Merrill] AND ITS MANAGEMENT

Ph.D. THESIS

by

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DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
INDIRA GANDHI KRISHI VISHWAVIDYALAYA
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STUDIES ON *Macrophomina phaseolina* (Tassi) Goid CAUSING CHARCOAL ROT OF SOYBEAN [*Glycine max* (L.) Merrill] AND ITS MANAGEMENT

Thesis

Submitted to the

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by

Pankaj Kumar Mishra

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

Doctor of Philosophy

In

Agriculture

(Plant Pathology)

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2017

CERTIFICATE - I

This is to certify that the thesis entitled "**Studies on *Macrophomina phaseolina* (Tassi) Goid causing charcoal rot of soybean [*Glycine max* (L.) Merrill] and its management**" submitted in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** of the Indira Gandhi Krishi Vishwavidyalaya, Raipur, is a record of the bonafide research work carried out by **Pankaj Kumar Mishra** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.

No part of the thesis has been submitted for any other degree or diploma or has been published/published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

Date: 5/5/2011

Chairman
(Advisory committee)

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

Chairman: (Dr. R.K. Dantre) ____________

Member: (Dr. G.K. Awadhiya) ____________

Member: (Dr. H.C. Nanda) ____________

Member: (Dr. R.R. Saxena) ____________

Member: (Head of the Department) ____________

CERTIFICATE - II

This is to certify that the thesis entitled "**Studies on *Macrophomina phaseolina* (Tassi) Goid causing charcoal rot of soybean [*Glycine max* (L.) Merrill] and its management**" submitted by **Pankaj Kumar Mishra** to the Indira Gandhi Krishi Vishwavidyalaya, Raipur, in partial fulfilment of the requirements for the degree of "**Doctor of Philosophy**" in the **Department of Plant Pathology** has been approved by the external examiner and Student's Advisory Committee after oral examination.

Date: 5.7.2017

External Examiner

(Name: M.N. KHARE **)**

Major Advisor ____________________

Head of the Department ____________________

Faculty Dean ____________________

Approved/Not approved

Director of Instructions ____________________

ACKNOWLEDGEMENT

Research is an evolving concept to understand the natures of nature. It implies the testing of nerves. It brings to light our patience, understanding and dedication. My work in the same spirit is just a step in the ladder. It is a drop in an ocean.

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Place: Raipur

Dated: 05/05/2017

Pankaj Mishra

Pankaj Kumar Mishra

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LIST OF ABBREVIATIONS

Abbreviations	Descriptions
%	Percent
°C	Degree Celcius
mm	Mili meter
viz.	Namely
CD	Critical difference
SEm±	Standerd error of means
cm	Centimeter
DAI	Days After Inoculation
et al.	And other
Fig.	Figure
ha	Hectare
i.e.	That is
m ha	Million hectare
Mt	Metric tone
No.	Number
L	Litre
BOD	Biological oxygen demand
ppm	Part per million
Kg	Kilogram
gm	Gram
/	Per, also means and or
T	Treatment
Ml	Millilitre
cv.	Cultivar
S.N.	Serial number
Trt.	Treatments
SC	Soluble concentration
WP	Wettable powder
EC	Emulsifible concentrate
@	At the rate
V	Variety

THESIS ABSTRACT

a)	Title of the Thesis	:	Studies on *Macrophomina phaseolina* (Tassi) Goid causing charcoal rot of soybean *(Glycine max* (L.) Merrill) and its management
b)	Full Name of the Student	:	Pankaj Kumar Mishra
c)	Major Subject	:	Plant Pathology
d)	Name and Address of the Major Advisor	:	Dr. R. K. Dantre, Professor Department of Plant Pathology
e)	Degree to be Awarded	:	Doctor of Philosophy in Agriculture (Plant Pathology)

Pankaj Mishra
Signature of the Student

Signature of Major Advisor

Date: 5/5/2017

Signature of Head of the Department

ABSTRACT

The charcoal rot disease of soybean caused by *Macrophomina phaseolina*, has emerged as a serious problem in most of the soybean growing area of India, leading to considerable yield loss in soybean (*Glycine max* L.). The intensive survey of major soybean growing area of Chhattisgarh recorded 42.63 percent disease incidence during *kharif* 2015 and 19.12 percent during *kharif* 2016. Symptoms of charcoal rot appeared on all the parts of soybean such as seedlings, adult plant, stem and root portion. Fifteen isolates of *M. phaseolina* belongs to Chhattisgarh, were characterized for cultural and morphological characteristics. The isolates showed varied pattern of mycelial growth rate; fast, medium and slow growing. Based on mycelial growth pattern, isolates were divided in to fluffy, partial submerged, submerged, and irregular, whereas colony colour; dark black, greyish and light greyish in nature. Sclerotia size of *M. phaseolina*, varied from

(71.5-102.4 x 62.4-85.7 μm). Based on the number of sclerotia per microscopic field, highest number of sclerotia per microscopic field was noted in MP4 (171.94) and least found in MP5 (75.33).

All the isolates of *M. phaseolina* were found to be pathogenic under artificially inoculated condition. Aggressiveness study of *M. phaseolina* under *in vitro* blotter paper technique and cut stem inoculation technique revealed that, isolates were variable in their aggressiveness. It was observed that isolate MP14 showed highly aggressive behavior.

Diagnosis of healthy looking soybean plant showed microsclerotia of *M. phaseolina.* The infection of *Macrophomina phaseolina* took place at early stage of plant growth, but did not show any symptom of charcoal rot and found hidden.

Screening of soybean germplasm and entries under natural field condition identified moderately resistant, moderately susceptible, susceptible and highly susceptible, that could be utilized for resistant breeding programme for development of resistance and tolerant varieties.

Management of charcoal rot under *in vitro* and *in vivo* condition. Under *in vitro* condition antagonist *Trichoderma viride* was found to be effective against *Macrophomina phaseolina.* Among the botanicals, neem oil recorded the maximum inhibition of mycelial growth followed by garlic (58.50%), neem oil + castor oil + karanj oil (29.93%) and karanj oil (17.03%). Efficacy of seven different fungicides was tested against virulent isolates of *Macrophomina phaseolina.* At 100 ppm concentration complete inhibition of *M. phaseolina* was observed with tebuconazole, hexaconazole, propiconazole and carbendazim+mancozeb and carbendazim, whereas complete inhibition of mycelium was observed with all the fungicides at 200 ppm.

In vivo sick pot method was conducted to test the performance of highly aggressive MP14 against five soybean varieties CG Soya-1, JS 97-52, JS 93-05, JS 335 and RSC 10-46. All the varieties were found to be susceptible against MP14. To test the efficacy of different biocontrol agents under glass house condition, minimum disease incidence 18.33 percent was found when seed was treated with *T. viride* followed by *P. fluorescens* 23.33 percent and *B. japonicum* 36.67 percent.

Field level management of charcoal rot using organic soil amendments, fungicides and biocontrol agents were evaluated. The percent disease incidence was low 10.60 percent, when the neem cake was used as organic amendment followed by karanj cake 15.60 percent, castor cake 16.00 percent, linseed cake 18.67 percent, FYM 19.82 percent and mustard cake 21.60 percent. Neem cake recorded highest yield (2166 kg/ha) followed by castor cake (1956 kg/ha), karanj cake (1937 kg/ha), mustard cake (1893 kg/ha) linseed cake (1883 kg/ha) and 1797kg/ha in FYM. Use of botanicals was found effective in disease management. The percent disease incidence was low 10.67 percent, when the neem oil used as seed treatment for disease management followed by neem oil + castor oil + karanj oil 13.13 percent, garlic extract 14.00 percent, karanj oil 16.00 percent, castor oil 18.33 percent and ginger extract 21.13 percent over control 23.13 percent. The highest yield (2165 kg/ha) was recorded in the case of neem oil. Seed treatment with systemic fungicides carbendazim + mancozeb, carboxin + Thiram and carbendazim resulted in significant reduction in the disease incidence 7.33 percent, 8.66 percent and 9.00 percent respectively. The highest yield (2552 kg/ha) was recorded in seed treatment with carbendazim + mancozeb. Biocontrol agent *T. viride*, *P. fluorescens* and *B. japonicum* used for seed treatment against charcoal rot of soybean. Disease incidence was reduced when seeds were treated with *T. viride* (7.09%) followed by *P. fluorescens* (8.70%). *T. viride* treated seed plots recorded highest yield (2274 kg/ha), followed by *P. fluorescens* (2253kg/ha). Foliar spray of systemic fungicides was also found effective for disease management. Minimum incidence of charcoal rot (14.67%) was observed, when foliar spray of carboxin + thiram was done. This was followed by hexaconazole (17.33%), carbendazim + mancozeb (18.33%), tebuconazole (19.00%) and trifloxystrobin + tebuconazole (19.17%). With regards to yield the highest yield (2100 kg/ha) was recorded in case of trifloxystrobin+ tebuconazole and lowest yield was recorded in tebuconazole (1925kg/ha).

The meteorological parameters played significant role on progress of disease charcoal rot. Disease incidence appeared to be high when low rainfall, maximum temperature and low relative humidity prevail during reproductive growth phase of soybean. Combined study of both year data showed that the prolonged dry period during *kharif* 2015 induce the disease (45%) compared to *kharif* 2016 (15%).

शोध ग्रंथ सारांश

शोध ग्रंथ का शीर्षक	:	*मैक्रोफोमिना फैसिओलिना* (टस्सी) गोइड पर अध्ययन जो कि सोयाबीन का चारकोल सड़न है (*ग्लाइसिन मैक्स* एल) मेरिल और इसके प्रबंधन
छात्र का पूर्ण नाम	:	पंकज कुमार मिश्रा
मुख्य विषय	:	पादप रोग विज्ञान
मुख्य सलाहकार का नाम और पता	:	डॉ. आर. के. दान्त्रे, प्राध्यापक पादप रोग विभाग, कृषि महाविद्यालय, रायपुर
प्रदाय की जाने वाली उपाधि	:	पी. एच. डी. पादप रोग विज्ञान

Pankaj Mishra

छात्र के हस्ताक्षर

मुख्य सलाहकार के हस्ताक्षर

विभागाध्यक्ष के हस्ताक्षर

दिनांक 5/5/2017

सारांश

मैक्रोफोमिना फैसिओलिना की वजह से सोयाबीन की लकड़ी का कोयला सडांध रोग भारत के अधिकांश सोयाबीन के बढ़ते क्षेत्र में एक गंभीर समस्या के रूप में उभरी है, जिसमें सोयाबीन (*ग्लाइसिन मैक्स* एल.) में काफी उपज का नुक्सान होता है। छत्तीसगढ़ के प्रमुख सोयाबीन क्षेत्र का गहन सर्वेक्षण के दौरान रोग का प्रतिशत खरीफ 2015 में 42.63 थी और खरीफ 2016 में 19.12 थी। कोयला सड़नें के लक्षण सोयाबीन के सभी हिस्सों में दिखाई देते हैं जैसे की पौध, वयस्क पौधे, तना और जड़ भाग पर माक्रोस्क्लेरोशिया और भूरे रंग का काला विसर्जन की उपस्थिति लकड़ी का कोयला सडांध के लक्षण दिखता था। *एम. फैसिओलिना* के पंद्रह विलगों छत्तीसगढ़ के विभिन्न भाग से लिया गया था सस्य क्रिया

lc/kh vkdkfjdh e fo"k'krk FkkA foyxk u dodh; of) d fofHkUu :i fn[kk,] dodh; of) :i d vk/kkj ij vyx&vyx jk;nkj] vkf"kdk tyeXu- vkj vfu;fer e foHkkftr fd;k x;k] tcdh dkykuh jx] idfr e v/kj dkyh] Hkjk vkj gYdk Hkjk jx dk FkkA LDyjkfI;k dk ,e- Qf l vkfyuk d foyxk d LDyjkfI;k dk ifj.kke ¼71-5&102-4 l 62-4&85-7 ekbØku½ tcfd vkdkj xky vkj vMkdkj FkkA ifr bdkbZ {k= e LDyjkfI;k dh l[;k d vk/kkj ij ifr l{e {k= ifr LDyjkfI;k dh lcl cMh l[;k ,e- ih- 4 ¼171-94½ e nt dh xbZ Fkh vkj de l de ,e ih 5 ¼77-33½ e ik;k x;k FkkA eØkQkfeuk Qf l vkfyuk d lHkh foxyk df=e #i l fuo"ku yxku okyh fLFkfr d rgr jkxtud ik, x,A bu foVk CykVj iij rduhd d rgr ,e- Qf l vkfyuk dk vkØed v/;;u vkj dV LVe bukD;y"ku rduhd l irk pyrk g fd foyxk mud vkØkedrk e FkA ;g ik;k x;k fd ,e- ih- 14 d vyx&vyx vkØked 0;ogkj fn[kkA

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CHAPTER-I

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] designated as miracle bean established its potential as an industrially vital and viable oilseed crop in many areas of India. It is the cheapest source of vegetable oil and protein. It contains about 40 percent protein, well balanced in essential amino acids, 20 percent oil rich with poly unsaturated fatty acid specially omega 6 and Omega 3 fatty acids, 6-7 percent total mineral, 5-6 percent crude fiber and 17-19 percent carbohydrates (Chauhan and Opena,1988).

Soybean is a world's first rank crop as a source of vegetable oil. Global area and production of soybean is 120.81 million hectare and 338 million tons respectively. In oilseed scenario of India it occupies first place and it is cultivated in area of 10.50 m ha, with production potential of 11.50 million tons. The major soybean producing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh, Karnataka and Chhattisgarh. In Chhattisgarh, soybean occupies 134.00 thousand ha area with a yield of 9.75 qt ha^{-1} (Anon., 2016). The major soybean growing districts are Rajnandgaon, Durg, Mungeli, Bemetara, and Kawardha (Anon., 2015)

Soybean crop can be attacked by more than 100 pathogens (Sinclair and Shurtleff, 1975). The major economically important diseases of soybean are rust, wilt, leaf spots, rots, web blight, powdery mildew, bacterial and viral diseases. Among the different soil borne pathogen which infect soybean, *Macrophomina phaseolina* is an important fungus that causes charcoal rot having broad host range like soybean, common bean, mung bean, sorghum, maize, cotton, peanut, sesame, cowpea, chickpea and cluster bean producing the symptoms of dry root rot, dry weather wilt, ashy stem blight and seedling blight (Su *et al*., 2001).

Macrophomina phaseolina is capable of infecting soybean at any crop growth stage, but usually, it infects at post flowering stage. The disease cycle of *M. phaseolinia* begins with germination of microsclerotia when temperatures are between 28 °C and 32 °C. Germinated microsclerotia produce germ tubes that develop hyphopodia which, in turn, either penetrate plant epidermal cell walls or through natural openings (Bressano *et al.*, 2010; Dhingra and Sinclair 1978). During the early stages of infection, hyphae are restricted to intercellular spaces of the root cortex, but subsequently, the fungus uses both mechanical pressure and chemical softening (Ammon *et al.*, 1974) to facilitate its intracellular colonization of xylem in vascular tissues (Wyllie, 1988). This fungus provokes disease development in plants by secreting one or more phytotoxins that facilitate host penetration, invasion, and colonization. Several phytotoxic metabolites produced by *M. phaseolina* have been identified in individual isolates (Bhattacharya *et al.* 1992). These phytotoxins include asperlin, isoasperlin, phomalactone, phaseolinic acid, phomenon, and phaseolinone (Dhar *et al.*, 1982; Mahato *et al.*, 1987). Phaseolinone is a non-host-specific toxin that causes wilting of seedlings and formation of necrotic lesions on leaves, similar to those incited by the pathogen (Bhattacharya *et al.,* 1987; Bilgrami and Jamalludin, 1979). In adult plants, the pathogen causes red to brown lesions on roots and stems, and produces dark mycelia and black microsclerotia. The stem shows longitudinal dark lesions and the plant becomes defoliated and wilted (Abawi and Pastor-Corrales, 1990).

The asexual structures formed by the fungus are pycnidia and microsclerotia. The black, 0.1–1.0 mm sized microsclerotia are formed in soil, infected seeds or host tissues constitute the primary inoculum source of the pathogen (Dhingra and Sinclair, 1978). Microsclerotia is the heat tolerant structure could withstand a temperature range of 60–65 ºC and could survive up to 15 years under different weather condition (Bega and Smith, 1962; Mihail and Alcorn, 1984).

In India, the charcoal rot, which is used to be a minor disease of soybean until 2004, became serious due to altered weather conditions particularly on the account of longer drought spells during crop growth period. There are report of few epiphytotics to occur in areas where temperature ranges from 35–40 °C during the crop season, disease can cause up to 80 percent yield losses. During the year 1997, charcoal rot caused substantial loss to plant stand and yield in soybean in Guna District of Madhya Pradesh State (Gupta and Chauhan, 2005). In Chhattisgadh, detailed survey of charcoal rot was conducted during *kharif* 2015 and the disease incidence recorded was 5.0 to 30.0 percent (Anon., 2015).

Macrophomina phaseolina exhibits variation in their morphological and pathogenic characters. Hence understanding the variability may be useful in devising novel management strategies. The disease can be managed to some extent by cultural, chemical and biological methods (Bristow and Wyllie, 1975; Gupta, 2004). To date, no charcoal rot-resistant variety of soybean is available. Development of any resistant variety, evaluation of soybean germplasm under natural condition is very essential for identification of resistance source. Information in this aspect is very limited and no report in the context of state Chhattisgarh.

A number of management approaches *viz.*, use of tolerant varieties, application of fungicides, cultural practices and combination of approaches leading to integrated management of the disease have been evaluated. Inspite of all these measures, charcoal rot continues to be one of the major constraints in soybean production. In addition, there are large variations among different genotypes and cultural practices in different regions. It is necessary to know the severity of the disease and factors associated with disease development, which will help in devising suitable and effective management practices feasible to each location, looking into the prevailing conditions.

Charcoal rot has become an important production constraint in the Chhattisgarh for last few years. The present investigations were, therefore, initiated on some of such neglected but important aspects of this disease and the pathogen. Epidemiological studies play an important role in developing prediction and forecasting models about disease progress in relation to incidence of disease. Host plant resistance is considered as most practical, feasible and an economical method of plant disease management.

Keeping in view, the severity and losses caused by dry root rot disease, present investigation is undertaken entitled "Studies on *Macrophomina phaseolina* (Tassi) Goid causing charcoal rot of soybean *(Glycine max* (L.) Merrill) and its management" with the following objectives:

1. To survey and collection of *Macrophomina phaseolina* isolates from Chhattisgarh and record the disease incidence of charcoal rot.
2. To study the morpho-cultural and pathogenic variability among the *Macrophomina phaseolina* isolates.
3. To identify the resistance source through the screening of soybean varieties/wild relatives against charcoal rot.
4. To evaluate different management practices against the charcoal rot of soybean.

CHAPTER – II

REVIEW OF LITERATURE

Soybean (*Glycine max*) is an important oil seed crop of India which is prone to several diseases. Among the fungal diseases, charcoal rot caused by fungus *Macrophomina phaseolina* (Tassi) Goid is a common, widespread, destructive and economically important disease in soybean, causing obstruction of xylem vessels and wilting leading to the yield loss, quality deterioration of seed and oil (Abawi and Pastor-Corrales, 1990). Various aspects related to pathogen and its management is reviewed below.

2.1Taxonomy and nomenclature

Macrophomina phaseolina (Tassi) Goid.(*Tiarosporella phaseolina* (Tassi) Van der Aa) is a soil borne plant pathogenic fungus. It belongs to the anamorphic Ascomycetes and is characterized by the production of both pycnidia and sclerotia in host tissues and culture media. The pycnidial state was initially named *Macrophoma phaseolina* by Tassi in 1901 and *Macrophoma phaseoli* by Maublanc in 1905. In 1927, Ashby maintained the name *Macrophomina phaseoli*, while Goidanich (1947) proposed *Macrophomina phaseolina. Tiarosporella phaseolina* (Tassi) Van der Aa was used in 1981 by Van der Aa to designate the species. The sclerotial state was described for the first time by Halsted as *Rhizoctonia bataticola* (Taub.) Butler on *Ipomoea batatas* in 1890.

Dhingra and Sinclair (1978) reported that the same fungus was isolated from cowpea in India in 1912 by Shaw and was then named *Sclerotium bataticola*. Recently Crous *et al.* (2006) demonstrated that although the telemorph is unknown, *M. phaseolina* is a member of the family *Botryo sphaeriaceae.* He pointed out the differences between *Tiarosporella* and *Macrophomina*, which produces in the pycnidia percurrently proliferating conidiogenous cells.

Short and Wyllie, 1978 identified the pycnidiospores which are ellipsoid to obovoid (16-) 20-24 (-32) × (6-) 7-9 (-11) µm. During the sclerotial formation, 50–200 individual hyphal cells aggregate to give multicellular bodies named microsclerotia. The size of microsclerotia are variable (50–150 µm), depending on the available nutrients of the substrate on which the propagules are produced.

2.2 Host Range and distribution

Macrophomina phaseolina is a pathogen of crops like soybean, common bean, mungbean, sorghum, maize, cotton, peanut, sesame, cowpea, chickpea and clusterbean (Diourte *et al.,* 1995). Softwood and other forest trees such as Abies, Pinus, Cassia (Lodha *et al.*1986; McCain and Scharpf 1989), fruit trees (*Citrus* spp., *Cocos nucifera*, *Coffea* spp., *Ziziphus mauritiana*, *Leucaena* spp.), medicinal plants and weed species (Lodha *et al*., 1986; Songa and Hillocks 1996) are also hosts. McGee (1991) reported the distribution of *Macrophomina phaseolina* throughout the continent in North and South America, Australia, Asia, Europe and African continents. In the USA, it usually occurs in Missouri, Mississippi, Alabama, Illinois and Indiana (Wyllie, 1988). In India, it commonly occurs in the states of Madhya Pradesh, Maharashtra, Rajasthan and Delhi (Gupta and Chauhan, 2005).

2.3 Disease cycle

M. phaseolina causes seedling blight, root rot and root and stem rot of more than 500 cultivated and wild plant species including economically important crops as soybean, common bean, sorghum, maize, cotton, peanut, cowpea (Dhingra and Sinclair, 1977; Hall, 1991 and Diourte *et al*., 1995).

M. phaseolina produces sclerotia in root and stem tissues of its hosts which enable it to survive adverse environmental conditions (Cook *et al*., 1973; Meyer *et al*., 1974; Short *et al*., 1980). In PDA, pycnidia are not produced except under some specific incubation conditions (Gaetan *et al*., 2006) and only sometimes in host crops (Mihail and Taylor, 1995) and their importance in the epidemiology of the fungus likely depends on the host involved as well as the fungal isolate

(Ahmed and Ahmed, 1969). On cowpea, pycnidia are produced at the end of the rainy season, but their epidemiological significance seems minor.

Microsclerotia in soil, infected seeds or host tissues serve as primary inoculum (Bouhot, 1968; Dhingra and Sinclair, 1977; Abawi and Pastor-Corrales, 1990). Root exudates induce germination of microsclerotia and root infection of hosts. The infective hyphae enter into the plant through root epidermal cells or wounds. During the initial stages of pathogenesis, the mycelium penetrates the root epidermis and is restricted primarily to the intercellular spaces of the cortex of the primary roots. As a result, adjacent cells collapse and heavily infected plantlets may die. At the onset of flower, the fungal hyphae grow intracellularly through the xylem and form microsclerotia that plug the vessels (Short *et al*., 1980; Mayek-Pérez *et al*., 2002) and disrupt host cells. The infected plants show necrotic lesions on stems, branches, and peduncles. From pod peduncles, the fungus spreads to the pods and invades grains. Heavily infected plants die prematurely due to the production of fungal toxins e.g. phaseolinone (Bhattacharya *et al*., 1994) and production of fungal tissue that plugs host vessels. In soybean, formation of microsclerotia is conditioned during flowering and pod setting (Wyllie and Cavert, 1969) and may be indicative of initiation of death of the host (Short and Wyllie, 1978). After plant death, colonization by mycelia and formation of sclerotia in host tissue continue until tissues are dry. The mycelium and microsclerotia produced in infected plant material, including plant residues are the means of propagation of the pathogen. Microsclerotia in soil, host root and stems are the main surviving propagules. After decay of root and plant debris, microsclerotia are released into the soil. They are distributed generally in clusters at the soil surface and are localized mainly at a depth of 0–20 cm (Alabouvette, 1976; Mihail, 1989; Campbell and Van der Gaag, 1993). They can survive for 2–15 years depending on environmental conditions and whether or not the sclerotia are associated with host residue (Cook *et al*., 1973; Papavizas, 1977; Short *et al*., 1980; Baird *et al*., 2003). Factors that adversely affect the survival of these propagules include repeated freezing and thawing of soil, low carbon: nitrogen ratios in soil and soil moisture content (Dhingra and Sinclair, 1974,1975).

2.4 Symptmatology

Macrophomina phaseolina causes a range of symptoms after a successful infection from restricted spindle-shaped lesions on the stem to extended lesions that result in the wilting of the plant. Deep and irregular necrotic lesions extending toward hypocotyls and root surfaces were observed in soybean (Ammon *et al.* 1974) chickpea (Singh and Mehrotra, 1982). As lesions coalesce they form larger patches on branches or entire plant, leading to premature senescence and plant death (Dhingra and Sinclair, 1978).

Seedlings can be infected in years when soils are exceptionally dry and soil temperatures are continuously above 35 °C for 2–3 weeks. After emergence, symptoms can be visible on cotyledons as brown to dark spots. Sometimes, the margins of the cotyledons become brown to black and shed at an early stage. From the unifoliate leaf stage onwards, the symptoms appear on emerging hypocotyls of infected seedlings as circular to oblong, reddish-brown, lesions that may turn dark brown to black after several days. These lesions may extend up the stem. Infected seedlings may die if hot and dry conditions persist.

The first above ground symptoms appear between 1 and 4 weeks before normal maturity. The pathogen causes lesions on the roots, stems, pods and seeds. From ground level upwards, superficial lesions, light brown to grey in colour, infrequently appear on the stem. Microsclerotia are formed in the vascular tissues and in the pith, giving a greyish-black appearance to the sub epidermal tissues of the stem. Such discolouration is first visible at nodes as profuse small, black, randomly distributed specks. A twin stem abnormality is usually observed in greenhouse infections (Bristow and Wyllie 1986).

Foliar symptoms progress from top of the plant downwards. Leaves of infected plants remain smaller than normal and subsequently turn yellow prior to wilting (Gupta and Chauhan 2005). A reddish-brown discolouration of the vascular elements of roots and lower stem precedes the premature yellowing as the fungus spreads up the stem during the season.

The infected mature and dry pods are covered with locally or widely distributed black bodies (microsclerotia).The fungus penetrates the pods and grains, inducing diverse symptoms. Diffuse black spots or blemishes appear on the seeds. Microsclerotia sometimes are produced in fissures and cracks in the seed coat.

After the death of the plant, numerous, minute, pinhead-sized microsclerotia appear, which can be seen readily when the epidermal tissue of the lower stems and roots is peeled from the affected parts. The infected crop in the field exhibits premature yellowing in scattered patches. Under severe disease conditions, the crop over a large area in the field may be affected. Normally, in the infected crop, the dead leaves remain attached to the petiole for several days after death.

2.5 Survey and Yield Loss

Charcoal rot was responsible for greater losses in soybean in comparison with other diseases from Central Mississippi and Alabamato Central Illinois and Indiana (Moore 1984). Wyllie (1988) estimated the average annual losses up to be 5% in Missouri (USA) with some growers experiencing 30–50% loss.

Almeida *et al.* (2001) correlate the disease incidence of charcoal rot caused by *Macrophomina phaseolina* on soybean with precipitation under conventional and no-till cropping systems.

Yang and Navi (2005) first reported the charcoal rot epidemics caused by *Macrophomina phaseolina* in Soybean in Iowa. It was observed that during 2003 growing season, a severe epidemic of charcoal rot was observed throughout the state. A systematic survey was conducted between late August and early September, 2003 to determine the prevalence and severity of charcoal rot in Iowa.

In India, epiphytotics of charcoal rot occur in areas where temperature ranges from 35–40°C during the crop season and the disease can cause up to 80% yield losses. During 1997 season, charcoal rot caused substantial loss to plant stand

and yield in soybean in Guna District of Madhya Pradesh State (Gupta and Chauhan 2005).

Anonymous (2015) conducted a survey of soybean growing area of Chhattisgarh *i.e*. Durg, Rajnandgaon, Mungeli, Bemetra, and Kawardha and observe the disease incidence of charcoal rot vary from 3-30%.

2.6 Factors affecting the infection and severity of charcoal rot disease

Gangopadhyay *et al.* (1970) reported the availability of *M. phaseolina* infection at the early seedling stage when grown in autoclaved soil. The stage of development at which a plant is most likely to exhibit symptoms of *M. phaseolina* infection varies to some extent with host species. Legume crops tend to be more susceptible at the seedling stage than cereal crops. In soybean, plant maturity is found to be the only factor affecting microsclerotial production, which is independent of moisture stress and temperature. Dhingra and Sinclair (1974) conducted experiment on viability of microsclerotia and found correlation with moisture and temperature. He concluded that reduction in the number of viable microsclerotia by keeping soil moisture above 60% of its moisture-holding capacity at 30°C or above for 3–4 weeks. Gupta and Gupta (1986) reported that in cowpea, *M. phaseolina* population in the soil was negatively correlated with soil moisture and positively correlated with maximum soil temperature.

Tosi and Zazzerini (1990) noticed that the disease index of charcoal rot disease was significantly low (2.0) when 14-day-old soybean cultivar Samsoy-1 seedlings inoculated with *M. phaseolina* were watered regularly to maintain a high (60–70%) soil moisture level. On the contrary, the disease index was high (5.0) when inoculated seedlings were water-stressed and grown under low (10–20%) soil moisture levels.

Diourte *et al.* (1995) found that the timing of host reproduction is another factor that has a strong influence on charcoal rot development and colonization of the pathogen was higher when plants were subjected to water stress and post flowering water stress resulted in greater intensity of charcoal rot.

Olaya and Abawi (1993) reported that high level of root infection can occur before reproductive development if there is a preponderance of hot and dry weather early in the growing season. Visible symptoms of the disease in the field are most apparent under conditions that reduce plant vigour, e.g., poor soil fertility, high seed rates (Sinclair and Backman 1989), low soil water content (Kendig *et al.,* 2000), high temperatures (Mihail, 1989) and root injury (Bowman *et al.,* 1986). The appearance of disease is also related to rainfall pattern but air temperature is the most critical factor.

Gupta and Chauhan (2005) reported the charcoal rot epiphytotics occur in India, areas where temperature ranges from 35–40°C during the crop season and the disease can cause up to 80% yield losses. During the 1997 season, charcoal rot caused substantial loss to plant stand and yield in soybean in Guna District of Madhya Pradesh State.

Kirkpatrick *et al.* (2006) reported the recovery of *M. phaseolina* from soybean significantly lower from plants flooded at the V4 growth stage when compared with the non flood treatment. Wrather *et al.* (2008) found that drought tolerance of the soybean genotypes and colonization by *M. phaseolina* were not related, and hence, they suggested that additional research is required to determine whether the effects of drought and infection by *M. phaseolina* are additive, synergistic or independent.

Wokocha (2000) under the humid tropical conditions of south-western Nigeria, high soil moisture levels were unfavourable for the growth and pathogenicity of *M. phaseolina*, while low soil moisture levels favoured these fungal traits.

Controlled environment experiments have shown that the maximum infection occurs in inoculated seedlings grown at 30-40 °C and temperature above 45°C reduced disease incidence (Meyer *et al.*, 1974). Management of water can limit, but not prevent the colonization of *M. phaseolina* (Kendig *et al.*, 2000). Dry conditions favour survival of microsclerotia in the soil, but mycelial growth and infection require moist conditions and are favoured by temperature above 27°C

(Hagedorn, 1991). Mihail (1989) observed a marked increase in mortality when soil temperature at 5-cm depth reached 28–30°C.

Mengistu *et al.* (2011) conducted experiment to measure the seasonal progress of charcoal rot (caused by *Macrophomina phaseolina*) over two growing seasons in four separate experiments: irrigated infested, irrigated non-infested, non-irrigated infested, and non-irrigated noninfested. Yield loss due to charcoal rot ranged from 6 to 33% in irrigated environments. Yield loss due to charcoal rot was consistently measured in all paired comparisons in irrigated environments, suggesting that charcoal rot can be an important disease in irrigated environments. Growth stage R7 was found to be the optimum stage for assessing disease using CFU. In addition, screening soybean genotypes under irrigation environment may have utility in breeding programs where there is a need for evaluating soybean genotypes for both disease resistance and yield.

2.7 Survival and recurrence

Ramakrishnan (1955) reported that the fungus does not exist in free soils, but inside vegetable debris, which it colonizes, and remains there either as mycelium, or in some resting stage. Short *et al*. (1980) studied the survival nature of microsclerotia. The fungus survives in soil and host crop debris generally through microsclerotia. After the harvest of the infected crop, the microsclerotia are protected in the fallen crop residues and are then released into the soil after crop residues break down. Microsclerotia which are distributed generally in clusters in the soil are confining mainly at a depth of 0–20 cm (Mihail, 1989) and persist within the soil up to3 years under adverse conditions such as low soil nutrient levels and temperatures above 30°C (Dhingra and Sinclair, 1977). Depending on environmental conditions and association of the microsclerotia with the host residue, microsclerotia can normally survive for 2–15 years, (Short *et al*., 1980; Baird *et al*., 2003).

The fungus is also known to survive for up to 3 years as mycelium in asymptomatic seeds or as microsclerotia in symptomatic seeds. Understanding the importance of soybean host debris, seed and soil as an inoculum source has special significance in disease management strategies (Hartman *et al.,* 1999).

Almeida *et al*. (2001) correlate the disease incidence of charcoal rot caused by *Macrophomina phaseolina* on soybean with precipitation under conventional and no-till cropping systems. In the 1997/ 98 and 2000/01 seasons, total precipitation between sowing and harvest reached 876.3 and 846.9 mm, respectively. For these seasons, disease incidence did not differ significantly between the no-till and conventional systems. In 1998/99 and 1999/00 precipitation totaled 689.9 and 478.3 mm, respectively. In 1998/99, in the no-till system, the disease incidence was 43.7% and 53.1% in the conventional system. In 1999/00 the final incidence was 68.7% and 81.2% for the no-till and conventional systems, respectively.

2.8 Diagnosis of *Macrophomina phaseolina*

Gangopadhyay *et al.* (1970) recovered the fungus from the radicles of a few seedlings. Seed infection of soybean with *M. phaseolina* at levels of 1.5–8.0% has been reported (Michail *et al.*, 1979). Kunwar *et al.* (1986) recovered the pathogen from all seeds showing symptoms of infection and also from 8 out of 1000 asymptomatic seeds harvested from naturally infected plants from Illinois. The fungus produced microsclerotia in 4% of symptomatic seeds after 2 day of incubation at 25 ± 2°C on acidified (pH 4.5) PDA. It was also observed that the microsclerotia developed near or adjacent to the seed coat, endosperm and hypodermis. The fungus, apparently, could penetrate and colonize soybean seeds without producing symptoms, but subsequently formed microsclerotia in asymptomatic seeds when conditions were favorable for seed germination.

Agarwal *et al.* (1972) found *Macrophomina phaseolina* to be associated with seeds of rice, wheat, blackgram, greengram and soybean when tested for seed-borne fungi. Kushi and Khare (1978) reported the seed-borne infection of *M. phaseolina* can be detected by blotter paper, agar plate and modified Potato-Sucrose-Agar [PSA + Penta Chloro Nitro Benzene (PCNB)] methods. He found that the blotter method was better than the agar plate method. The three incubation conditions, namely 20°C black light fluorescent tubes, 28°C near ultraviolet and 28°C artificial day light proved to be equally effective. Pretreatment with NaClO in the agar plate method reduced the percentage incidence of some pathogens including *M. phaseolina*.

Nicholson and Sinclair 1973, observed during experiment at Jabalpur, M.P., India, soybean seeds harvested from the first three planting dates in rainy season 1971 and stored in a refrigerator (1–5°C) had greater frequency of internally borne fungi including *M. phaseolina* and a lower germination than those stored at room temperature at 15–45°C. Infected seeds have indefinite black spots and blemishes on the seed coat and reduced germination (Gangopadhyay *et al*., 1970), and thus the pathogen can be carried on and in the seed coat and is capable of infecting the radicle (Dhingra and Sinclair 1978).

Gangopadhyay *et al*. (1973) observed *M. phaseolina* in cotyledons and seed coats from artificially infected seeds, which were plated separately on PDA following surface sterilization.

Kunwar *et al.* (1986) observed that microsclerotia were formed in the cotyledons of asymptomatic seeds after 3–4 day of incubation and in the hypocotyl radical axis, after 4–5 day. Kumar and Singh (2000) reported that the fungus was invariably present in the seed coat of all the infected seeds and moved into the cotyledons (including embryonal axis) of the 40% infected seeds. The pathogen remained viable for 15 months in seeds at room temperature and was transmitted to seedlings during germination by local contact. Histological examination of symptomatic seeds showed that hyphae and microsclerotia were ecto- and endophytic, while hyphae were inter- and intracellular in tissues of the seed coat, endosperm and embryo. During germination of the asymptomatic seeds, the inoculum could invade the cotyledons and embryo within 3–5 day, and microsclerotia developed on such seeds.

Babu *et al*. (2010) developed the species-specific primers and probes for the identification and detection of *M. phaseolina* at molecular level by exploiting rDNA gene cluster as a target. The conserved sequences of the ITS region was highly specific and yielded a specific 350 bp products. An oligonucleotide probe, MpKH1, has been designed at the ITS region, and it can identify the target sequences even at minute concentration. Babu *et al.*, (2010) were able to detect the Macrophomina *phaseolina* even from the soil DNA with the use of Real time PCR. The quantification of *M. phaseolina* from soil and plants was done by real-time PCR by `Taq Man and SYBR Green assay techniques.

2.9 Cultural, morphological and pathogenic variability

Much work has been done to elucidate the variability in morphology, physiology, pathogenicity, and genotype of *M. phaseolina*. Philip *et al.* (1969) noticed the differences in morphology of *Macrophomina phaseolina* isolates from various parts of host plants. They include the pycnidial development *in vitro* on mung bean roots infected with *Macrophomina*, as black, globose or depressed and 150-200 µm in diameter. Pycnidiospores were oval or elliptical, hyaline, non-septate, thin walled with 10-24 x 6-10 µm in size. The pycnidiospores develop into *R. bataticola* in culture at room temperature.

Jain *et al.* (1973) studied isolates of *R. bataticola* obtained from urd plant parts (root, stem, leaf, pod and seeds) and soil. The isolates from various plant parts and soil showed differences in virulence. The soil isolate was most pathogenic. The isolates differed in their growth pattern and sclerotial size. The leaf isolate developed the largest sclerotia and the seed and soil isolates developed the smallest sclerotia. When grown on different media the isolates varied in growth pattern and growth rate. The soil isolate showed the least amount of growth in almost all media.

Dhingra and Sinclair (1973) studied the variability in *R. batoticola*. Colour of cultures on PDA, ability to sporulate in infected host plants and pycnidial size also have been reported to vary greatly in *M.phaseolina*. Also noticed variation in growth rate among the isolates from same plant and also between isolates from different plants. Owing to the highly polykaryotic characteristics of the mycelium, *R. batoticola* (*M. phaseolina*) appears to be highly variable in nature.

Dhingra and Sinclair (1977) studied fifteen isolates of *M. phaseolina* from root, stem, petiole, pod and seed of soybean plants. They observed that sclerotia from root isolates ranged maximum from 122- 188 x 77-127 µm, while stem isolates range was minimum 84-100 x 60-71 µm.

Byadgi and Hegde (1985) obtained the isolates of *R. bataticola* from different plants, which differed in cultural characterestics, growth rate, morphology of sclerotia and virulence. Gliricidia (*Gliricidia muculata*) isolate produced biggest sclerotia 101.51 µm while cowpea isolate the smallest sclerotia 66.88 µm.

Jharia and Khare (1985) isolated *R. bataticola* from different hosts. The species of *R. bataticola* from different hosts differed in their morphological and cultural characters and even differences were observed in the isolates, isolated from various parts of the same host. Variation was recorded in their growth rate. The soil isolate was fast (90 mm) followed by root (89 mm) and seed (67 mm) isolates on potato dextrose agar medium. Maximum width of hyphae (4.5 µm) was found in the root isolate followed by soil isolate (3.5µm) and seed isolate (3.0 µm). The sclerotia were largest in the root isolate (84x93 µm) in comparison to soil isolate (90 x75 µm) and seed isolate (85x70 µm).

Pearson *et al*. 1987(a) compared the growth aptitude of more than 2000 isolates from 13 states in medium containing potassium chlorate. They classified isolates of *M. phaseolina* from maize as chlorate-resistant and those from soybean as chlorate sensitive. The growth of the last group was inhibited by chlorite produced in the medium. In contrast, Zazzerni and Tosi (1989) reported that *M. phaseolina* isolates from four host species varied widely in chlorate-utilization irrespective of their original host and concluded that there was no evidence for host specialization within *M. phaseolina*.

Raut and Ingle (1989) studied isolates of *R. bataticola* from fifteen host crops (mung, chickpea, guar, black gram, cowpeas, pigeon peas, soyabean, sorghum, safflower, sunflower, cotton, okra, sesamum, chilli and citrus. Pathogen varied in their growth rate in terms of sclerotial size, production of pycnidia and size of pycnidiospores. Differences were evident between isolates from crops belonging to the same family. On observation it was found that cotton and cowpea isolates formed comparatively more sclerotia. The sclerotia of cotton isolate were the largest (mean diameter 115.06 µm) and soybean isolate the smallest (56.33 µm). Cluster bean, pigeonpea and black gram isolates measured between 61 and 70 µm. Safflower, okra, green, chillies, sunflower and citrus isolates between 71 and 80 µm and cow pea sorghum, chickpea and sesamum isolates between 81 and 90 µm.

Than *et al.* (1991) conducted colony compatibility tests on thirty seven isolates of chickpea along with two isolates from different hosts, collected from several locations in Myanmar and reported the possible occurrence of more than one type of *R. bataticola* isolate in a field and the existence of closely related isolates at different places.

Bhattacharya *et al.* (1992) developed the microtiter plate-based enzyme immunoassay for phaseolinone, a phytotoxin isolated from the culture filtrate of the plant-pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid. The smallest amount of phaseolinone detectable by the method is 5 pg per well. The method is validated by comparison with high-performance liquid chromatography and used to confirm and estimate phaseolinone production in seeds infected with the fungus. The degree of seed germination inhibition correlated well with the amount of toxin produced in infected seeds, 50% inhibition being observed at a toxin concentration of 0.60 ug/g of wet tissue.

Monga and Sheo (1994) studied seven isolates of *R. bataticola* and observed that the isolates were distinct in cultural characteristics, sclerotial number and size. The size of sclerotial varied from 58.83 to 126.63 µm.

Mihail and Taylor (1995) studied the variability of 114 *M. phaseolina* isolates from different host species, soils and continents clearly indicated that *M. phaseolina* is a heterogeneous species that cannot be partitioned into distinct subspecies groups based on pathogenicity, pycnidium production and chlorate utilization. Su *et al.* (2001) also pointed out that the host specialization in *M. phaseolina* occurs with maize, no clear evidence for the occurrence of *formae* *speciales*, subspecies or physiological races has been reported so far. Various recent studies were devoted to the genetic and pathogenic variability of *M. phaseolina*.

Diourte *et al*. (1995) reported that isolates of *M. phaseolina* obtained from resistant sorghum genotypes were more pathogenic on susceptible sorghum than two other isolates originally obtained from susceptible sorghum genotypes.

Ratnoo *et al*. (1997) observed the large variation in *R. bataticola*, they differ in cultural, morphological and pathogenic characters. Studied two isolates, from which it was found that M1 isolate showed dark gray mycelium, fluffy growth, hyphae thin, sclerotia formation abundant, blackish globose to round and measure 49- 76 µm in diameter. M2 isolate was gray cottony and fast growth of mycelium, hyphae thin dark brown, round and measured 45-53 µm in diameter.

Mandal *et al.* (1998) studied the sclerotial morphology and pathological reaction of thirteen isolates of *M. phaseolina* obtained from jute growing regions of India. They found that sclerotial size ranged from 66.14 to 128.25 µm and the colour varied from light to dark brown. Sclerotial shape in most cases was irregular except for few which were round to elongate.

Prameela and Singh (1998) collected 56 isolates of *M. phaseolina* from root, stems, pod and seed of black gram and green gram from eleven different locations of India. Divided the fifty six isolates into six morphologically different groups.

Lokesha *et al*. (2004) evaluated cultural variability of isolates of *M. phaseolina*. The differences in the growth of four isolates, upon incubation exhibited in terms of the maximum mean mycelial weight i.e. 231.0 mg was recorded, on 18th day of incubation and the isolate from ICRISAT recorded lowest mycelial weight (190.9 mg) and rest remained significantly superior to other isolates. Potato Dextrose Agar (PDA) and Czapek‘s 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), followed by Bidar isolate (68.68).

Aghakhani and Dubey (2009) studied morphological and pathogenic variation among twenty three isolates of *R. bataticola,* collected from ten different major chickpea growing state of India. They found isolates were highly variable in their morphology. The majority of isolates (nineteen) produced suppressed mycelium and rest produced aerial mycelium. Cultural characters were found to varied in colour, as white to dull white creamy, gray and black. The sclerotia formed in different isolates were dark black to black and highly variable in size (40-600 µm).

Kanchan and Biswas (2009) evaluated morphological variability of *R.bataticola*, causal agent of leaf spot and blight disease of pigeon pea. The results revealed that the nature of mycelium varied from fluffy dark brown to partially fluffy mycelium colony with smooth margin.

Manjunatha and Naik (2011) studied the cultural and morphological diversity, in isolates of *R. bataticola,* causing dry root rot of chickpea. The results revealed that the isolates were highly variable. Based on cultural and morphological characters, the isolates were categorized into three groups. The first group comprised of all isolates from Bidar (RB7) which exhibited light brown with fluffy growth and formed dark brown sclerotia centrally with clustering growth pattern. The second group comprised of all isolates from Raichur and Gulbarga and also an isolate from Bidar (RB7) showed brownish black with flat sclerotia which were uniformly distributed. Third group comprised of only RG3 isolates from Gulbarga which showed flat colony and brown coloured sclerotia formed at the center of the culture.

Twizeyimana *et al.* (2012) deveoped the cut-stem inoculation technique for evaluation of soybean for resistance to *M. phaseolina*. They found that the cut stem inoculation technique, has several advantages over field tests, successfully distinguished differences in aggressiveness among *M. phaseolina* isolates and relative differences among soybean genotypes for resistance to *M. phaseolina* comparable with results of field tests.

Bellaloui *et al*. (2012) observed susceptible (S) or moderately resistant (MR) to charcoal rot, but the mechanism of resistance is not known. Significantly higher levels of phenolics, seed coat lignin, isoflavones, sugars, and total boron were observed in MR genotype than in (S) genotype. Charcoal rot disease in soybean is caused by the fungus *Macrophomina phaseolina*, which is believed to infect plants from soil through the roots by a toxin-mediated mechanism.

Mohan and Balabaskar (2012) conducted survey on the incidence of groundnut root rot disease in Cuddalore district of Tamil Nadu and assessed the cultural characters and pathogenicity of *Macrophomina phaseolina*. The isolates of *M. phaseolina* exhibited cultural and pathogenic variability among them. The isolate (MP18) with faster mycelial growth, maximum sclerotial production and maximum sclerotial size, recorded the maximum incidence of root rot disease under pot culture condition.

Gupta *et al.* (2012) evaluated diversity in isolates of *R. bataticola* causing dry root rot in chickpea collected from four major chickpea growing states of central India. The isolates were highly variable, in their morphological and cultural characteristics and were grouped into 24 groups, out of which six isolates produced highest number of sclerotia (18-23 µm) of bigger size (103.3 to 117.2 x 90.1-106.5µm). Whereas 23 isolates produced minimum number of sclerotia (8-13) that too with smaller size (72.7-87.5 x 57.1-73.5 µm). Based on sclerotial morphology, two groups of isolates could be formed, one with oblong shape having irregular edges and the other being round with regular edges. Culturally the isolates varied in terms of mycelial growth (submerged, partially submerged and fluffy), colour (dark black to greyish cottony) and size of colony (growth rate).

Sharma *et al*. (2012) made attempt to find out the diversity in *R. bataticola* populations in India, a total of ninety four isolates collected from *R. bataticola* infected chickpea plants from different agro climatic regions of India, and were analyzed for different morphological properties. *R. bataticola* isolates were found to be very diverse with respect to their cultural and morphological parameters like colony colour (light black to black and light grey to grey), growth pattern (radial to irregular), growth rate (fast and slow), mycelial characters (colour, low and high), sclerotial initiation time, sclerotial intensity (high, moderate and low) and morphology of the sclerotia (oblong, ellipsoid, irregular and round).

Varma and Pathe (2013) evaluated morphological and cultural variability of *R.bataticola* responsible for charcoal rot of soybean twenty two isolates of *R.bataticola* obtained from soybean from different places of Madhya Pradesh state showed considerable variability in cultural and morphological characters. Cultural studies of all twenty two isolates of *R. bataticola* revealed that isolates do not differ in the type of mycelial growth and growth rate on potato dextrose agar medium to be favorable for luxuriant growth for all the isolates. Morphological studies of different isolates of *R. bataticola* revealed variability in size of sclerotia (72.1-99.9x57.1-106.5 µm) and number of sclerotia (9.2-22.2 µm). On the basis of measurement, the sclerotia were categorized into three groups i.e small, medium and large.

Iqbal and Mukhtar (2014) studied the morphological and pathogenic variability among the 65 isolates of *Macrophomina phaseolina* from different agro ecological regions of Punjab and Khyber Pakhtunkhwa provinces of Pakistan. Characters taken for study were radial growth, sclerotial size, weight and pathogenicity. Sixteen isolates were 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 as medium sized. Ten fungal isolates appeared to be least virulent, whereas eight isolates of diverse origin proved to be highly virulent against mungbean cultivars. The remaining isolates were regarded as moderately virulent. No relationship was found among the morphological characters and pathogenicity of the isolates.

2.10 Charcoal rot management strategies

Most of the described control methods aim to reduce the number of sclerotia in soil or to minimize the contact of the inoculum and the host.

Solarization (Usmani and Ghaffar, 1982), addition of organic amendments, maintenance of high soil moisture content (Dhingra and Sinclair, 1975) and fumigation (Watanabe *et al*., 1970) have been suggested as possible methods to manage soil borne pathogens. Solarization alone was not effective for controlling *M. phaseolina* in forest and field (Mihail and Alcorn, 1984) soils.

Soil moisture content greatly affects the sensitivity of resting structures to heat treatment (Lodha *et al*., 2003), and one summer irrigation was sufficient to reduce the population of *M. phaseolina* by 25–42 % (Lodha and Solanki, 1992; Lodha, 1995). Amendments with nitrogen-enriched pearl millet residues significantly reduced the population of *M. phaseolina* within 45 days by 94% (Sharma *et al*., 1995). Combined effects of amendments, irrigation and polyethylene mulching resulted in almost complete eradication of the population of *M. phaseolina* (93–99 % reduction) at 0–30 cm soil depth within 15days. A considerable reduction (75–95 %) was also achieved by natural heating of irrigated soil for 15 days after amending with cruciferous residues (Lodha *et al*., 1997). Tillage is a crucial cultural measure that could affect the inoculum potential of soilborne pathogens. If the pathogen requires high inoculum density to infect plants, then increased dispersal over the soil profile could reduce disease severity. If, however, a low inoculum density is sufficient for infection, then dispersal may aggravate incidence and severity (Olanya and Campbell, 1988).

Tillage reduces the stratification of organic residue on the surface, which in turn can influence soil temperature and moisture (Campbell and Van der Gaag, 1993), soil chemistry (Blevins *et al*., 1980), population of soil animals, and the structure of microbial communities (Franchini *et al*., 2006). These changes in physical and biological factors may in turn also affect disease incidence and severity of *M. phaseolina*. Irrigation at any time during the cropping season reduces disease infection in soybean (Kendig *et al*., 2000). The density of soil sclerotia and the number of diseased melon plants was higher in drip irrigated plots than in furrow irrigated plots (Nischwitz *et al*., 2004).

2.10.1. Biological control

Management strategies to control charcoal rot also include the use of biocontrol agents to prevent host infection or to suppress the growth of the pathogen (Ghaffar *et al*., 1969; Siddiqui and Mahmood, 1993). In jasmine, *Trichoderma harzianum* and *T. viride* were effective against *M. phaseolina* (Alice *et al*., 1996). *T. harzianum* and *Pseudomonas fluorescens* significantly reduced the germination of sclerotia by 60 % in natural field soil (Srivastava *et al*., 1996).

Chakraborty and Purkayastha (1984b) reported the antagonistic property of *Rhizobium japonicum*. It reduced the severity of charcoal rot disease in soybean on account of the fungitoxic action of rhizobitoxine.

Arora *et al*. (2001) and Deshwall *et al*. (2003) reported that the strains of *Bradyrhizobium* sp. and *Rhizobium meliloti* were antagonistic against *M. phaseolina* and to have plant growth promoting properties in groundnut.

Adekunle *et al*. (2001) treated cowpea seeds with three *Trichoderma* spp. and planted in soils amended with *M. phaseolina.*They found significantly greater plant stands percentage than the controls. In tomato and eggplant *Saccharomyces cerevisiae* the biological control agent reduced disease percentage, improved root lengths and fresh and dry weights (Attyia and Youssry, 2001).

Hashem, 2004 worked on three antagonists (*Trichoderma harzianum*, *Epicoccum nigrum* and *Paecilomyces lilacinus*) significantly suppressed *M. phaseolina in vitro* and *in vivo* by producing an inhibition zone while *T. harzianum* suppressed them by overgrowing.

Seed treated with *T. harzianum* at sowing was effective to provide a considerable reduction of the disease caused by *M. phaseolina* in sesame (Pineda, 2001). Pan *et al.* (2001) screened the antagonistic properties of 4 *Gliocladium virens* strains against *M. phaseolina*. All the strains were highly antagonistic against *M. phaseolina*. Black gram seeds treated with *Trichoderma viride* significantly reduced the sclerotial germination of *M. phaseolina* (Rettinassababady *et al.*,2000). The incidence of root rot in blackgram was significantly reduced by 50 % when treated with *Trichoderma* spp. alone or in combination with biofertilizer both under glass house and field conditions. Soil application of talc based formulation of *T. harzianum*, *T. polysporum* and *T. viride* effectively controlled the root rot (*M. phaseolina*) of egg plant under field condition (Ramezani, 2008). Seed treatment with both *Trichoderma virens* and *Pseudomonas fluorescens* along with soil application supported the maximum plant stand and less root rot incidence in pigeon pea (Lokesha and Benagi, 2007).

Gupta and Chauhan (2005) recommended the soybean (4–5 g/kg seed) treatment with *T. viride* or *T. harzianum.* Singh *et al.* (2012) evaluated the antifungal potential of different strains of *Trichoderma* species against *Macrophomina phaseolina.* All strains of *Trichoderma* showed inhibitory effects on *M. phaseolina* and they were ranked according to the degree of inhibition. It was found that *T. harzianum* showed maximum inhibition of *M. phaseolina* at 40 % concentration of culture filtrate followed by other screened culture filtrates of *Trichoderma* strains.

Choudhary (2011) characterized plant growth-promotion activities of Rhizobacteria A5F and FPT721 and *Pseudomonas* sp. strain GRP3 for their antagonistic activities against *M. phaseolina*. Dubey *et al.* (2015) evaluated six isolates *Bradyrhizobium* sp. from *Vigna mungo* for their plant growth promoting (PGP) attributes and antifungal properties *in vitro*. All the isolates produced IAA but none of them produced HCN. The number of sclerotia producing less hyphae got increased with increasing the concentration of culture filtrate of strains VR1 than VR2. These results suggest that the presence of inhibitory properties in culture filtrates of *Bradyrhizobium* strains help to act as potential biocontrol agent for control of *M. phaseolina*.

Triveni *et al.* (2015) studied the influence of bio filmed formulations composed of *Trichoderma viride* and *Anabaena torulosa* against *Macrophomina phaseolina* (Tassi) Goid. infected cotton crop, in terms of plant growth and biocontrol parameters. Scanning electron microscopy revealed significant colonisation of biofilms on the root surface, which could be correlated with lowest mortality of 5.67%, recorded using *T. viride–B. subtilis* biofilm.

Manjunatha and Naik (2010) evaluated several isolates of two bio-control agents, *T. viride* and *P. fluorescens*. Bio-control agents were assessed for their ability to reduce the growth of *M. phaseolina* under laboratory conditions and subsequently used for field studies. The most effective isolate of each bio-control agent and the common chemical for seed treatment were too used as experimental trail. It was found that trial combining with soil application through bioagent enriched farm-yard manure, along with seed treated with the bio-control agent showed maximum germination, least root rot incidence and highest yield, as compared to the other biological or chemical seed treatments.

Govindappa *et al.* (2010) isolated three biocontrol agents such as *T. harizianum*, *P. fluorescens* and *Bacillus subtilis* from safflower rhizosphere soil and tested individually for their effectiveness in controlling root-rot of safflower. Talc based formulations were prepared and treated the seeds at different concentrations, for assessing their ability to induce plant growth and in turn control root rot disease. Among bioagents, *P. fluorescens* and *T. harzianum* (10 g/kg) proved to be effective in controlling disease under laboratory, greenhouse and field conditions. The efficacy of these biocontrol agents are equivalent to the standard fungicide Bavistin (carbendazim). Seed treatment with these biocontrol agents enhanced the seed germination and growth parameters against root-rot disease.

Jyothirmai *et al.* (2011) evaluated the plant growth promoting microorganisms viz., *Trichoderma* spp. and fluorescent pseudomonas which was isolated from the rhizosphere of groundnut, exhibited the suppression of *R. bataticola*. *Trichoderma* spp. and fluorescens Pseudomonas isolate 1 effectively restricted the radial growth of *R. bataticola* by 69.92 % and 67.31 % respectively.

Patel *et al.* (2011) tested *in vitro* antagonistic acitivity of *P. fluorescens* via dual culture which exhibited significant reduction in the radial growth of *R. bataticola* over control (90 mm colony diameter) and in the field trial study of chickpea seed treatment with four strains significantly reduced the disease incidence of dry root rot in all treatments in comparison to uninoculated control, however *P. fluorescence* strain BHUPf4 was found more effective in mycelial growth reduction as well as in disease reduction followed by strains BHUP5, BHUP6 and BHUPsb.

Govindappa *et al.* (2010) screened thirty eight isolates of *Pseudomonas fluorescens* for biological control of *M. phaseolina* root-rot of safflower. These results indicated that the seedling assay is more important for selection of promising biocontrol agents besides they also increased the plant growth by controlling the disease as evidenced by induction of defense enzymes.

Devadason and Subramanian (2012) evaluated the effects of biocontrol agents on *M. phaseolina* infecting *Gloriosa superba* a medicinal plant. Under *in vitro* conditions a commercial formulation of *T. viride* and *P. fluorescens* inhibited the mycelial growth of *M. phaseolina* isolates. Seven isolates of *M. phaseolina* were completely inhibited by the commercial formulation of *T. viride*. Inhibition by other isolates ranged from 22% to 83%. The other native isolates (1–10) were not effective against the seven isolates of *M. phaseolina*. Commercial formulation of the bacterial antagonist *P. fluorescens* was tested against *M. phaseolina* isolates under *in vitro* condition. Among the seven isolates of *M. phaseolina* 88.63 % inhibition was recorded over the control whereas, *P. fluorescens*, isolate on other hand inhibited 47.95% to 73.75%.

Doley and Jite (2012) tested biological efficacy of *T. viride* against soil borne plant pathogen *M. phaseolina* under *in vitro* condition. The dual culture technique was followed in which *T. viride* showed significant antifungal activity by inhibiting the mycelial radial growth of *M. phaseolina* by 71.42%. The results showed variable mycelial growth rate for all fungal isolates which was determined after 6 days of incubation.

Gejera *et al.* (2012) evaluated *in vitro* potentialities of seven species of *Trichoderma* against phytopathogen *Macrophomina phaseolina* by dual culture techniques. The maximum growth inhibition of test pathogen was observed by antagonist *T. koningi* MTCC 796 (T4) (74.3%) followed by *T. harzianum*

NABIITh 1 (T1) (61.4%) at 7 days after inoculation (DAI). Further, mycoparasitism by antagonists was observed up to 14 DAI. Pattern of growth and inhibition of test fungus was continued with maximum 14.7% increase in T4 (85.2%) followed by T1 (65.6%) antagonists during 7 to 14 DAI. Microscopic study showed that these two antagonists were capable of overgrowing and degrading *M. phaseolina* mycelia, coiling around the hyphae with appressoria and hook-likestructures. At 14 DAI, *T. koningi* MTCC 796 completely destroyed the host and sporulated. The growth inhibition of the pathogen during antagonism was positively correlated with coiling pattern of antagonists at 14 DAI.

Belkar and Gade (2013) isolated fifteen isolates of *Pseudomonas fluorescens* from acidic and alkaline rhizospheric soil from Vidarbha and Konkan region. All the cultural and biochemical studies confirmed them to be *P. fluorescens*. In vitro antagonistic study showed 67.04 per cent inhibition by *P. fluorescens* (Pf3) against *R. bataticola*.

Hegde *et al*. (2013) made an attempt for biological management of *Jatropha curcas*, root rot caused by *R. bataticola*. Efficacy of bio agent was evaluated. Among different bioagents evaluated against *R. bataticola*, *T. virens* (57.78%) was the most effective and at par with *T. harzianum* (57.23%), *T.viride* (55.83%) and *T. koningii* (54.45%) followed by *P. fluorescens* (30.11%).

Kumar *et al*. (2013) evaluated three bio agents, *T. harzianum*, *T. Viride* and *T. atroviride*, and one bacterium, *Bacillus subtilis* isolated from moth bean fields at Bikaner, were tested for their antagonistic activity against eight isolates of *M. phaseolina* causing dry root rot of moth bean. Inhibition of the mycelium growth of *M. phaseolina* isolates by *T. harzianum*, *T. viride*, *T. atroviride* and *B. subtilis* varied from 61.1 to 70.1%, 58.6 to 66.6%, 52.0 to 63.1% and 45.8 to 54.8%, respectively. Maximum growth inhibition was caused by *T. harzianum*. In a greenhouse study, these antagonists reduced root rot incidence in a susceptible moth bean cv. Rmo- 225 up to 69.0, 65.0, 62.5 and 58.3%, respectively, as compared to control where 100 % mortality was observed. *T. viride* was most effective among the antagonists tried in minimizing the disease followed by *T. harzianum*, *T. atroviride* and *B. subtilis*.

Ashwini *et al*. (2014) evaluated the efficacy of different antagonists viz. *Trichoderma viride* and *Pseudomonas fluorescens* against *M. phaseolina* of blackgram by dual culture technique. Among these bioagents, *Pseudomonas fluorescens* proved best antagonist against *M. phaseolina* (62.41%) by inhibition of mycelia growth whereas, lowest mycelial growth was observed against *T. viride* (44.94%).

Veena *et al*. (2014) studied the mode of parasitism between *Trichoderma viride* and *R. bataticola* under a microscope. Observed the formation of several loops and coiling around the hyphae of the pathogen, forming a thick compact rope like structure followed by rupturing, twisting and leakage of hyphal protoplasm, air bubbling inside the cytoplasm, breaking of cytoplasmic continuity, aggregation of cytoplasm within cell leading to severe vacuolation.

Nagamani *et al*. (2014) conducted a study for efficient management of dry root rot caused by *R. bataticola* in chickpea. The results revealed that seed treatment with carbendazim and *T. viride* along with soil application of FYM (FarmYard Manure) fortified with *T. viride* as the most effective in decreasing the mortality per cent.

Marisa *et al*. (2014) evaluated *Pseudomonas chlororaphis* subsp. *aurantiaca* for control of *M. phaseolina*. The bio agent caused a significant inhibition of *M. phaseolina in vitro* and reduced damping-off in the *in vivo* assays.

2.10.2. Botanical and organic amendements

The essential oil actinidine isolated from *Nepeta clarkei* was effective *in vitro* against *M. phaseolina* (Saxena and Mathela, 1996). Kazmi *et al.* (1995) and Alice *et al*. (1996) reported that in *in vitro* neem oil was more or equally effective compared to benomyl and carbendazim. However, neem seed extracted samples of different locations showed variable suppression of growth of the pathogen. More effective inhibition of the growth of *M. phaseolina* was obtained by aqueous extracts of *Cymbogon citratus* (Bankole and Adebanjo, 1995). Powder of *Datura fastulosa* (Datura) was also reported to be effective against *M. phaseolina* and *Meloidogyne javanica* infection in a pot experiment (Ehteshamul *et al*., 1992). The aqueous extracts of *Tephrosia candida* and *Boehmeria nivea* could well inhibit the formation of sclerotia of *M. phaseolina* (Anuradha *et al.* 2003). Extracts of pulverized bark of *Prosopis africana* and leaves of *Nauclea latifolia* 100 % inhibited both radial mycelial growth and sclerotial formation of *M. phaseolina* (Oluma *et al.* 2002). Datar (1999) found that aqueous extracts of *Polyalthia longifolia*, *Allium sativum* and *Parthenium hysterophorus* were found most effective in reducing mycelial growth of *M. phaseolina*. Neem leaf extract, Marigold leaf extract and Garlic bulb extract at 5 % as seed treatment significantly reduced the charcoal rot incidence and increased yield (Sinha and Sinha, 2004).

Some plants have shown to exert high antimicrobial ⁄ antifungal activities. This might be due to their chemical constituents which have higher water solubility and diffusion coefficient and also due to their higher hydrogen bonding potential (Bisht *et al*., 2010). The botanicals found as effective as chemical fungicides against *M. phaseolina* were as follows: (i) essential oil actinidine isolated from *Nepeta clarkei* (Saxena and Mathela 1996), (ii) neem oil (Alice *et al*., 1996), (iii) aqueous extract of *Cymbogon citratus* (Bankole and Adebanjo 1995), (vi) powder of *Datura fastulosa* (Ehteshamul- Haque *et al*., 1992) and (v) dry hot water extract of *Cleome viscoasa* and *Mentha longifolia* and dry methanol extract of *Berberis aristata*, *Conyza bonariensis*, *Cleome viscoasa*, *Lantana camera* and *Vitax negunda* (Arora and Kaushik 2003). Seed infection of *M. phaseolina* was completely inhibited by dipping the seeds for 5 min in ginger, garlic and neem extracts (Hossain *et al*., 1999).

Muthusamy and Mariappan (1992) observed that neem or gingelly cake at 3%, inactivated 90 and 80% microsclerotia from soybean, by stimulating germination and lysis as compared to the untreated control. They also observed maximum germ tube production and lysis in coconut cake extracts. Rathore (2000) conducted experiment to see the effect of organic amendments on incidence of seed rot and seedling blight of moth bean. They found that the Charcoal rot disease can be controlled by organic amendments such as farmyard manure, neem and mustard cake effectively. Lodha *et al.* (2002) observed that 63–72% reduction in *M .phaseolina* induced plant mortality at harvest in cluster bean by soil amendment with pearl millet compost.

Dubey and Kumar (2003) conducted experiment on the efficacy of neem oil and fungicides on growth and survival of sclerotia of *Macrophomina phaseolina* causing charcoal rot of soybean. He reported that all the sclerotia lost their viability after 96h of treatment with neem oil, mancozeb and bavistin as compared to control where 83% sclerotia germination was noted. Neem-based product, azadirachtin, possessed equally good fungicidal properties as mancozeb and bavistin.

2.10.3. Chemical control

Ilyas *et al.* (1976) observed when benomyl, thiophanatemethyl, thiram, thiobendazole, triforine and captan mixed with soil decreased the viability of microsclerotia in soil, and in soybean stem pieces in laboratory. In field studies with inoculated soil, fungicides did not affect emergence, but microsclerotia numbers were greatly reduced by benomyl and to a lesser extent by the other fungicides. Soil fumigation with sodium methyl dithiocarbamate reduced populations of the pathogen on soybean residue and in the roots of plants grown in field plots (Kittle and Gray 1982). Fumigation with methyl bromide significantly increased soybean yields and reduced the number of viable microsclerotia and prevalence of *M. phaseoli* (Watanabe *et al.*, 1970).

Seed treatment by fungicides is effective to some extent in reducing losses caused by *M. phaseolina* in crops, which are particularly vulnerable at the seedling stage. Vir *et al.* (1972) reported that, thiophanate-methyl and furcarbanil (each at 1000 ppm) provided the best degree of control against *M. phaseolina* on soybean. Seed treatment with carbendazim 50 WP (2.0 g ⁄ kg seed) and thiophanate-methyl (1.0 g⁄kg seed) was effective in eliminating the pathogen *M. phaseolina* from infected seeds of soybean (Kumar and Singh 2000). Gupta and Chauhan (2005) also recommended seed treatment with captan at 3 g⁄kg or thiram at 3 g⁄kg or thiram + carbendazim (2 : 1) at 3 g⁄kg or thiram+ carboxin at 2 g / kg seed. Seed treatment with captafol and mancozeb was effective in control of root rot and soybean seedling emergence (Singh *et al.*1990). Gibberellic acid (GA) and 2,3,5-triiodobenzoic acid (TIBA) reduced the severity of charcoal rot in soybean, while indole-3-acetic acid and kinetin gave inconsistent responses (Oswald and Wyllie 1973). The effect of TIBA was attributed to a change in the pattern of vessel elements that impeded fungal colonization and not to fungal toxicity (Kroll and Moore1981).

Kirkpatrick and Sinclair (1973) reported that growth rate of *M. phaseolina* (*Rhizoctonia bataticola*) on Potato Dextrose Agar medium containing 5, 10 or 50 μg/ml of fungicides BD 18654 and topsin M (thiophanate-methyl) was significantly less than the respective controls.

Chakraborty and Purkayastha (1984b) reported that foliar sprays of GA reduced and of kinetin increased the disease severity. *In vitro* tests showed that both the hormones increased the growth of the pathogen. Soybean grown in the growth chamber and in the field was treated with indole-3-acetic acid and kinetin, prior to inoculation with *M. phaseolina*. Either increased, did not alter, or decreased disease severity, depending on the applied concentration and growth conditions, while gibberellins and triiodobenzoic acid limited disease severity under all experimental conditions.

Dubey (1989) isolated *M. phaseolina* from diseased soybean nand cultured on Czapex Dox medium individually amended with1000g⁄ml of each tetracycline, ampicillin, grisofulvin and agrimycin 100, which inhibited fungal growth by 73.9, 50.9, 40.7 and 13.0%, respectively.

Dubey (1991) conducted the efficacy experiment of fungicides, herbicides and insecticides on survival of *Macrophomina*. Fungicides Bavistin 50 WP (carbendazim at 5000 μg/g), dithane M-45 75 WP (mancozeb at 10000 μg/g) and PCNB 75 WP (quintozene at 10000 μg/g) were effective in reducing the survival of *M. phaseolina* than the herbicides Fernoxone 80 WP (2,4-D), Benthiocarb 50 EC (thiobencarb) and Machete 50 EC (butachlor) or the insecticides namely BHC 50 WP (HCH), endocel 35 EC (endosulfan) and Nuvacron 36EC (monocrotophos), each at 10000 µg/g concentration. High pesticide concentration (5000–10000 µg/g) was required to kill the inoculum from pre colonized soybean stem pieces in soil. Using PCNB, Anahosur *et al*. (1983) reported 87% inhibition of *M. phaseolina* growth in vitro.

Kumar and Singh (2000) conducted experiments with different fungicides as a seed treatment. They found that carbendazim 50 WP (2.0 g/kg seed) and thiophanate-methyl (1.0 g/kg seed) were effective in eliminating the pathogen *M. phaseolina* from infected seeds of soybean. Seed treatment with captan at 3 g/kg or thiram at 3 g/kg or thiram + carbendazim (2:1) at 3 g/kg or thiram + carboxin at 2 g/kg (Gupta and Chauhan, 2005).

Dubey and Kumar (2003) tested the efficacy of azadirachtin, carbendazim and mancozeb against the viability of *Macrophomina phaseolina* sclerotia. At 30 ppm and above, the growth of the pathogen was completely inhibited by azadirachtin and carbendazim, whereas mancozeb inhibited the growth by 87.3%. A gradual decline in the viability of microsclerotia was recorded with an increase in incubation time. All the microsclerotia lost the viability after 96 h of treatment with azadirachtin, mancozeb and carbendazim.

Ammajamma *et al*. (2009) screened systemic and non- systemic fungicides against *R. bataticola*, among systemic fungicides hexaconazole and metalaxyl have given cent per cent inhibition. Thiram was found most effective among the contact fungicides and thiram + carboxin was also found effective.

Khodke and Raut (2010) observed that seed treatment with thiram (3g) + carbendazim (1g) + *Trichoderma* (4g/kg) has resulted in the least pre emergence mortality of soybean.

Sangeetha and Jahagirdar (2013) reported that for *R. bataticola* the comboproduct carbendazim + mancozeb gave maximum inhibition of mycelia growth (96.41%) which was at par with carboxin+thiram (94.53% inhibition) in the *in vitro* evaluation of combiproducts against *Rhizoctonia bataticola*.

Kumar *et al*. (2016) studied the efficacy of nanoform of a commercial fungicide Trifloxystrobin 25% + Tebuconazole 50% (75 WG) against *Macrophomina phaseolina* using various concentrations, namely 5, 10, 15, and 25 ppm using poisoned food technique. The fungicidal potential of nano form was better in comparison to the conventional ones. Nanoform of the fungicide was effective at 10 ppm and it exerted hyphal abnormality, hyphal lysis and abnormality of sclerotial formation on *M. phaseolina* when tested under *in vitro* than control.

2.11 Screening and host resistance

Gangopadhyay *et al.* (1973) reported that the soybean cultivar Hill was least susceptible and Harosoy most susceptible to the infection. Pearson *et al.* (1984) reported soybean cultivars Bay, Essex, Forrest and Sprite had the slowest rates of *M. phaseolina* colonization.

Gupta and Chauhan (2005) screen the soybean varieties inIndia against the charcoal rot of soybean. He found that soybean varieties, namely NRC 2, NRC 37, JS71 05, LSb 1 and MACS 13 with low susceptibility to charcoal rot, have been identified as either moderately resistant or tolerant under field conditions.

Mengistu *et al.* (2007) evaluated the soybean genoytpes and proposed a classification system based on a colony-forming unit index (CFUI) and identified four soybean genotypes out of 24 genotypes as moderately resistant to *M. phaseolina* in USA.

Wrather *et al*. (2006) also found variation in root tissue colonization among soybean genotypes. At the R6 growth stage, root tissue colonization was significantly lower for PI416937 (2640 cfu/g root) and N987288 (1525 cfu/g root) than Hutcheson (4000 cfu/g root).

Wani *et al.* (2010) reviewed the role of RNA interference (RNAi) in the development of disease-resistant varieties and further suggested that such goal can be achieved in a more selective and robust manner with the success of genetic engineering techniques. In this regard, RNAi has emerged as a powerful tool to overcome the threats posed by viruses, fungi and bacteria. The application of tissue-specific or inducible gene silencing in combination with the use of appropriate promoters to silence several genes simultaneously will result in the protection of crops against destructive pathogens. RNAi application has resulted in successful control of many economically important diseases in plants. This molecular technique can be utilized to protect soybean from charcoal rot.

Sources of resistance to some soil borne pathogens have been identified, but highly resistant cultivars are often not available for polyphagous and unspecialized pathogens like *M. phaseolina*. Pastor-Corrales and Abawi (1988) reported some selected bean lines with stable resistance to the fungus. Resistance in beans to *M. phaseolina* has been associated with drought tolerance (Pastor-Corrales and Abawi,1988). In common bean resistance to *M. phaseolina* is controlled by two dominant complementary genes (Olaya and Abawi, 1996). Grezes-Besset *et al*. (1996) reported resistance to *M. phaseolina* in *Ricinus persicus* and incorporated this in cultivated castor bean.

The improved lines showed high seedling resistance to the disease.Based on seed yield and the levels of lower stem and taproot colonization, Smith and Carvil (1997) identified four resistant cultivars among 24 soybean cultivars screened for resistance to *M. phaseolina*. Research on sources for resistance to *M. phaseolina* in sorghum has led to breeding lines and cultivars with stable performance. Resistance in sorghum was associated with delayed leaf senescence (Ducan, 1984; Diourte *et al*., 1995). The stability of this resistance, however, was influenced by water stress. In soybean, resistance factors to *M. phaseolina* do not protect plants against infection, but more likely restrict the growth rate of the fungus within plant tissues (Smith and Carvil, 1997). Reduced growth of the pathogen within host tissues may be due to lower levels of the stress related free amino acids proline and asparagine in resistant than in susceptible cultivars (Pearson *et al*., 1987b).

Mengistu *et al.* (2011) suggested growth stage R7 should be taken as the optimum stage for assessing disease using CFU to evaluate soybean genotypes.

Gupta *et al.* (2012) assessed one hundred seventy chickpea genotypes for locating new and better sources of resistance against dry root rot through blotter paper technique and in multiple disease sick fields at JNKVV, Jabalpur during 2007 to 2010. The susceptible cultivar BG 212 was used which exhibited 100 percent mortality *i.e.* rating 9 in 1-9 scale. Out of 170 accessions, sixty eight genotypes exhibited resistant reaction (<10% mortality), out of which twenty eight were the promising lines namely (JG 1-14, 2-125, 2-4-110, 14-11, 14-10, 2001-13,2001-13, 2001-18, 2001-80, 2001-115, 2002-20, 2003-95, 2003-14-16, 2004-110,210, 9605, 1-9, 99-115, 2001-04, 2003-14-2, JG 2000-07, JSC 37, MPJG 89-11551, MPJG 89-9023, CSJ 592 and Rajas) from JNKVV, Jabalpur. These lines further evaluated for their performance in sick field for three consecutive years and revealed six lines *viz*., JG 2000-07, JSC 37, MPJG 89-11551, MPJG 89-9023, CSJ592 and Rajas as resistant exhibiting <10 per cent mortality, however fourteen lines showed moderately resistance reaction. Resistant genotypes had white healthy root system with greater number of lateral roots. All other lines having >20% mortality showed necrotic root lesions leading to extensive root rotting.

CHAPTER – III
MATERIALS AND METHODS

The present studies entitled "Studies on *Macrophomina phaseolina* (Tassi) Goid causing charcoal rot of soybean (*Glycine max* (L.) Merrill) and its management" were carried out at the Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur, (C.G.). The materials used and methods / techniques followed during the course of there studies are narrated as under –

3.1 Materials

3.1.1 Glass wares and plastic wares

Whenever required, the glass wares of Borosil make, blotter paper of standard grade and chemicals of standard grade (Merck, Qualigens, S.D. fine etc.) were used during the course of investigation. All the glass wares, polythene bags, ethyl alcohol, formalin, chemicals and other materials were procured from the Department of Plant Pathology, College of Agriculture, I.G.K.V., Raipur (C.G.).

3.1.2 Equipment

The following equipments and/or materials were used in present investigation-

1. Autoclave for sterilization.
2. BOD incubator for incubation of the pathogen.
3. Compound and stereoscopic microscope for identification of the pathogen.
4. Hot air oven for glassware sterilization.
5. Laminar Air Flow for isolation, purification & inoculation of pathogen.
6. An amid Electronic Digital Balance for weighing.
7. Forceps, needles, blades, cork borer & inoculation needle.
8. Spirit lamp for sterilization.
9. Microwave oven for melting of media.

10. pH meter.
11. Desiccators for growth of pathogen.
12. Micro pipette
13. Funnel etc.

3.1.3 Experimental site

All the field experiments were conducted during *kharif* 2015 and 2016 at the soybean experimental field of Department of Plant Pathology situated in the research farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). Besides this field experiment, all the laboratory experiment were carried out at the Department of Plant Pathology, IGAU, Raipur.

3.1.4 Experimental designs

The designs used in all field experiments and laboratory experiments were done by Randomized Block Design (RBD) and Complete Randomized Block Design (CRD) respectively.

3.1.5 Source of seed and their utilization

Soybean seeds were procured from AICRP (All India Coordinated Research Project) on Soybean. IGKV, Raipur for studying the pathogenic variability and management against charcoal rot.

3.1.6 Chemicals

Analytical grade chemicals were procured from Department of Plant Pathology, College of Agriculture, I.G.K.V, Raipur (C.G.).

3.1.7 Cleaning and sterilization of materials

Before use, glass wares were cleaned with detergent powder and washed with tap and or distilled water as per requirement of the experiment. The dried glass wares were sterilized in hot air oven at 180 °C for 2 hours. The forceps, inoculation needle and other metallic instruments were sterilized by dipping them in alcohol and heating over the flame of spirit lamp before using. Sterilization of media was done by autoclaving at 1.21 Kg cm^{-2} pressure for 20 minutes. The plastic plates were surface sterilized with ethyl alcohol and air dried before use.

3.1.8 General procedure followed

For each set of treatment different replications were used in all *in vitro* studies. In general, in each Petri dish about 15-20 ml of potato dextrose agar medium was poured, supplemented with streptomycin in order to check the unwanted bacterial contamination. Wherever growth studies were conducted five mm disc of pure culture of *Macrophomina phaseolina* by the help of cork borer, was used for inoculation of medium in Petri dish. The inoculated plates were incubated in the 25°C for three days. Observations for the growth and sporulation were recorded at 10 to 15 days after inoculation.

Table 3.1 Composition of different media used in present investigation

Media	Composition	Quantities
Potato Dextrose	Potato (peeled and sliced)	200 g
Agar	Dextrose	20 g
	Agar-Agar	20 g
	Distilled water	1000 ml
	Potato (peeled and sliced)	200g
Potato Dextrose	Dextrose	20g
Broth	Distilled water	1000ml
	Agar-Agar	20g
Water Agar	Distilled water	1000ml

3.2 Survey and collection of diseased sample

An intensive survey was conducted during *kharif* 2015 and 2016 on the incidence of charcoal rot in soybean growing districts of Chhattisgarh. Soybean plants showing typical symptoms on stems bearing microsclerotia of *Macrophomina phaseolina* and characteristic symptoms of charcoal rot were collected from the infected plants from farmer’s field at different locations of Chhattisgarh (Rajnandgaon, Durg, Mungeli, Bemetra, Kabirdham and Raipur). Samples of 3–5 plants were sampled per field. Total 10-12 spots were selected randomly for taking root samples representing the whole field. Wherever required, the complete infected plants were also collected for isolation of the pathogen and other studies. The per cent disease incidence was calculated by using the following formula.

$$\text{Per cent} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

3.2.1 Preservation and transportation of specimens

The diseased samples were first packed in paper bags and then in 15×20-cm polyethylene bags, labeled, brought to the lab and stored at 4°C until processed for identification.

3.2.2 Isolation of pathogen *M. phaseolina*

For isolation of *M. phaseolina*, the samples were cut into small pieces (5-10 mm long), surface sterilized with 1 % sodium hypochlorite for 2 minutes and then rinsed twice in sterilized distilled water. The pieces were placed on Potato Dextrose Agar (PDA) medium containing antibiotics to avoid bacterial contamination. Inoculated petridishes were incubated in dark at 25 ± 1°C for 2-3 days. A small portion of the fastest growing colony of *M. phaseolina* was taken from the periphery of 90 mm diameter petri dish and spread on petridishes containing potato dextrose agar medium. A small portion of the colony having sclerotia was taken into a drop of sterilized water and agitated with a sterilized needle to separate the sclerotia from the mycelia. Sclerotia were then transferred to 90 mm dia. petridishes containing PDA medium. Colonies appearing from single sclerotium were again transferred to PDA medium containing petriplates, incubated and identified on the basis of standard key (Barnett and Hunter, 1972).

3.2.3 Identification

Colony colour of *Macrophomina phaseolina* varies in culture from black to brown or gray and becomes dark in color with age. Abundant aerial mycelium is produced in the culture plate with sclerotia imbedded within the hyphae or engrossed in the agar or on the agar surface with smooth precincts. Hyphae are septate, initially hyaline turning to a honey or black color. Numerous dark brown to black colored sclerotia can be seen on the reverse side of the culture plate. The vegetative mycelium is characterized by the formation of monilid or barrel-shaped cells and the formation of septum near the branching of the mycelium. Branching occurs at right angle to parent hyphae, but branching at acute angles is also common (Dhingra and Sinclair, 1977). Microsclerotia are formed from the aggregation of hyphae with 50 to 200 individual cells coupled by a melanin pigment. The microsclerotia of *M. phaseolina* are black in color and their size varies (50-150μm) with the host and the media used (Short and Wyllie, 1978). Isolates were given to name on the basis of genus and species name of *M. phaseolina* i.e. MP.

Table 3.2 Isolates of *Macrophomina phaseolina* collected from different locations of Chhattisgarh

S.No.	Name of District	Block	Name of the Isolates
1	Raipur	Dharsiwa	MP1
2	Bemetara	Ranka	MP2
3	Bemetara	Jaibra	MP3
4	Bemetara	Bemetra	MP4
5	Bemetara	Patharra	MP5
6	Bemetara	Saigona	MP6
7	Kawardha	Kawardha	MP7
8	Kawardha	Kawardha	MP8
9	Kawardha	Lohara	MP9
10	Kawardha	Lohara	MP10
11	Rajnandgaon	Gandai	MP11
12	Rajnandgaon	Khairagarh	MP12
13	Rajnandgaon	Salebharii	MP13
14	Durg	Dhamdha	MP14
15	Durg	Dhamdha	MP15

3.2.4 Storage of pure culture of *M. phaseolina*

The purified culture (5mm disc) from each isolate growing on PDA was transferred to 10 ml culture tubes and incubated in dark at 25±1°C for 6 days, until the surface of PDA was covered with dense sclerotial layer of the fungal culture. The culture tubes were labeled and stored at 4°C.

3.3 Mass multiplication of *M. phaseolina*

The isolates of the pathogen were multiplied on rice grain (Fig.3.1). Rice grain one hundred gram were mixed with 10 ml of water in 250 ml glass flask. Autoclaved at 121°C temperature and 15 lbs pressure for 20 minutes. Actively growing mycelia discs (9 mm) of *M. phaseolina* was inoculated into each flask under aseptic condition and the flasks were incubated at temperature 25±2°C for 30 days. Repeatedly shaking after every alternate day for proper mixing of inoculum on the surface of rice seed. For confirmation the colonized rice seeds were examined under stereoscope microscope for minute sclerotia (Iqbal, 2010).

3.4 Proving the pathogenicity

Pathogenicity of isolated fungus was tested using cut stem inoculation technique and *in vivo* sick pot method.

3.4.1 Cut stem inoculation technique

Artificial inoculation of the fungus was carried out to prove the pathogenicity by cut stem inoculation technique (Twizeyimana *et al.* 2012), using soybean variety JS 97-52. The stem apex of each six week- old V2-stage soybean plant was cut 25 cm above the unifoliolate node with a sharp razor blade. The open end of a 10- to 200-µl pipette tip (Fisher Scientific) was pushed into the margin of an actively growing *M. phaseolina* culture growing on potato dextrose agar, and a circular disc of fungal mycelium and agar was cut and removed. The pipette tip containing the agar disc with *M. phaseolina* mycelium was immediately placed over the cut stem and pushed down as far as possible in order to embed the stem into the medium and to secure the tip onto the stem. Three days after inoculation,

Fig. 3.1: Mass multiplication of inoculum of *M. phaseolina* on rice grains

A. **Flask containing rice grains before inoculation of *M. phaseolina***
B. **Flask containing rice grains after one month of inoculation**
C. **Microsclerotia observed on the individual rice grains**

the pipette tips were removed from each plant and discarded. Linear stem necrosis (in millimeters) and microsclerotia produced by *M. phaseolina* infection was observed.

3.4.2 *In vivo* sick pot method

The mass multiplication of *M. phaseolina* on rice seed was used for the pot experiments (Fig. 3.2). Seeds of variety JS 97-52 were disinfested by immersing them in 2.5% NaOCl for 5 min, rinsed in sterilized water to remove chemical residues and air dried. Soybean seed were sown in 2kg pots containing 1500 gm sterilized soil infested with *M. phaseolina* isolate @ 2 gm/kg soil. Pots without inoculum served as control. The pots were then placed in a glasshouse at 30 ± 2°C. Observations are recorded on the symptoms of germinated seedling.

3.5 Variability studies

3.5.1 Morpho-cultural variability

3.5.1.1 Radial growth

For studying variability in radial growth, the isolates were grown on Potato Dextrose Agar. Fifteen ml of autoclaved PDA was poured in 90 mm diameter petriplates and allowed to solidify. Five millimeter plug from the actively growing culture of each isolate of the fungus was placed in the centre of PDA plates separately and incubated at 25±1°C for five days. Each isolate was replicated five times. After stipulated period, the growth of each isolate was measured. On the basis of radial growth, the isolates were categorized as fast (full growth after 72hr), medium (full growth after 96hr) and slow (requires more than 96hr).

3.5.1.2 Colony colour

The colour of the colony was observed from the bottom side of the culture with the help of the Munsell' soil colour Chart (Munsell' Colour Company, Inc., 1954). The observations were taken on the mycelia growth after 10 days of incubation. Based on the colony pigmentation the cultures were assigned to different groups.

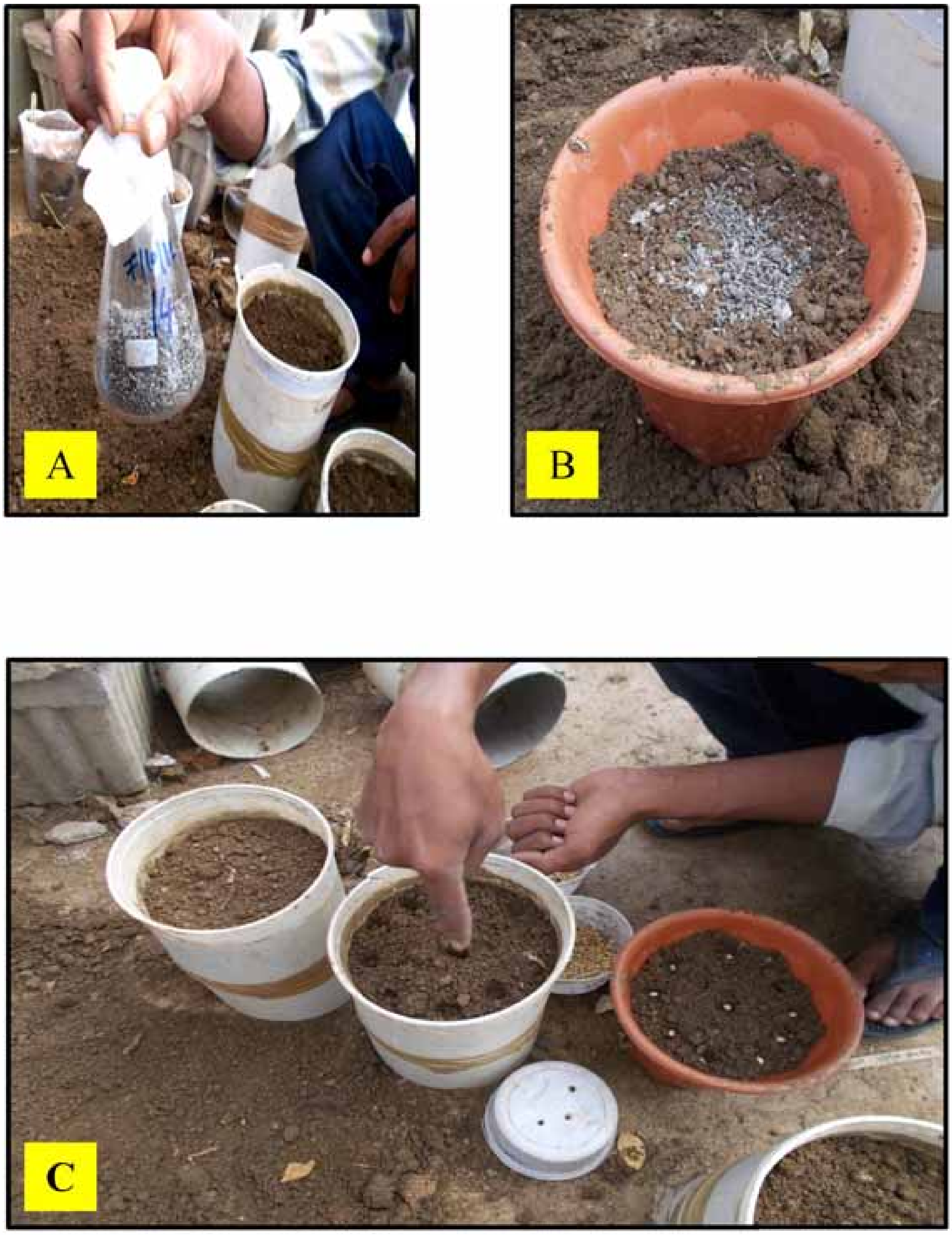

Fig. 3.2: Sick pot preparation using inoculum of *M. phaseolina* multiplied on rice grains

A. **Flask containing *M. phaseolina* infected rice grains**
B. **Pot containing sterilized soil added with rice grains having microsclerotia**
C. **Sowing of soybean seed in sick pot containing inoculums of *M. phaseolina***

3.5.1.3 Colony texture

Observations for the colony texture were made on the 7th day of growth when the colony growth is at its full. The isolates were designated to different groups based on the nature of the texture of their mycelial growth and appearance of the respective isolates.

3.5.1.4 Sclerotial characteristics

The slides of various isolates were prepared in lactophenol for morphological studies. For morphological characteristics *viz.,* hyphal width, size of sclerotia and shape of sclerotia were recorded after ten days of incubation (Dhingra and Sinclair, 1978). For measuring sclerotial size, slides from seven days old pure culture of *M. phaseolina* isolates were prepared and examined under microscope. Size of ten randomly selected sclerotia was measured using ocular micrometer and their means were found.

3.5.2 Pathogenic variability

3.5.2.1 *In vitro* Blotter paper technique (Nene *et al.,* 1981)

Five different varieties of soybean such as CG Soya-1, JS 97-52, JS 93-05, JS 335 and RSC-10-46 were used during experiment and screened against *M. phaseolina* by blotter paper technique (Nene *et al*., 1981). Seeds of all the five varieties were surface sterilized (5 min. in 2.5% sodium hypo chlorite) and sown on sterilized sand. Pure culture of all the isolates of *M. phaseolina* were obtained on potato dextrose agar medium from infected soybean plants collected during field survey. Culture of the fungus was multiplied on 100 ml potato dextrose broth in 250 ml flasks and incubated as stationary culture for 10 days at 25°C (Fig.3.3a A & B). Mycelial mats of the flasks were added in the sterilized water at the rate of two flacks in 100 ml, macerated for 5 min (Fig. 3.3a D). Fresh inoculum was used after every ten blotters each containing 10 seedlings. Seven days after sowing, the seedlings were uprooted and roots were carefully washed in running tap water and then rinsed in sterilized water. Roots of ten seedlings of each test accession were dipped in the inoculums Fig.3.3a E with an up and down movement for about ten seconds and placed side by side on a paper towel (Fig.3.3b A and B). Each paper

Fig. 3.3a: Preparation of *M. phaseolina* inoculum solution for blotter paper technique

A,& B. Multiplication of *Macrophomina phaseolina* on potato dextrose broth to prepare mycelium mat

C. Filtration of potato dextrose broth

D. Crushing of mycelium mat

E. Dipping of 7 days old soybean seedling in the inoculum solution

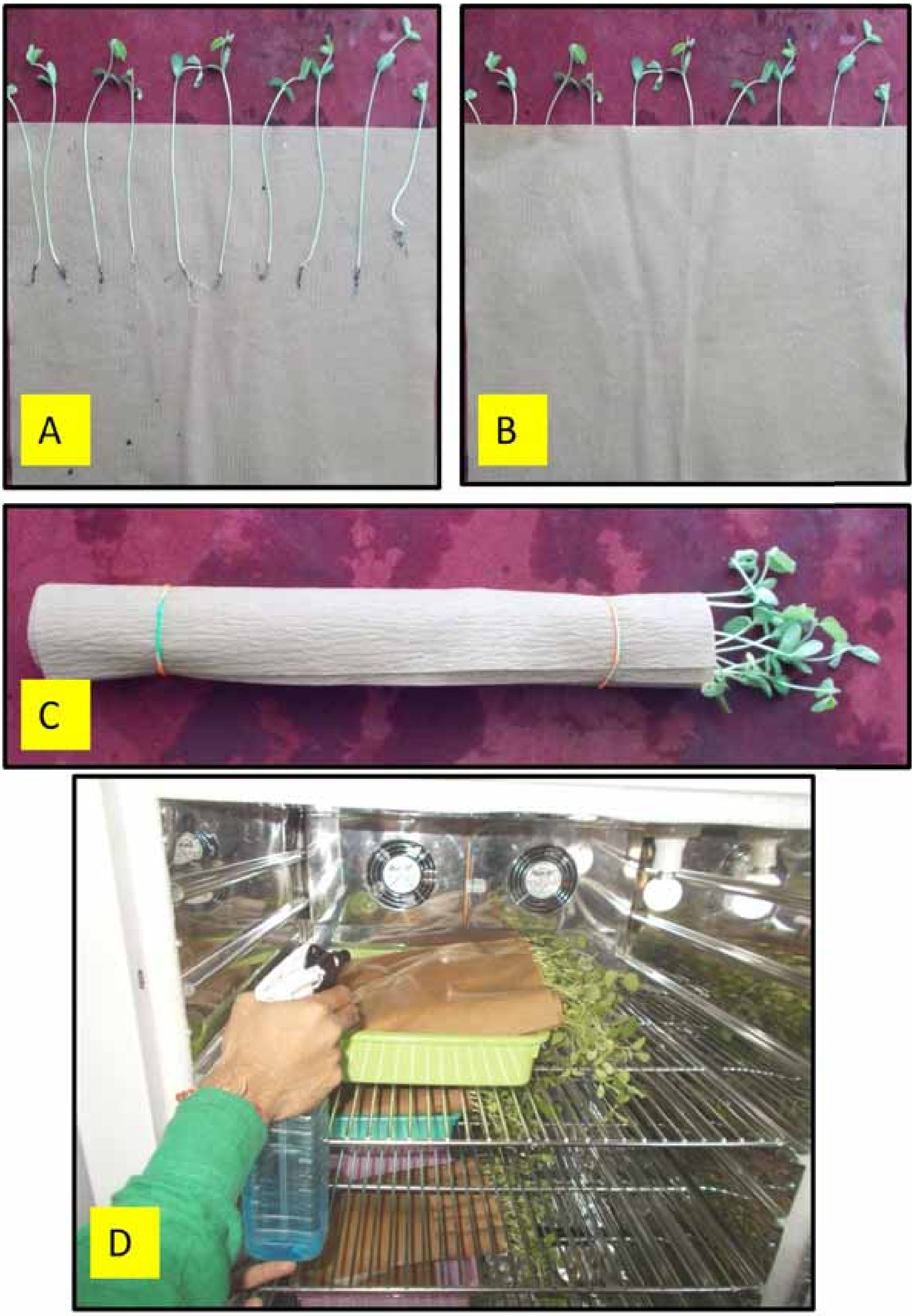

Fig. 3.3b: A –C. Rolling of suspension dipped seedling in the paper towel D. Incubation of rolled paper towel under controlled condition

towel was then folded, covering the roots and leaving the green tops outside (Fig. 3.3b C). Control plants were dipped in sterile, distilled water. Paper towels were then kept one over the other in heaps of 5 on a tray and placed in the incubator at 30±2ºC for 7 days with 12 h artificial light per day for better growth of pathogen (Fig.3.3b D). Paper towels were kept moist by adding sterile water as needed. Symptoms were observed on ten seedlings of each variety. Seedlings were examined for the extent root damage using a 0-9 rating scale (Nene *et al*., 1981).

Table 3.3 Description of the rating scale for scoring (Nene *et al.,* 1981)

Rating	Symptoms of Charcoal rot
1	No infection on roots
3	Very few small lesions on roots
5	Lesions on roots clear but small, new roots free from infection
7	Lesions on roots many, new roots generally free from lesions
9	Roots infected and completely discoloured

3.5.2.2 Cut stem inoculation technique (Twizeyimana *et al*., 2012)

Artificial inoculation of the fungus was carried out to test the aggressiveness by cut stem inoculation technique (Twizeyimana *et al.,* 2012). Adopted soybean varieties CG Soya-1, JS 97-52, JS-335 were used. The stem apex of each sixweek- old V2-stage soybean plant was cut 2.5cm above the unifoliolate node with a sharp sterilized razor blade (Fig.3.4 C). The open end of a 10- to 200-µl pipette tip (Fisher Scientific) was pushed into the margin of an actively growing *M. phaseolina* culture growing on potato dextrose agar, and a circular disc of fungal mycelium and agar was cut and removed (Fig. 3.4 B). The pipette tip containing the agar disc with *M. phaseolina* mycelium was immediately placed over the cut stem and pushed down as far as possible in order to embed the stem into the medium and to secure the tip onto the stem (Fig. 3.4 D). Three days after inoculation, the pipette tips were removed and discarded. Linear stem necrosis (in cm) caused by individual isolates of *M. phaseolina* on different variety was observed during study to test the aggressiveness (Fig.3.4 E).

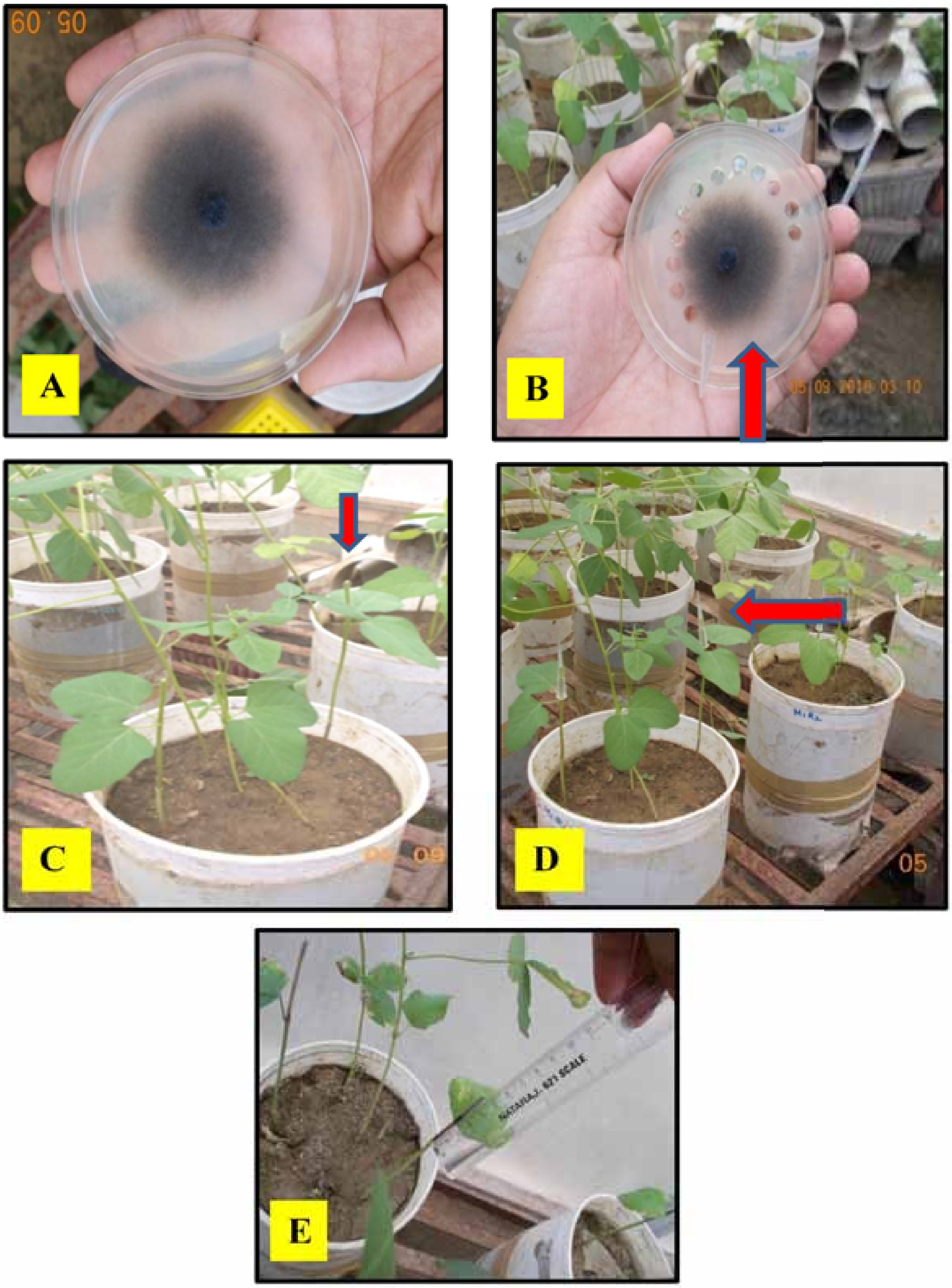

Fig. 3.4: Steps performed during cut stem inoculation technique

A. Pure culture of *M. phaseolinae*
B. Pipette tip loaded with fungal inoculum
C. Cutting of 45 days old healthy soybean plant, 2.5cm above unifoliolate node
D. Transfer of pipette to the cut portion of soybean plant
E. Measurement of necrosis lesion in cm

3.6 Diagnosis

Early diagnosis of charcoal rot is difficult. Infection of *M. phaseolina* occurs at the seedling stage, but the sign and symptom appear between 1 and 4 weeks before normal maturity. Microsclerotia is the primary source of inoculum, germinate on the root surface, germ tubes form appresoria that penetrate the host epidermal cell walls by mechanical pressure and enzymatic digestion or through natural openings.

From ground level upwards, superficial lesions, light brown to grey in colour, infrequently appear on the stem. Microsclerotia are formed in the vascular tissues and in the pith, giving a greyish-black appearance to the sub epidermal tissues of the stem. Leaves of infected plants remain smaller than normal and subsequently turn yellow prior to wilting (Gupta and Chauhan 2005).

Early diagnosis is important to manage this disease. The healthy looking soybean plants were collected from the field. Individual plant for the diagnosis was cut into pieces. Initially plants were washed in running tap water, after that root portion was separated from stem. Stem of soybean plant cut in to the small size of 15cm, surface sterilized with 1 % sodium hypochlorite for 2 minutes and then rinsed twice in sterilized distilled water. All root and stem pieces, were placed in desiccators for 72 hr. After that the plant parts were removed from desiccators and put in butter paper labeled and placed inside the hot air oven at 35°C for 48 hr. Later the samples were removed from the oven and examined using the stereoscopic binocular microscope for the microsclerotia and grey black colour on plant tissue (Fig.3.5).

3.7 Screening studies

3.7.1 Field Screening of soybean germplasm and entries

A field experiment was conducted to know the resistance levels in the promising varieties of soybean during *kharif* 2015 and 2016. The experiment was conducted with randomized block design with four replications with 30 cm spacing (3 rows of 3 m length). All the agronomic practices were followed except plant

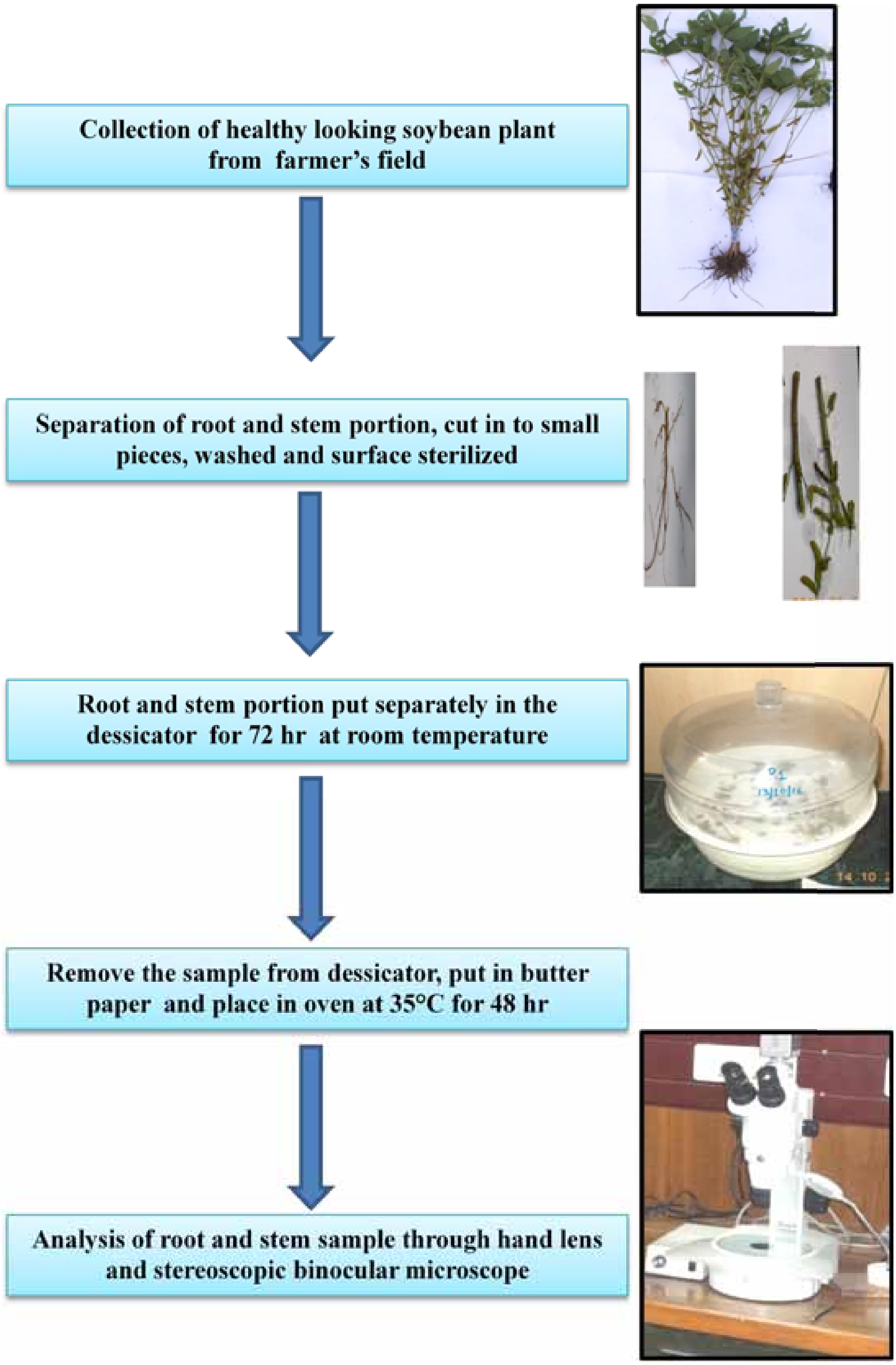

Fig. 3.5: Steps performed during diagnosis of soybean plant for disease charcoal rot

protection measures. The severity of charcoal rot was recorded at the R7 stage of the crop using a disease rating scale 0 to 9.

Table 3.4 Disease rating scale of Charcoal rot of soybean (Anon., 2012)

Charcoal rot mortality (%)	Category	Rating
0	Absolute resistant	0
1	Highly resistant	1
1.1 -10	Moderately resistant	3
10.1 – 25	Moderately susceptible	5
25.1 - 50	Susceptible	7
> 50	Highly susceptible	9

3.8 Management of charcoal rot

3.8.1 *In vitro* condition

3.8.1.1 *In vitro* evaluation of *T. viride* against *M. phaseolina* isolates

The experiment was conducted to study the efficacy of biocontrol agent *T. viride* against fifteen isolates of *M. phaseolina* pathogen. *Trichoderma viride* was used to test antagonistic performance in dual culture with a test pathogen *M. phaseolina.* The *Trichoderma viride* culture was obtained from Department of Plant Pathology I.G.K.V. Raipur, multiplied and maintained on PDA medium and kept in BOD incubator at 25°C. Twenty ml sterilized melted PDA medium was poured in 90 mm diameter petriplates. After solidification of the medium, 5mm disc of the antagonist and the test pathogen were separately cut with the help of a sharp sterilized cork borer from the edge of 4 days old culture. Plates without antagonist served as control. Three replications were maintained. The inoculated petriplates were incubated at 25°C. Observations were made on radial growth of antagonist and test pathogen in control plate. The observation was taken after 5 days of inoculation. The per cent growth inhibition of test pathogen in presence of antagonist was calculated over control as given below:

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

Whereas

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

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

3.8.1.2 In vitro evaluation of botanicals against *M. phaseolina* isolate

Plant based pesticides which are relatively economical, safe and non-hazardous can be used successfully against the plant pathogenic fungi. The following plant extracts/botanicals Table 3.5 were selected to know their efficacy in inhibition of *M. phaseolina* (as virulent one). Antifungal activity of different plant extracts were studied under *in-vitro* condition. The five medicinal plant species viz., neem, castor, karanj, garlic and ginger were used for antifungal activity.

Fresh plant materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized mixer & grinder by adding 100 ml sterile water (1:1 w/v). The extract was filtered through Whatman filter paper No.1, thereafter centrifuged at 8000-10000 rpm and filtered through bacteria-proof MF-Millipore membrane filters (thickness, 0.22 µm) under aseptic conditions. The filtrate was used as stock solution. five and ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of sterilized molten Potato Dextrose Agar (PDA) medium respectively, so as to get 5, 10 and 15 per cent concentrations, respectively.

Table 3.5 Botanicals used in the experiment

S. No.	Botanical name	Common name	Family
1	*Azadirachta indica*	Neem	Meliaceae
2	*Ricinus communis*	Castor	Euphorbiaceae
3	*Pongamia pinnata*	Karanj	Fabaceae
4	*Allium sativum*	Garlic	Liliaceae
5	*Zingiber officinale*	Ginger	Zingiberaceae

3.8.1.3 *In vitro* evaluation of fungicides against *M. phaseolina* isolate

Poisoned food technique was employed for the evaluation of fungicides in the laboratory**.** Seven fungicides viz. Teubeconazole, Hexaconazole, Propiconazole 25EC, Trifloxystrobin and Tebuconazole, Carbendazim + Mancozeb, Carboxin + Thiram, Carbendazim, were evaluated against *M. phaseolina.* Two concentrations i.e., 100 ppm and 200 ppm of each treatment were used. The required quantity of fungicide was mixed with PDA at the time of pouring. Three replications were maintained for each fungicide for each of its concentration in CRD. The medium was shaken well so as to enhance proper mixing of the fungicides. To avoid bacterial contamination a little amount of streptomycin was added in each flask before plating; five mm disc was cut with the help of sterilized cork borer from seven days old culture of the test fungus (isolate MP14) and was placed in the center of the medium in the reversed position to maintain continuous contact of the pathogen with poisoned medium. PDA plates without fungicide served as control. The radial growth of the colony was measured when the growth in control plates reached the rim of the Petri plates. Percent growth inhibition under the influence of different fungicides was calculated on the basis of the control. Observation was recorded from 3rd day onwards.

Percent inhibition of radial growth were calculated by the following formula,

$$Inhibition\ (\%) = \frac{C - T}{C} \times 100$$

Whereas

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

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

Table 3.6 Common name, Chemical name and trade name of fungicides used during experiment

S. No.	Common Name	Chemical Name	Trade Name
1	Tebuconazole 250 EC	(RS)-1-(4 Chlorophenyl)- 4,4-dimethyl-3-(1H, 1,2,4-triazol-1-ylmethyl)pentan- 3-ol	Folicur
2	Hexaconazole 5% SC	RS-2-(2, 4-D)-1-(1H-1, 2, 4 Trizole-1-yl) hezan 2-ol	Contaf
3	Propiconazole 25% EC	1-[[2-(2,4-dichlorophenyl)-4-	Tilt

		propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole	
4	Trifloxystrobin 50% + Tebuconazole 25% WG	Methyl(E)-alpha-methoxyimino-2-[(E) 1(3trifluoromethylphenyl) ethylidenaminooxymethyl+(RS)-1-(4Chlorophenyl)- 4,4-dimethyl-3-(1H,1,2,4-triazol-1-ylmethyl) pentan- 3-ol	Nativo
5	Carbendazim 12% + Mancozeb 63% WP	Methyl *1H*-benzimidazol-2-ylcarbamate+manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt	Saaf
6	Carboxin 37.5 % + Thiram 37.5% WP	6-methyl-N-phenyl-2,3-dihydro-1,4-oxathiine-5-carboxamid+ Dimethylcarbamothioylsulfanyl N,N-dimethylcarbamodithioate	Vitavax Power
7	Carbendazim 50% WP	Methyl 1H-benzimidazol-2-ylcarbamate	Bavistin

3.8.2 Under glass house condition

3.8.2.1 *In vivo* sick pot method to test the performance of *M. phaseolina*

The mass multiplication of virulent isolate of *M. phaseolina* on rice seed was used for the pot experiments. Five varieties of soybean *viz*., CG Soya-1, JS 97-52, JS- 93-05, JS 335 and RSC-10-46 were used during experiment and screened against *M. phaseolina*. Each variety was treated as treatment having four replications, pot without inoculum was consider as control. Before sowing of the crop seeds of all the five varieties were surface sterilized (5 min. in 2.5% sodium hypo chlorite) and the soil used for pot experiment was sterilized. The inoculum of. *M phaseolina* applied per pot was @2gm/kg of soil. After the sowing of soybean seed, pots were then placed in a glasshouse at 30 ± 2°C. Observation was recorded on germinated seedlings and survived seedlings.

3.8.2.2 *In vivo* sick pot method to test the efficacy of different biocontrol agents

Pot experiments (CRD) were carried out under glass house conditions. The plastic pots of 2kg capacity filled with sterilized sandy loam soil, and infested with one month old mass multiplied culture of *M. phaseolina* (MP14) (prepared on rice seed) @ 2g/kg soil. Seeds of soybean variety JS 97-52 were surface sterilized with 2 per cent sodium hypochlorite for three minutes and dried aseptically. In first treatment the seeds were coated with microbial antagonists *T. viride* by using 2 per cent sticker for 1 hour and 10 seed were planted per pot in four replications with control. In second treatment the seeds of soybean coated with biocontrol agent *P. fluorescence* by using 2% sticker for 1 hour and sown 10 seed per pot in four replications along with control. Third treatment was conducted by using the nitrogen fixing bacteria *Bradyrhizobium japonicum.* The soybean seed treated with *Bradyrhizobium* used for sowing, 10 seed per pot were sown maintaining four replications with control. In 4^{th} treatment, control soybean seeds grown in sick pot containing inoculum of *M. phaseolina* after that 10 seeds per pot were sown with four replications as control. Pots were kept randomly in a green house. The percent mortality was observed from 7 days to 30 days of sowing. Then plants were uprooted for measurement of root length and shoot length.

3.8.3 Under natural field conditions

3.8.3.1 Organic soil amendments against charcoal rot of soybean

Soil application of six different organic amendments at their recommended dose was used along with control on disease incidence and severity of charcoal rot. In field, organic amendment was applied in furrow before sowing of soybean crop. The field experiment was laid out in RBD with seven treatments and three replications during *kharif* 2015 and 2016 at Plant Pathology Research Farm, IGKV Raipur (C.G.). Seed of JS 97-52 variety was sown with 30cm × 10cm spacing in plots measuring 4m x3 m. All other cultural and pest control practices were followed as recommended in package of practices.

3.8.3.1.1 Observations

Data of the following parameters were recorded, total plant population, pre and post-emergence mortality caused by the disease, plant height (cm), branches/plant, pods/plant, chaffy pods/plant, percent disease incidence (PDI), percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight (seed index) and yield per plot kg/ha.

Table 3.7 The dose of organic soil amendments according to treatments

Treatments	Organic soil Amendments	Dosage (tone/ha)
T1	FYM	10
T2	Neem Cake	1
T3	Mustard Cake	1
T4	Karanj Cake	1
T5	Linseed Cake	1
T6	Castor Cake	1
T7	Control	_

3.8.3.2 Soybean seed treatment with botanicals

Seed treatment with six different botanicals were taken at their recommended dose along with control (without treated). The disease incidence and severity of charcoal rot were observed. After the seed treatment with different botanicals, shade drying of seeds was done for 30 minutes. The field experiment was laid out in RBD with seven treatments and three replications during *kharif* 2016 at Plant Pathology Research Farm, IGKV Raipur (C.G.). Seeds of JS 97-52 variety were sown with 30cm × 10cm spacing in plots measuring 4m x 3 m. All other cultural and pest control practices were followed as recommended in package of practices.

3.8.3.2.1 Observation

Data on the following parameters were recorded, total plant population, pre and post-emergence mortality caused by the disease, plant height (cm), branches/plant, pods/plant, chaffy pods/plant, percent disease incidence (PDI), percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight and yield per plot kg/ha.

Table 3.8 The doses of botanicals according to treatments

Treatments	Botanicals	Dosage/ kg seed
T1	Neem oil	5ml
T2	Castor oil	5ml
T3	Karanj Oil	5ml
T4	Garlic Extract	4g
T5	Zinger Extract	4g
T6	Neem oil+ Castor oil+ Karanj oil (1:1:1)	5ml
T7	Control	_

3.8.3.3 Soybean seed treatment with fungicides

Seed treatment with seven different fungicides was done at their recommended dose along with a control (without treatment) used on disease incidence and severity of charcoal rot. The field experiment was laid out in RBD with eight treatments and three replications during *kharif* 2016 at Plant Pathology Research Farm, IGKV Raipur (C.G.). Seeds of JS 97-52 varietiy were sown with (30cm × 10cm) spacing in plots measuring 3m x 4m. All other cultural and pest control practices were followed as recommended in package of practices.

3.8.3.3.1 Observations

Data on the following parameters were recorded, total plant population, pre and post-emergence mortality caused by the disease, plant height (cm), branches/plant, pods/plant, chaffy pods/plant, percent disease incidence (PDI), percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight (seed index) and yield per plot kg/ha.

Table 3.9 The doses of fungicides used for seed treatments

Treatments	Fungicides	Dosage/ kg seed
T1	Tebuconazole	0.5ml
T2	Hexaconazole	0.5ml
T3	Propiconazole	0.5ml
T4	Trifloxystrobin and Tebuconazole	1gm
T5	Carbendazim 12%+ Mancozeb 63%	3gm
T6	Carboxin 37.5% + Thiram 37.5%	3gm
T7	Carbendazim	2gm
T8	Control	-

3.8.3.4 Seed treatment with biocontrol agent

Seed treatment was done with different biocontrol agents viz., *Trichoderma viride, Psedomonas fluorescens* and *Bradyrhizobium japonicum*, commercial product of IGKV, Raipur. Before sowing of the crop seeds were treated with biological agent *Bradyrhizobium japonicum* culture @4gm/kg, *Pseudomonas fluorescens* @10gm/kg, *Trichoderma viride* 10gm/kg of soybean seed. Seed treatment with Carbendazim @2gm/kg is used as control. The field experiment was laid out in RBD with four treatments and five replications during *kharif* 2016 at Plant Pathology Research Farm, IGKV Raipur (C.G.). Seeds of JS 97-52 variety were sown with 30cm × 10cm spacing in plots measuring 4m x 3m. All other cultural and pest control practices were followed as recommended in package of practices.

3.8.3.4 .1 Observations

Data on the following parameters were recorded, total plant population, pre and post-emergence mortality caused by the disease, plant height (cm), branches/plant, pods/plant, chaffy pods/plant, percent disease incidence (PDI), percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight and yield per plot kg/ha.

Table 3.10 The doses of biocontrol agents according to treatments

Treatments	Fungicides	Dosage (g/kg seed)
T1	*T. viride*	10
T2	*P. fluorescens*	10
T3	*B. japonicum*	4
T4	Carbendazim	2

3.8.3.5 Foliar spray of systemic fungicides

Effect of different systemic fungicides on disease incidence and severity was evaluated against charcoal rot of soybean. The field experiment was laid out in RBD with seven treatments and three replications during *kharif* 2016 at Plant Pathology Research Farm, IGKV Raipur (C.G.). Seeds of JS 97-52 variety were sown with 30× 10cm^2 spacing in plots measuring 4m x 3m. All other cultural and pest control practices were followed as recommended package of practices. The schedule of spray was 30 days, 60 days and 90 days interval using Knapsack sprayer. For recording the observation, ten plants of each treatment were randomly selected and tagged to observe the disease severity.

3.8.3.5.1 Observations

Data on the following parameters were recorded, total plant population, pre and post-emergence mortality caused the disease, plant height (cm), branches/plant, pods/plant, chaffy pods/plant, percent disease incidence (PDI),

Table 3.11 The doses of fungicides according to treatments

Treatments	Fungicides	Dosage/ hactare
T1	Tebuconazole	400 ml
T2	Hexaconazole	500ml
T3	Propiconazole	500ml
T4	Trifloxystrobin and Tebuconazole	500gm
T5	Carbendazim 12%+ Mancozeb 63%	1000gm
T6	Carboxin 37.5% + Thiram 37.5%	1000gm
T7	Control	Water spray

percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight and yield per plot kg/ha.

3.9 Epidemiological study on progress of charcoal rot

Epidemiology encompasses the study of all the factors associated with the development of disease. The three major components of the disease i.e., susceptible host, virulent pathogen and favourable environment in time and space causes epidemic. A field experiment was conducted during *kharif* 2015 and 2016.

Observation of weather parameter such as maximum and minimum temperature, rainfall and relative humidity were recorded during *kharif* 2015 and 2016. Disease progress was recorded from the control plot of experiment without any application of treatment.

3.9.1 Progress of charcoal rot during crop season under natural conditions

The progress of charcoal rot during crop growth stages of soybean. Observations were recorded from the V5, R3, R5, R6, and R7 stage of crop. The experiment was conducted in control plots of experimental plot. Single variety JS 97-52 taken during study and normal agronomic practices were adopted to raise the crop except spraying of fungicides. The severity of charcoal rot was recorded using scale 0 to 9. The meteorological data for the experimental period was collected and correlated with charcoal rot disease incidence.

3.9.2 Correlation of charcoal rot incidence with weather parameters

To determine the influence of various physical factors of environment on the development of the charcoal rot, the disease incidence data were correlated with different meteorological parameters *viz*., effect of rainfall (RF), maximum temperature (Max.T), minimum temperature (Min.T) and morning relative humidity (RH) through analysis of correlation. The favourable weather condition for the disease development and progress of the disease was worked out. The data on various weather parameters were collected from the Department of Agrometeorology, College of Agriculture, IGKV, Raipur (table 3.12 & 3.13).

Table 3.12: Weekly meteorological observations during *kharif* 2015

Weeks	Max T(°C)	Min T (°C)	RF(mm)	RH (%)
Jul 22-28	31	25	23.7	80.30
Jun 29-5 Jul	33.2	24.4	8.0	76.45
Jul 6-12	32.9	25.6	8.4	77.35
Jul 13-19	33.4	25.8	2.5	77.65
Jul 20-26	30.1	25.1	6.5	85.15
Jul 27-2 Aug	32	25.7	0.7	72.40
Aug 3-9	29.2	24.3	6.7	85.25
Aug 10-16	32.3	25.3	15.9	82.70
Aug 17-23	31.9	25.6	7.1	78.60
Aug 24-30	31.6	25.3	7.7	83.90
Aug 31-6 Sep	32	25.8	1.1	79.25
Sep 7-13	33.5	25.5	1.4	76.45
Sep14-20	31.9	25.3	23.8	84.60
Sep 21-27	30.8	24.5	5.3	80.30
Sep 28-4 Oct	33.3	25.3	0	73.80
Oct 5-11	33.7	21.9	0	67.90
Oct 12-18	33.6	23.1	0	68.60
Oct 19-25	34.1	22.1	0	65.40

Table 3.13: Weekly meteorological observation during *kharif* 2016

Weeks	Max T(°C)	Min T (°C)	RF(mm)	RH (%)
Jul 22-28	35.8	26.1	5.9	70.55
Jun 29-5 Jul	32.9	26.0	6.7	79.50
Jul 6-12	28.8	24.5	16.9	90.85
Jul 13-19	30.4	24.7	11.2	85.35
Jul 20-26	31.5	25.3	23.9	83.00
Jul 27-2 Aug	31.6	25.4	3.8	82.05
Aug 3-9	28.7	21.9	16.9	86.65
Aug 10-16	30.1	25.3	2.2	81.85
Aug 17-23	29.1	24.2	1.7	79.75
Aug 24-30	32.3	26.3	3.8	81.35
Aug 31-6 Sep	31.4	25.2	7.9	79.40
Sep 7-13	30.9	24.5	19.1	82.80
Sep14-20	31.8	24.7	13.9	84.20
Sep 21-27	31.2	25.0	12.3	86.40
Sep 28-4 Oct	29.3	24.6	13.1	88.40
Oct 5-11	31.2	24.4	2.2	81.05
Oct 12-18	32.3	20.3	0.0	62.10
Oct 19-25	31.5	18.3	0.0	61.60

CHAPTER – IV
RESULTS AND DISCUSSION

The study on the survey for the incidence of charcoal rot of soybean, isolation of pathogen, its pathogenicity, diagnosis, variability studies on *Macrophomina phaseolina* , screening of soybean genotypes against the disease and the integrated management of the disease were carried out during 2015-16 and 2016-17.The laboratory and pot experiments were conducted in the Department of Plant Pathology, College of Agriculture, Raipur (C.G.) and the field experiment was conducted in research farm, IGKV, Raipur (C.G.) during *kharif* 2015-16 and 2016-17.

4.1 Survey of soybean growing area to assess the incidence of charcoal rot

The intensive survey was conducted at the R7 (Beginning Maturity i.e. Physiological maturity) to record the incidence of charcoal rot in major soybean growing district of Chhattisgarh, Raipur, Bemetara, Kawardha, Rajnandgaon and Durg. Among the five district, twenty two locations (Dharsiwa, Ranka, Jaibra, Bemetara, Saigona, Kanhera, Kodia, Patharra, Chorbhatti, Chimagondi, Maharajpur, Lohara, Udiya Khurd, Ataria, Narmada, Kanhar, Salebharri, Bhulatola, Parpondi , Basni, Pendri and Rajpur during *Kharif* 2015-16 and 2016-17. Observations were recorded from farmer’s fields under natural condition.

Results of survey; conducted during *kharif* 2015-16 are presented in table 4.1 and Fig.4.2 which indicated the appearance of disease in all the soybean growing areas of Chhattisgarh. Disease incidence ranged from 30 to 60 per cent during the year 2015-16 with average of (42.63%). The maximum percent of disease incidence (60%) was observed in Jaibra, followed by Chorbhatti (55.30%), Parpondi (50.50%), Udiya Khurd (50.40%), Patharra (50.00), Kodia (45.80%), Ataria (45.60%), Maharajpur (45.55%), Chimagondi (45.50%), Narmada (40.70%), Kanhera and Bhulatola (40.60%), Ranka (40.50%), Saigona (40.40%), Pendri (40.30%), Salebharri (40.10%), Kanhar and Rajpur (40.00%), Lohara (35.50%), Dharsiwa (30.70%). The lowest percent of incidence was found in Bemetara and Basni (30.00%). The soybean crop gets damaged substantially in the Chhattissgarh state due to the disease charcoal rot (Fig.4.1). The average percent disease incidence in different soybean growing areas of Chhattisgarh was about 42.63%.

Survey of soybean charcoal rot was also carried out during the *kharif* 2016-17. The results presented in table 4.2 and Fig. 4.2 indicate that less disease incidence was recorded on compared to the previous year crop. It ranged from 15 to 25.10 percent with average disease incidence of 19.12 percent as compared to the last year's average (42.63%). The maximum percent of disease incidence (25.10%) was observed in Ataria followed by Jaibra (25.00%), Lohara and Kodia (20.70%), Saigona (20.60%), Chorbhatti (20.50%), Ranka, Bhulatola, Basni and Pendri (20.40%), Dharsiwa (20.30%), Chimagondi, Udiya Khurd, Narmada and Salebharri (20.00%), Kanhera (15.70%), Rajpur (15.60), Kanhar (15.30%), Maharajpur (15.20%) and Patharra (15.10%). Bemetara and Parpondi recorded the minimum charcoal rot disease incidence (15.00%).

Data of disease incidence presented in table 4.1 & 4.2 indicate the seasonal variation of disease. It was reported that during *kharif* 2015-16 Chhattisgarh recorded less rainfall and high temperature that favours the high disease incidence without consideration of variety. During *kharif* (2016-17) less disease incidence was reported, because during this period state reported the high rain fall and low temperature which is not suitable for disease charcoal rot.

A survey of soybean growing areas of Chhattisgarh was conducted in the districts i.e. Durg, Rajnandgaon, Mungeli, Bemetara, and Kawardha where the disease incidence of charcoal rot varied from 3-30% (Anonymous, 2015). Similar report was also made by Gupta and Chauhan (2005) about the charcoal rot epiphytotics in India, where temperature ranges from 35–40°C during the crop season and the disease can cause up to 80% yield losses. During the 1997 season, charcoal rot caused substantial loss to plant stand and yield in soybean in Guna District of Madhya Pradesh State. Wrather *et al*. (1997) estimated yield loss due to charcoal rot in soybean in the top 10 soybean-producing countries during 1994 to

Table 4.1: Incidence of soybean charcoal rot in different locations of Chhattisgarh during *Kharif* 2015

S. No.	District	Block	Location	Variety sown	% Disease incidence
1.	Raipur	Dharsiwa	Dharsiwa	JS 97-52	30.70
2.	Bemetara	Bemetara	Ranka	JS 335	40.50
3.	Bemetara	Bemetara	Jaibra	JS 335	60.00
4.	Bemetara	Bemetara	Bemetara	JS 335	30.00
5.	Bemetara	Bemetara	Saigona	JS 335	40.40
6.	Bemetara	Bemetara	Kanhera	JS 335	40.60
7.	Bemetara	Bemetara	Kodia	JS 335	45.80
8.	Bemetara	Bemetara	Patharra	JS 335	50.00
9.	Bemetara	Bemetara	Chorbhatti	JS 335	55.30
10.	Kawardha	Kawardha	Chimagondi	JS 335	45.50
11.	Kawardha	Kawardha	Maharajpur	JS 335	45.55
12.	Kawardha	Lohara	Lohara	JS 335	35.50
13.	Kawardha	Lohara	Udiya Khurd	JS 335	50.40
14.	Rajnandgaon	Ataria	Ataria	JS 97-52	45.60
15.	Rajnandgaon	Narmada	Narmada	JS 335	40.70
16.	Rajnandgaon	Kanhar	Kanhar	JS 335	40.00
17.	Rajnandgaon	Salebharii	Salebharri	JS 97-52	40.10
18.	Rajnandgaon	Bhulatola	Bhulatola	JS 335	40.60
19.	Durg	Dhamdha	Parpondi	JS 335	50.50
20.	Durg	Dhamdha	Basni	JS 335	30.00
21.	Durg	Dhamdha	Pendri	JS 97-52	40.30
22.	Durg	Dhamdha	Rajpur	JS 335	40.00
	Mean				**42.63**

Table 4.2: Incidence of soybean charcoal rot in different location of Chhattisgarh during *kharif* 2016

S. No.	District	Block	Location	Variety sown	% Disease incidence
1.	Raipur	Dharsiwa	Dharsiwa	JS 335	20.30
2.	Bemetara	Bemetara	Ranka	JS 335	20.40
3.	Bemetara	Bemetara	Jaibra	JS 335	25.00
4.	Bemetara	Bemetara	Bemetara	JS 97-52	15.70
5.	Bemetara	Bemetara	Saigona	JS 335	20.60
6.	Bemetara	Bemetara	Kanhera	JS 335	15.70
7.	Bemetara	Bemetara	Kodia	JS 335	20.70
8.	Bemetara	Bemetara	Patharra	JS 97-52	15.10
9.	Bemetara	Bemetara	Chorbhatti	JS 335	20.50
10.	Kawardha	Kawardha	Chimagondi	JS 335	20.00
11.	Kawardha	Kawardha	Maharajpur	JS 97-52	15.20
12.	Kawardha	Lohara	Lohara	JS 335	20.70
13.	Kawardha	Lohara	Udiya Khurd	JS 335	20.00
14.	Rajnandgaon	Gandai	Ataria	JS 97-52	25.10
15.	Rajnandgaon	Gandai	Narmada	JS 335	20.00
16.	Rajnandgaon	Gandai	Kanhar	JS 335	15.30
17.	Rajnandgaon	Khairagarh	Salebharri	JS 335	20.00
18.	Rajnandgaon	Khairagarh	Bhulatola	JS 335	20.40
19.	Durg	Dhamdha	Parpondi	JS 97-52	15.00
20.	Durg	Dhamdha	Basni	JS 335	20.40
21.	Durg	Dhamdha	Pendri	JS 97-52	20.40
22.	Durg	Dhamdha	Rajpur	JS 335	15.60
	Mean				**19.12**

Fig. 4.1: A, B- Charcoal rot causing severe damage to soybean field

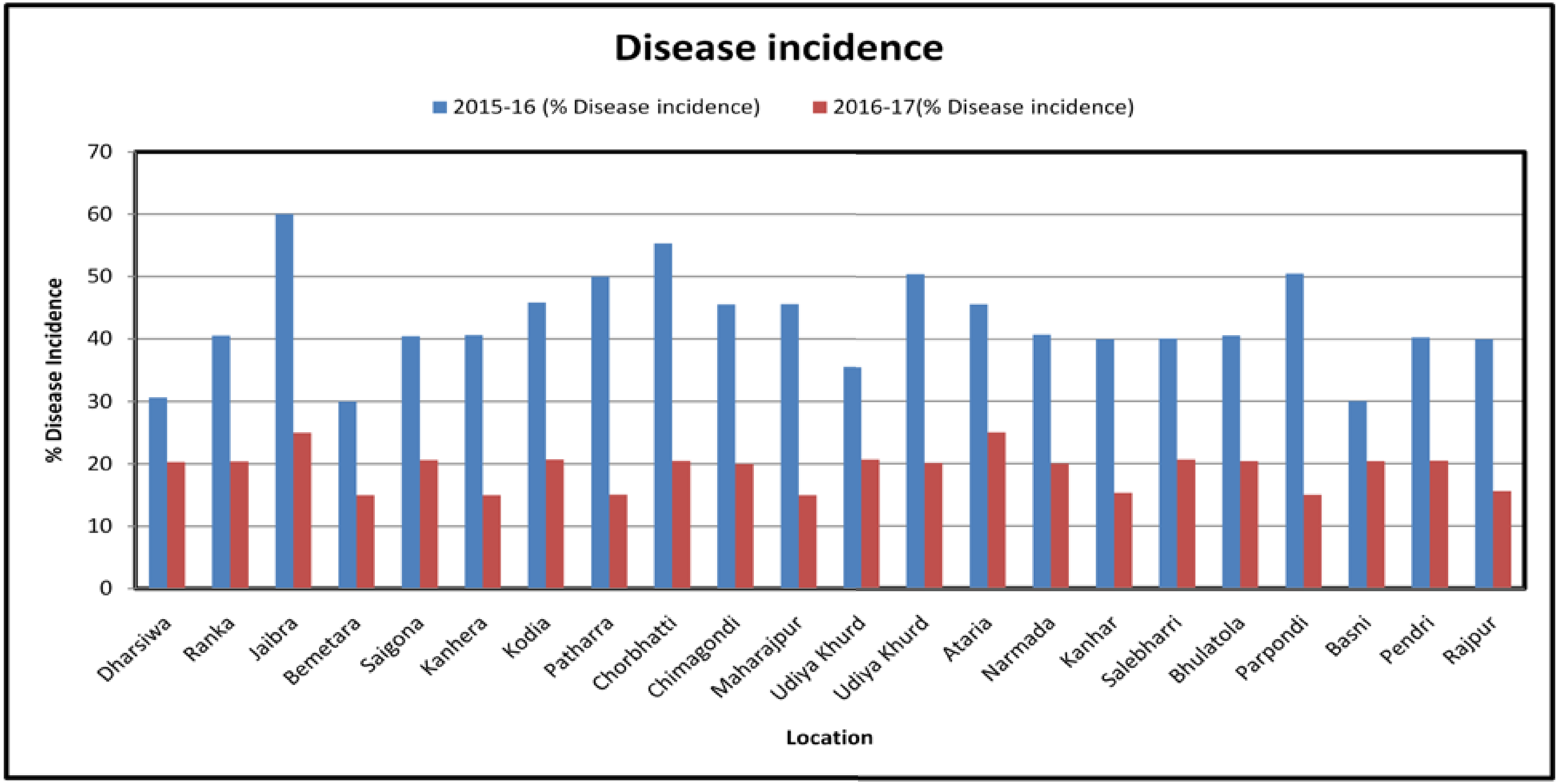

Fig. 4.2: Yearly variation of charcoal rot disease incidence from soybean growing areas of Chhattisgarh

be 1.234 million metric tones. Yang and Navi (2005) also reported the charcoal rot epidemics caused by *Macrophomina phaseolina* in Soybean in Iowa. It was observed that during the 2003 growing season, a severe epidemic of charcoal rot was observed throughout the state. A systematic survey was conducted between late August and early September, 2003 to determine the prevalence and severity of charcoal rot in Iowa.

4.2 Symptomatology

Following symptoms of charcoal rot were observed in soybean under natural field condition (Fig. 4.3a D).

- After emergence of seedlings, symptoms can be visible on cotyledons as brown to dark spots (Fig. 4.3a A and B). Sometimes, the margins of the cotyledons become brown to black and shed at an early stage.

- From the unifoliate leaf stage onwards, the symptoms appear on emerging hypocotyls of infected seedlings as circular to oblong, reddish-brown, lesions that may turn dark brown to black.

- The first aboveground symptoms appear between one and four weeks before normal maturity (Fig. 4.3a C). The infected crop in the field exhibits premature yellowing in scattered patches. Under severe disease conditions, the crop over a large area in the field may be affected.

- Normally, in the infected crop, the dead leaves remain attached to the petiole for several days after death. Leaves of infected plants remain smaller than normal and subsequently turn yellow prior to wilting (Fig. 4.3a D).

- A reddish-brown discolouration of the vascular elements of roots and lower stem showing grey to black discoloration with lesions on the stem above the soil line, precedes the premature yellowing as the fungus spreads up the stem during the season.

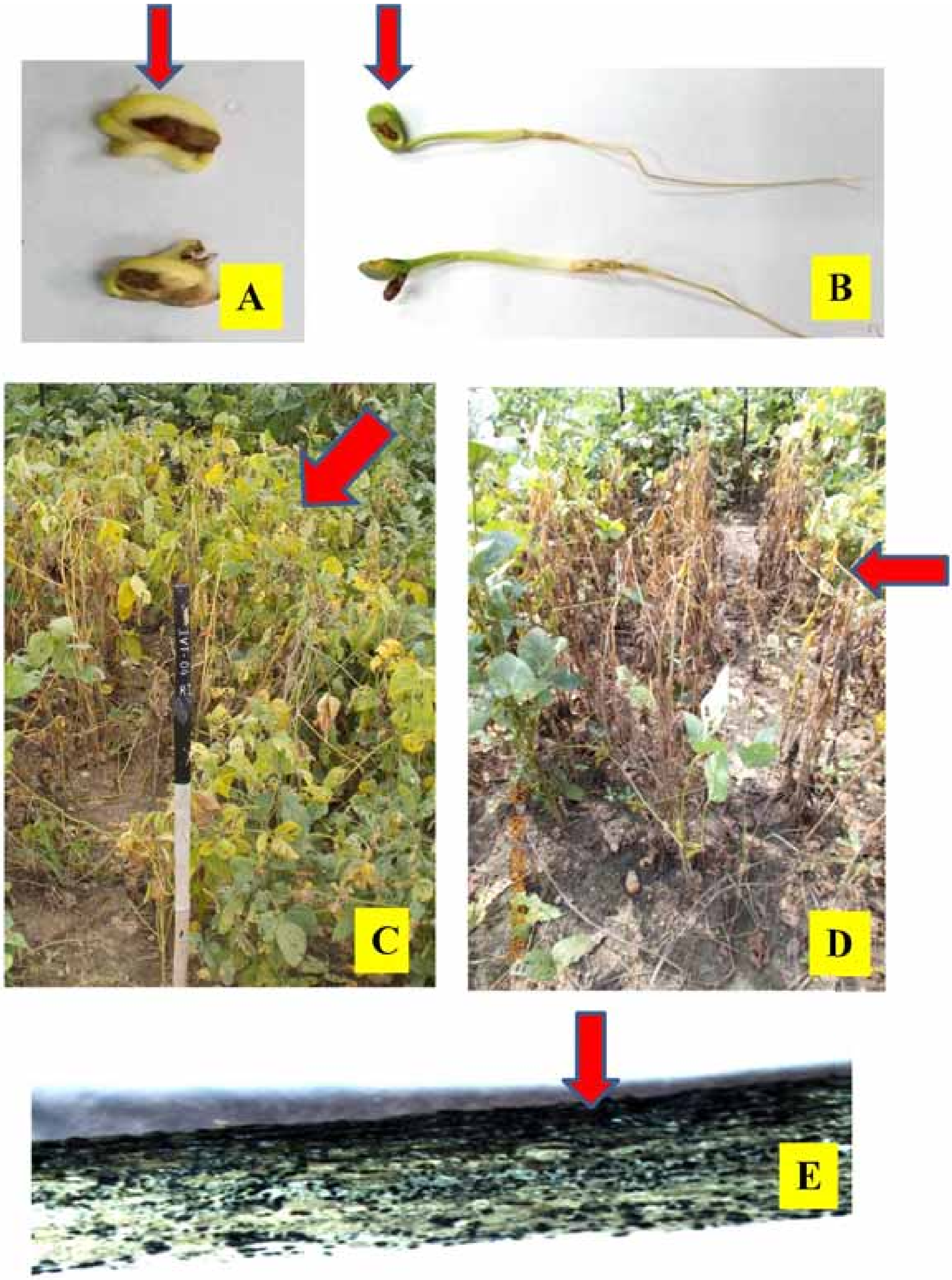

Fig. 4.3a : Symptoms of Charcoal rot of soybean
A & B- Brown to dark spots on the cotyledons of seedling
C- Premature yellowing of soybean leaf
D- Wilting and drying of soybean plant
E- Minute sclerotia found on the dead soybean plant

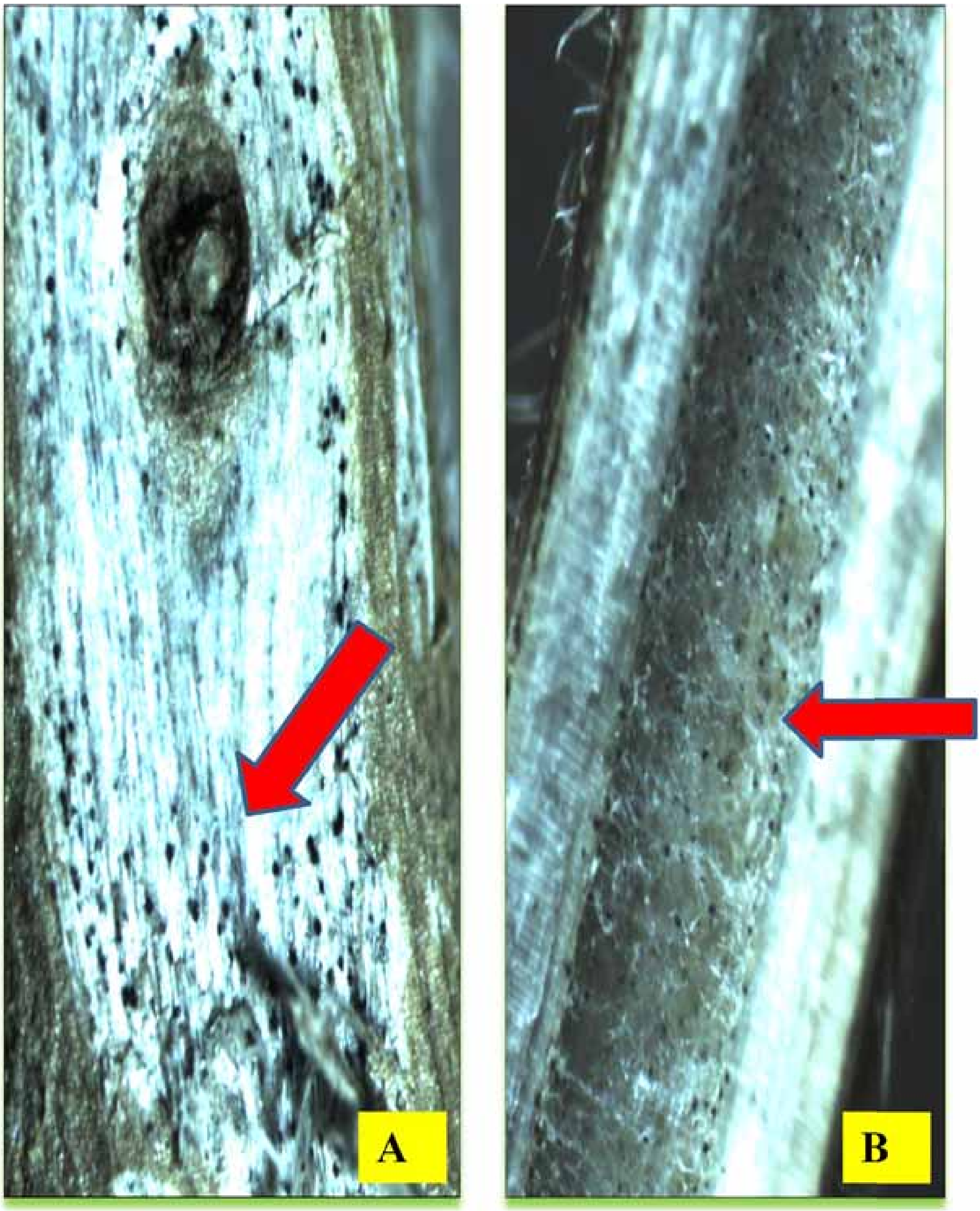

Fig. 4.3b, A. Charcoal rot infected root showing dark grey and black discoluration with minute black microsclerotia.
B. Microsclerotia found inside the plant tissue

- Microsclerotia are formed in the vascular tissues and in the pith, giving a greyish-black appearance to the sub epidermal tissues of the stem (Fig. 4.3b A and B).

- The infected mature and dry pods are covered with locally or widely distributed black bodies (microsclerotia). The fungus penetrates the pods and seeds, inducing diverse symptoms. Diffuse black spots or blemishes appear on the seeds. Microsclerotia sometimes are produced in fissures and cracks in the seed coat.

- After the death of the plant, numerous, minute, pinhead-sized microsclerotia appear, which can be seen readily when the epidermal tissue of the lower stems and roots is peeled from the affected parts.

- Longitudinal streaks along the main stem, wilting of branches, and shriveled pods are the characteristic symptoms.

4.3 Identification of *M. phaseolina*

4.3.1 Hyphal branching

Branches of mature hyphae that served to identify isolated pathogen *M. phaseolina* arose at right and acute angle that is near to 45 degree to main hyphae (Fig. 4.4 D). A septum in the branch near the point of origin was also observed (Fig. 4.4 C and D). In addition to ordinary vegetative hyphae moniloid cells, barrel shaped cells, short cell, sclerotial cell were also observed. Moniloid cell arose as bud or blown out ends of pre existing cells (Parmeter, 1970). The colony colour of *M. phaseolina* appeared as black and greyish (Fig. 4.4 A).

4.3.9 Sclerotial development

Sclerotial initial were formed by coiling and branching of hyphae followed by many more septa (Fig.4.5 A). The abundant septation resulted in the formation of short barrel shaped cells filled with dense content (Fig.4.5 B). Initial increased in size by repeated branching and formation of new cell by more septa (Fig.4.5 E). There was no rind formation and zonation in the mature sclerotium. Mature sclerotia were much less closely interwoven and there were no well identified zones (Fig.4.5 F). The basic colour of the mature sclerotia was typically brown to dark black (Fig.4.5 F).

4.4 Cultural and morphological variability

Fifteen isolates of *M. phaseolina viz.,* MP1 to MP15 collected from different locations of Chhattisgarh were taken for the present study depicted in materials and methods section (table 3.2). Variability studies among these isolates of *Macrophomina phaseolina* were done on the basis of morphology, mycelial growth pattern and other cultural characteristics. The cultural characteristics *viz*, mycelial growth, hyphal width, type of mycelia growth, colony colour, sclerotial size, number and shape of sclerotia. Variations in cultural and morphological characteristics were observed in all the isolates of *M. phaseolina* and result is represented in table 4.3, Fig. 4.6a,b and 4.8.

4.4.1 Mycelial growth

The data in table 4.3 indicates that significant variations were observed in mycelial growth of *M. phaseolina* isolates collected from different location of Chhattisgarh. Isolates of *M. phaseolina* fungus categorized in to three classes on the basis of complete radial mycelium growth. Isolates wise completion of radial growth was recorded at the interval of 72h, 96h and more than 96h and then classified into 3 groups: fast (72h), medium (96h), slow growing (>96h). Two fast growing isolates MP1 and MP14 were completed their radial growth within (72h) of inoculation. Seven medium growing isolates *viz.,* MP3, MP4, MP5, MP6, MP8, MP12 and MP13 were completed their radial growth within (96hr) of inoculation. Six isolates namely MP2, MP7, MP9, MP10, MP11 and MP15 were growing very slow and didn’t complete the full radial growth even after (96hr) of inoculation considered as slow growing. On the basis of growth pattern, Iqbal and Mukhtar (2014) reported the significant variation among the isolates of *Macrophomina phaseolina* on the basis of mycelia growth pattern and categorized the isolates in to three group fast growing, medium growing and slow growing. Gupta *et al*. (2012) also categorized the isolates of *R. bataticola* with respect to colony diameter (growth rate) at 4th day of incubation as, slow growing and and fast growing.

Table 4.3: Cultural and Morphological characteristics of various isolates of *M. phaseolin*

S.No.	Isolates	Location	Mycelial growth (mm)				Hyphal width (µm)*	Type of mycelial growth	Colony colour
			24 hr	48hr	72hr	96 hr			
1	MP1	Dharsiwa	37.33	58.33	90.00	90.00	5.73	Submerged	Greyish
2	MP2	Ranka	12.33	39.00	59.00	75.67	6.20	Partial submerged	Dark Black
3	MP3	Jaibra	18.33	51.67	76.67	90.00	5.70	Submerged	Light greyish
4	MP4	Bemetra	16.67	47.67	69.67	90.00	7.27	Fluffy	Dark Black
5	MP5	Patharra	21.00	52.33	71.67	90.00	7.53	Submerged	Dark Black
6	MP6	Saigona	23.33	43.33	63.33	90.00	5.83	Partial Submerged	Dark Black
7	MP7	Chimagondi	19.67	44.67	61.67	73.33	6.50	Submerged	Greyish
8	MP8	Maharajpur	32.33	54.00	77.67	90.00	7.63	Submerged	Dark Black
9	MP9	Lohara	11.33	29.67	40.67	65.67	5.97	Irregular	Dark Black
10	MP10	Udiya Khurd	18.67	43.00	60.67	74.00	6.50	Fluffy	Greyish
11	MP11	Gandai	19.00	44.67	62.00	72.33	6.67	Fluffy	Greyish
12	MP12	Kanhar	22.00	49.33	72.00	90.00	5.60	Partial submerged	Dark Black
13	MP13	Bhulatola	22.67	49.33	71.00	90.00	5.50	Fluffy	Greyish
14	MP14	Pendri	34.00	62.00	90.00	90.00	5.60	Submerged	Dark Black
15	MP15	Dhamdha	14.33	42.33	61.00	72.33	5.67	Partial Submerged	Light Greyish
	SEm(±)		**2.727**	**1.288**	**1.774**	**0.993**	**0.162**		
	CD(5%)		**7.876**	**3.720**	**5.125**	**2.867**	**0.467**	*Average of 10 hyphae	

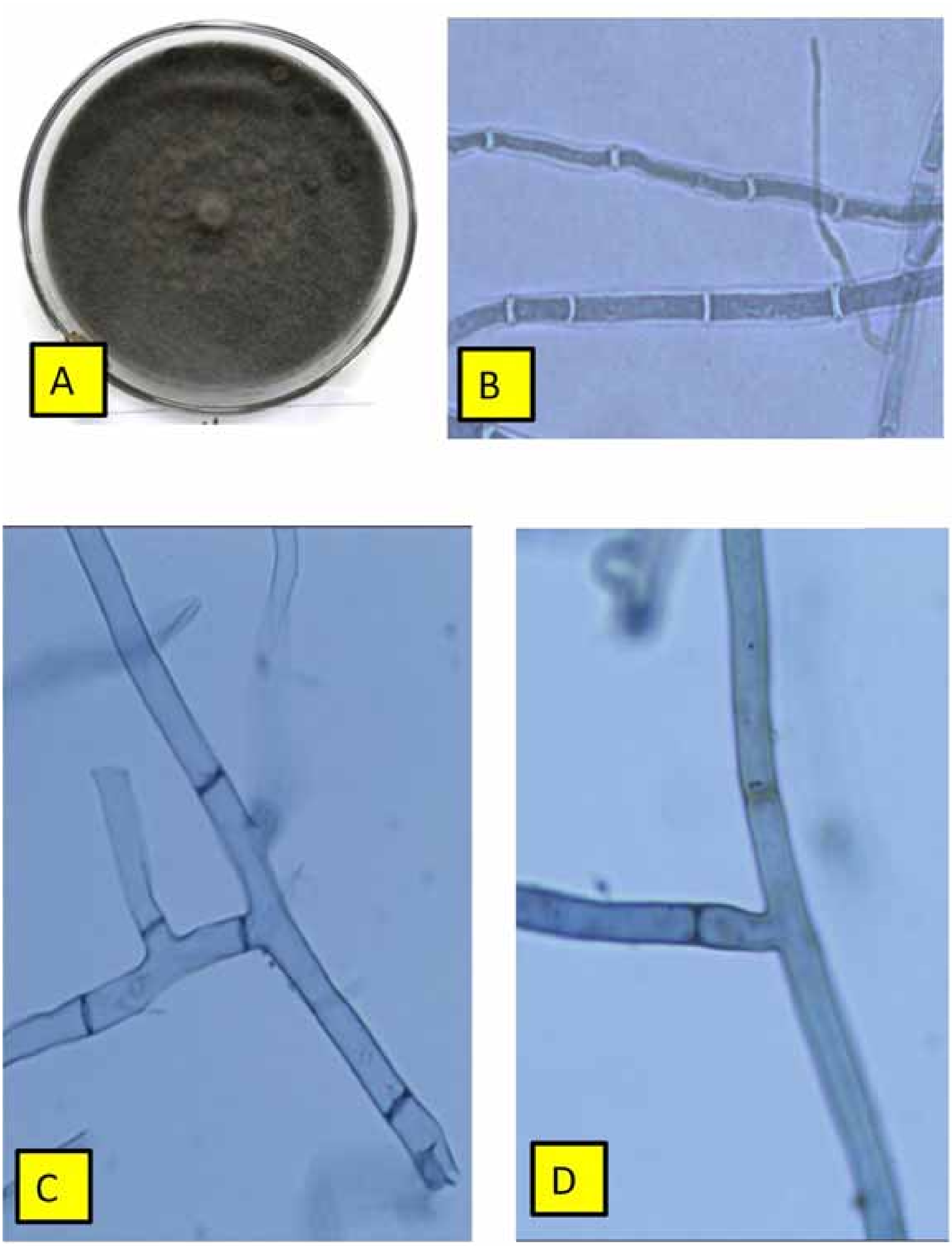

Fig. 4.4: Identification of *M. phaseolina*
A. Black colony colour of *M. phaseolina*
B. Pattern of hyphal septation
C and D. Branching pattern and septation

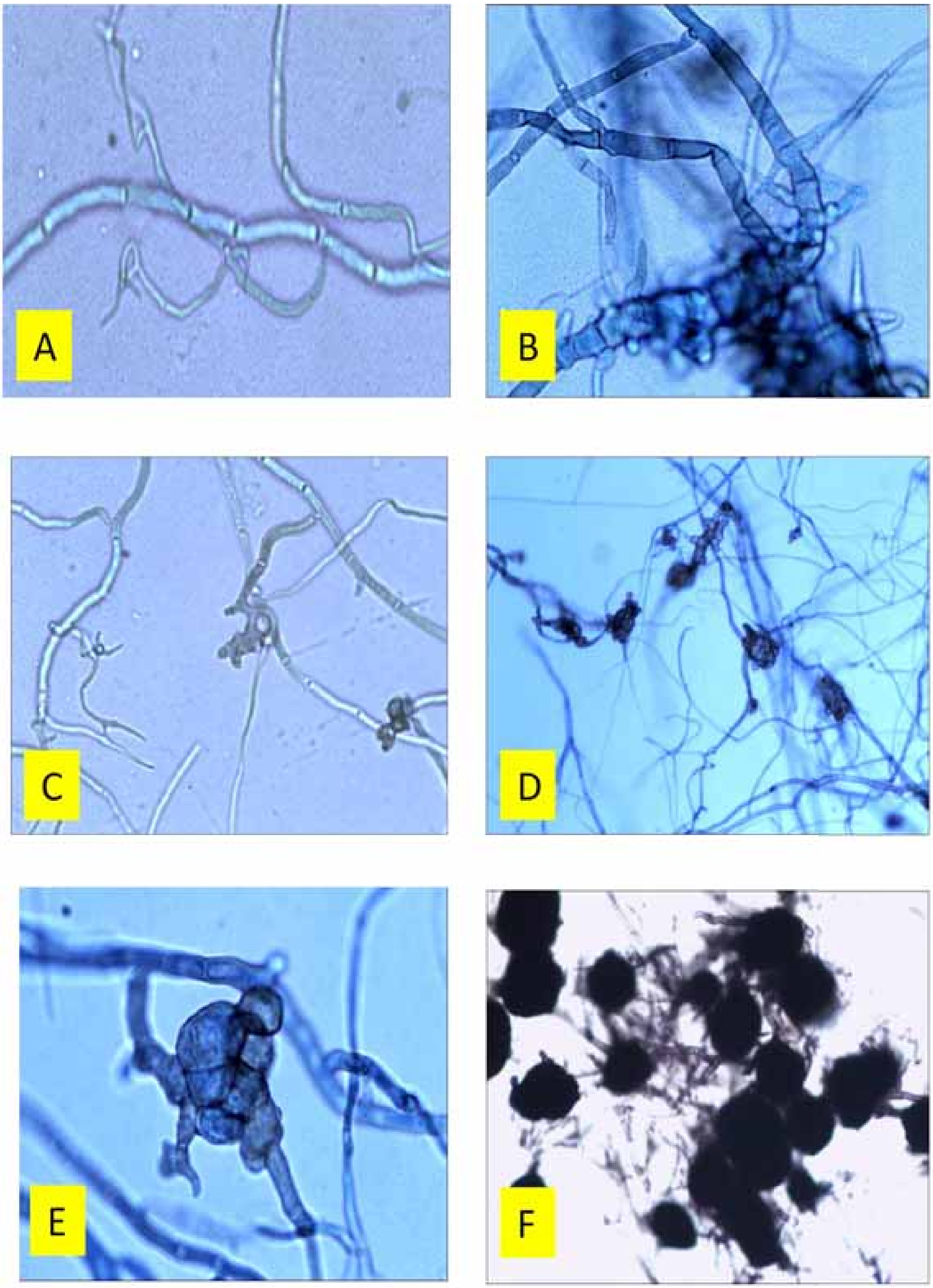

Fig. 4.5 Microphotograph of *M. phaseolina* sclerotial development (40X)
A.Initiation of coiling at hyphal tip
B - E. Aggregation of hyphae and sclerotial development
E. Mature sclerotia of *M. phaseolina*

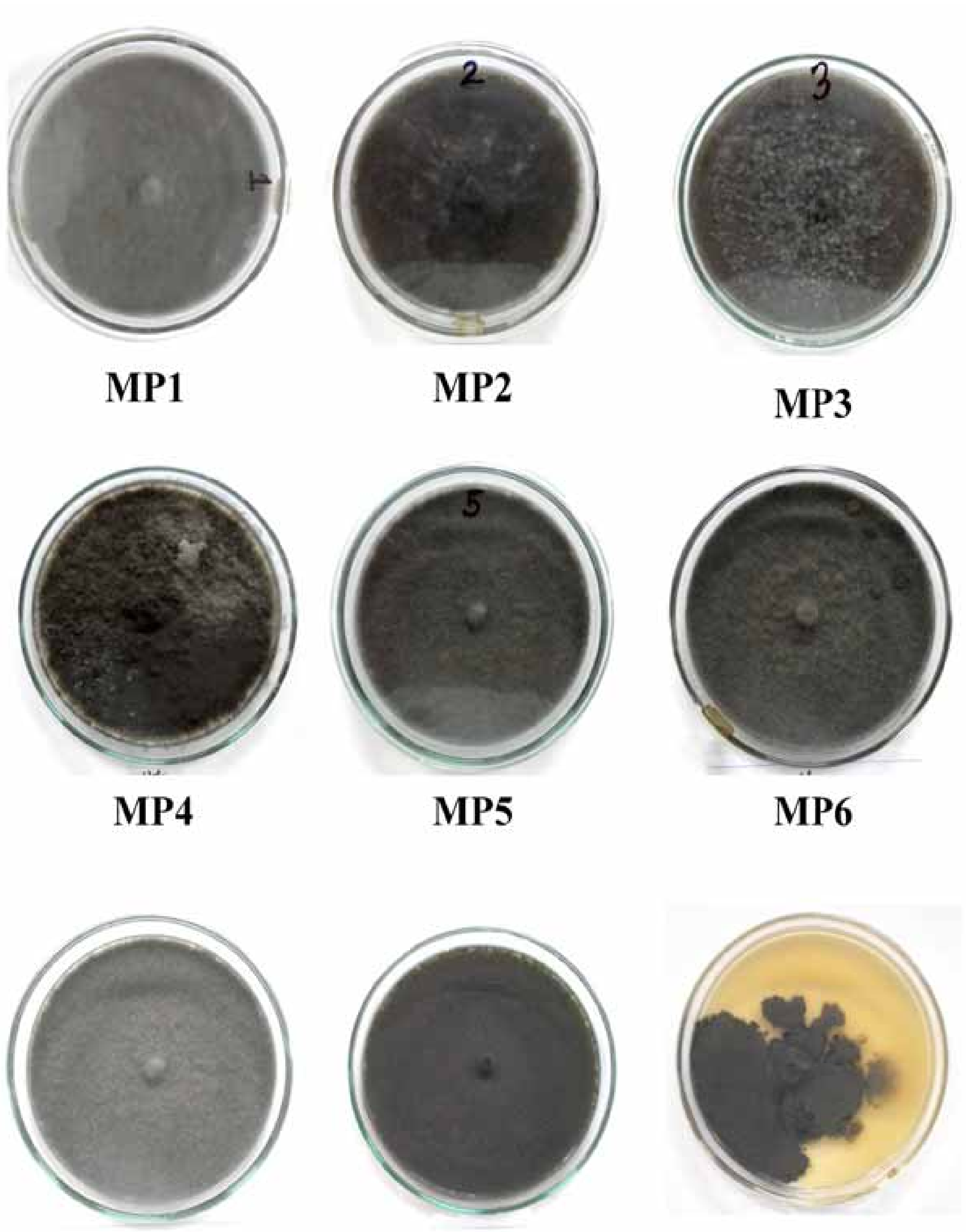

Fig. 4.6a: Cultural and morphological varibility of *M. phaseolina* isolates on PDA medium

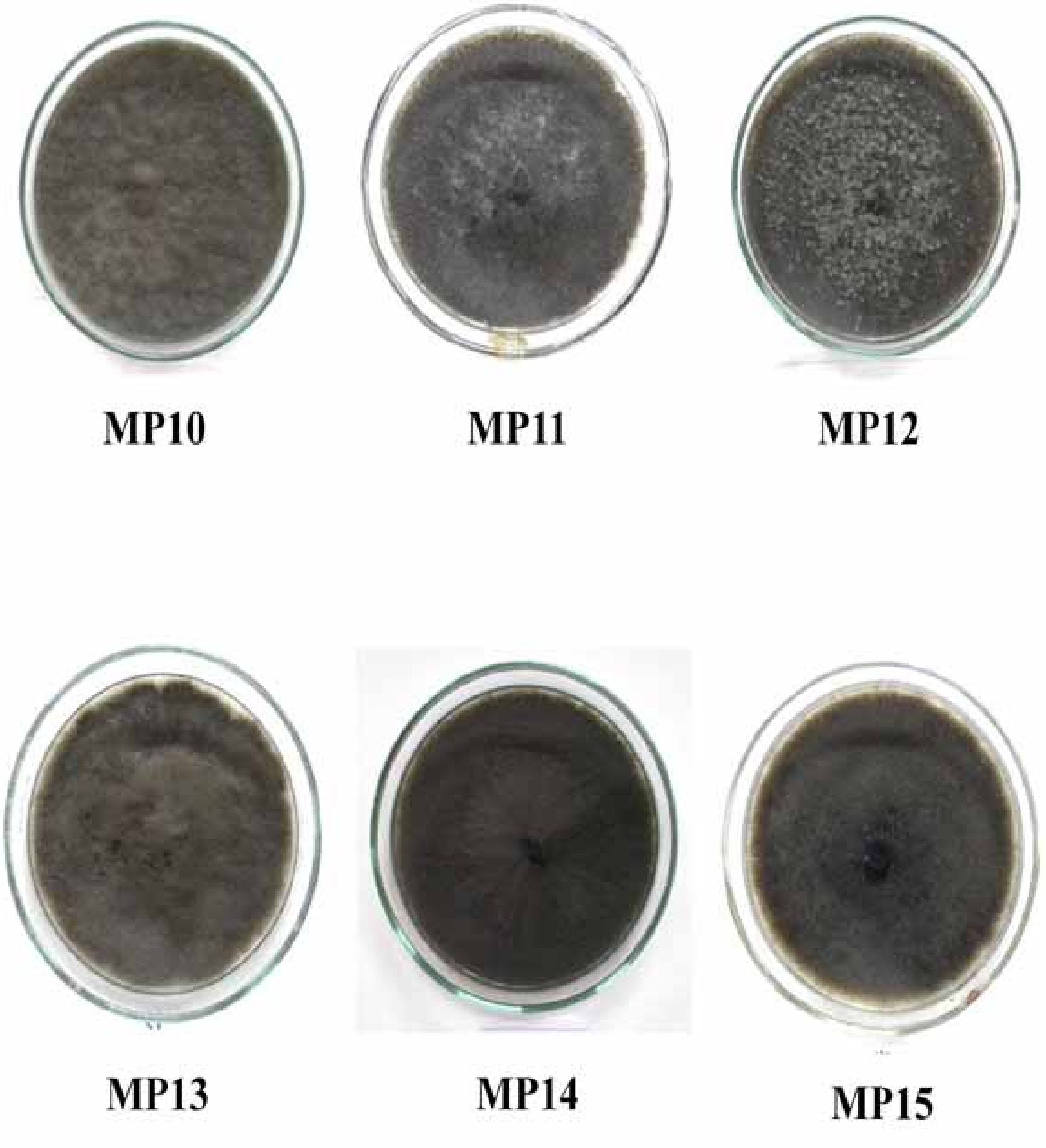

Fig. 4.6b: Cultural and morphological varibility of *M. phaseolina* isolates on PDA medium

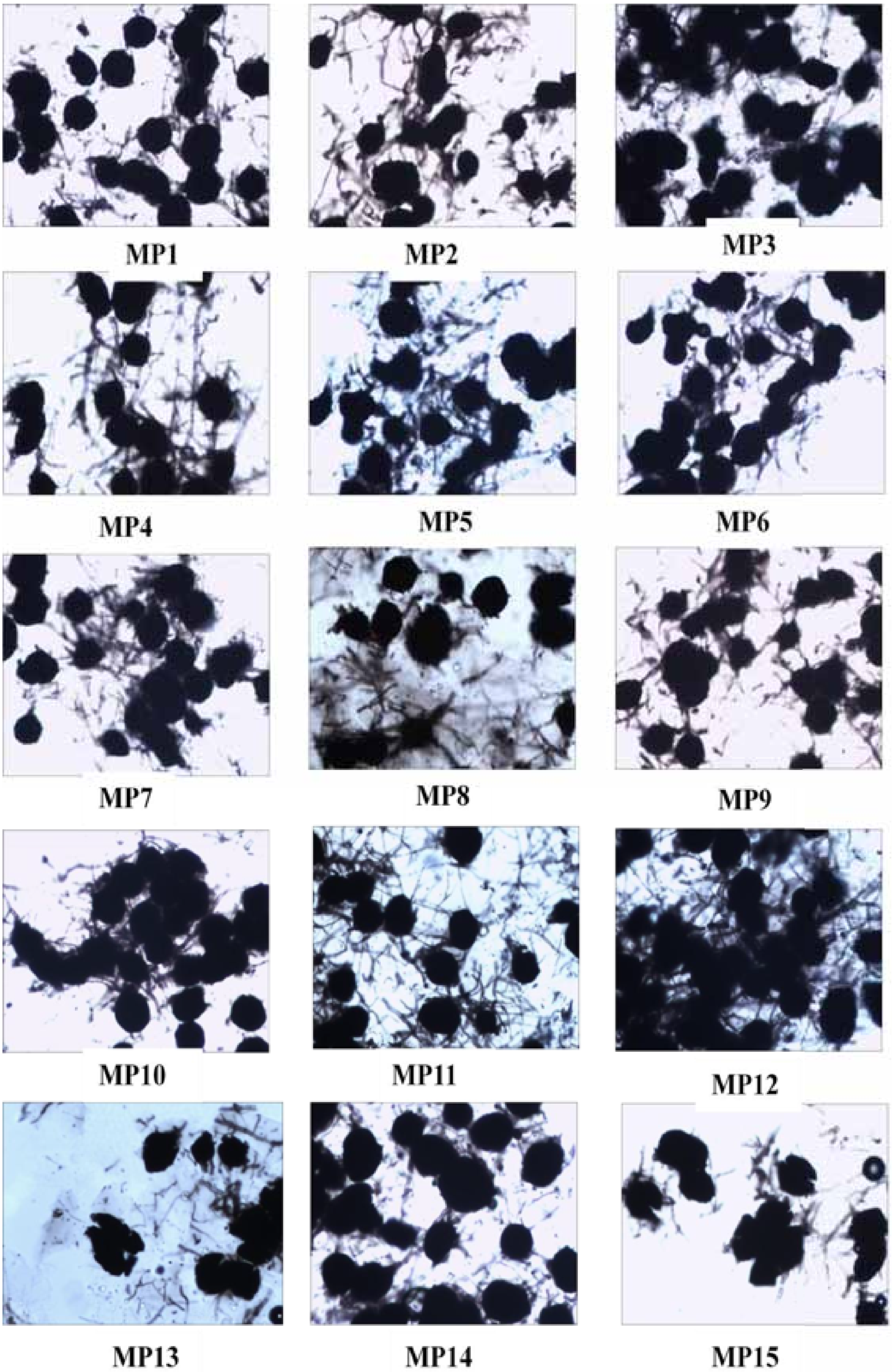

Fig. 4.7: Sclerotial microphotograph of *M. phaseolina* isolates (40X)

Table 4.4: Variability in morphology of sclerotia produced by different isolates of *M. phaseolina*

S.No.	Isolate	Size of sclerotia (µm)**	Shape	No. of sclerotia per microscopic field
1	MP1	87.3x83.2	Oval	141.95
2	MP2	82.2x70.1	Round	140.09
3	MP3	90.3x83.1	Oval	128.14
4	MP4	71.5x62.4	Round	171.94
5	MP5	81.5x70.5	Round	75.33
6	MP6	85.6x76.2	Oval	100.15
7	MP7	77.5x66.2	Oval	117.36
8	MP8	90.5x83.3	Round	87.32
9	MP9	102.4x73.6	Round	112.73
10	MP10	78.3x66.0	Round	119.70
11	MP11	88.0x85.7	Oval	155.23
12	MP12	95.0x80.1	Round	144.96
13	MP13	80.3x70.3	Oval	134.85
14	MP14	85.8x75.3	Round	150.49
15	MP15	86.2x72.7	Oval	169.64
SEm±				**16.350**
CD (5%)				**47.223**

** Average of 10 sclerotia

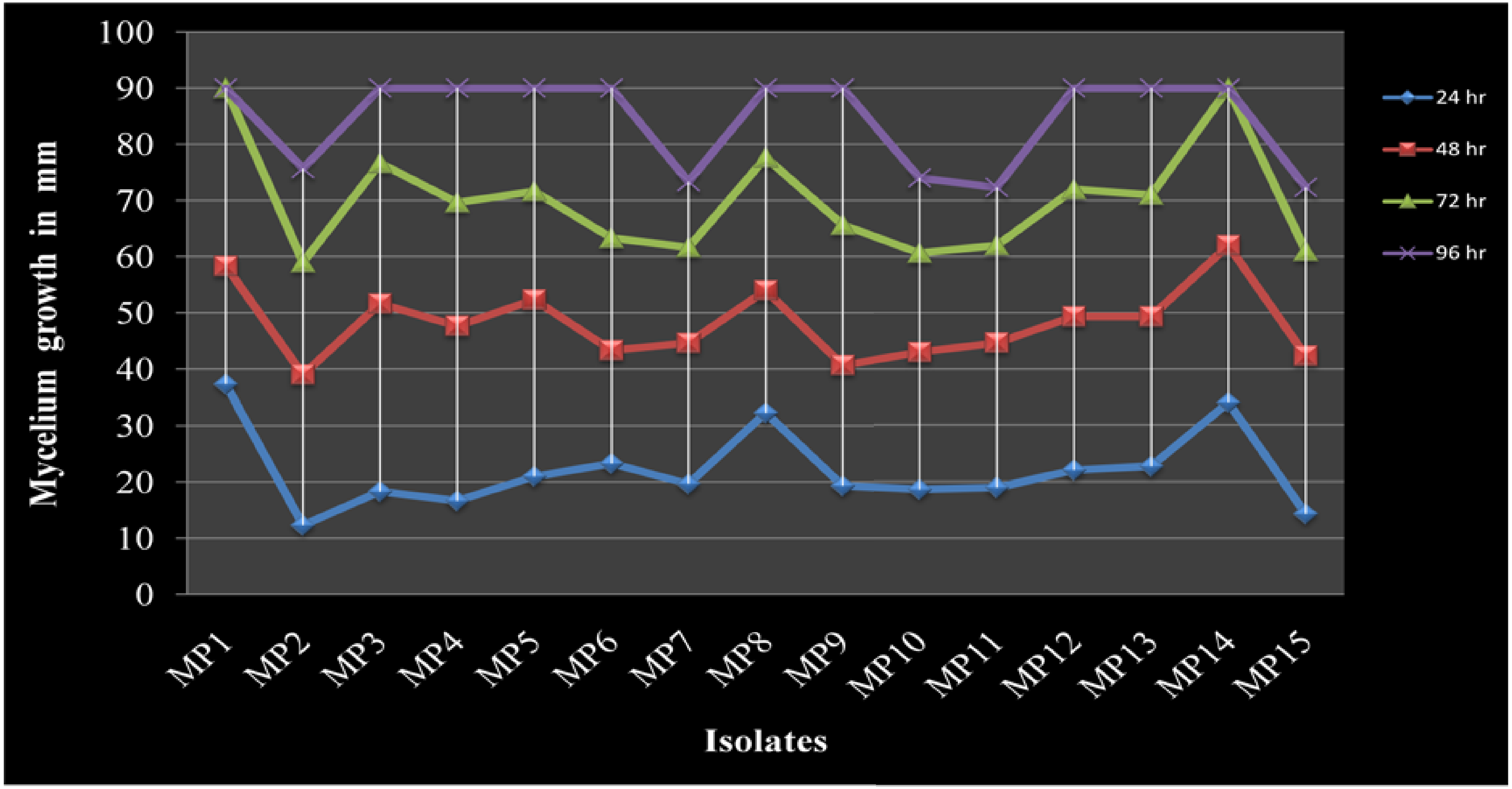

Fig. 4.8 Radial growth rate of different isolates of *M. phaseolina*

4.4.2 Hyphal width

Based on the hyphal width of isolates, the pathogen was categorized in three groups. First group comprised of eight isolates namely MP1, MP3, MP6, MP9, MP12, MP13, MP14 and MP15 with hyphal width ranging between (5-6 µm). Second group isolates having hyphal width ranging (6-7 µm), MP2, MP7, MP10 and MP11. Third group consists of only three isolates *viz.,* MP4, MP5 and MP8 with hyphal width more than 7µm. However, maximum and minimum hyphal width was observed in MP8 (Maharajpur) and MP13 (Bhulatola) respectively. The results were in agreement with the findings of Gupta *et al.* (2012). They observed a wide range of hyphal diameter and classified in three groups very thin (5.2-6.5 µm), thin (6.6-7.9µm) and thick (8.0-9.3µm). These observations coincided with the observations of Sobti and Sharma (1992) who reported that the width of hyphae varied from (F6-8.48µm). Jharia and Khare (1985) observed the hyphal width and found variation among the isolates of root 4.5 µm, soil (3.5µm) and seed (3.0 µm).

4.4.3 Type of mycelial growth

Based on mycelial growth pattern on PDA after seven days of incubation, all the fifteen isolates were placed in four groups *viz*., fluffy, partial submerged, submerged, and irregular. The first group includes, MP4, MP10, MP11 and MP13 isolates having fluffy mycelial growth. Whereas, MP2, MP6, MP12 and MP15 isolates formed in second group partially submerged. Submerged mycelial growth was identified in six isolates of *M. phaseolina*, *viz.,* MP1, MP3, MP5, MP7, MP8 and MP14. Only single isolate MP9 collected from Lohara fall was kept in the fourth group having irregular mycelium growth. In agreement to present finding Kanchan and Biswas (2009) evaluated morphological variability of *R. bataticola*, revealed that the nature of mycelium and colony varied from fluffy dark brown to partially fluffy mycelium colony with smooth margin. In accordance to present investigation Gupta *et al.* (2012) also reported mycelial growth pattern of fourty *R. bataticola* isolates collected from Chhattisgarh, Madhya Pradesh and Maharastra, showed significant variation in the type of mycelial growth (submerged, partially submerged and fluffy). Isolates collected from Chhattishgarh showed significant variation submerged, partial submerged and fluffy confirmed the existence of diversity within the state. Varma and Pathe (2013) also categorized the *R. bataticola* isolates on the basis of mycelial growth pattern into two broad group fluffy and submerged.

4.4.4 Colony colour

Based on the observation on the the colony colour all the fifteen isolates were classified into three groups *viz.,* dark black, greyish and and light grayish in appearance (Fig. 4.6 a & b). Eight isolates *viz.,* MP2, MP4, MP5, MP6, MP8, MP9, MP12 and MP14 produced dark black colony colour. Out of fifteen isolates five isolates produced greyish colony *i.e.* MP1, MP7, MP10, MP11 and MP13. Rest two isolates MP3 and MP15 produced light greyish colony. The results were in agreement with Mandal *et al.* (1998) in which they found that the colony colour of *R. bataticola* varied from light to dark brown appearance. Sulaiman and Patil (1966) also reported the variation in the colony colour of *R. bataticola* isolates *i.e.* dark black, dark grey and partial grey appearance. Similar results also observed by Aghakhani and Dubey (2009) in colony colour of *R. bataticola* isolates, white, dull white, creamy, grey and black. Sharma *et al.* (2012) found variation in colony colour like light black to black and light grey to grey. Present results confirm the observations made by Gupta *et al.* (2012) they reported that colony colour of *R. bataticola* varied from dark black to greyish cottony.

4.4.5 Sclerotial size

On the basis of microscopic observations of the sclerotia size varied from 71.5-102.4 x 62.4-85.7 µm (table 4.4). Where MP9 found to have largest sclerotial size (102.4 x 73.6 µm) followed by MP12 (95.0 x 80.1 µm), MP8 (90.5 x 83.3 µm), MP3 (90.3 x 83.1µm), MP11 (88.0 x 85.7 µm) MP1 (87.3 x 83.2 µm), MP15 (86.2 x 72.7 µm), MP14 (85.8 x 75.3 µm), MP6 (85.6 x 76.2 µm), MP2 (82.2 x 70.1 µm), MP5 (81.05 x 70.5 µm), MP13 (80.3 x 70.3 µm), MP10 (78.3 x 66.0 µm) and MP7 (77.5 x 66.2 µm). The smallest sclerotial size was observed in MP4 (71.5 x 62.4 µm). Gupta *et al.* (2012) also experienced the variation pattern in the sclerotial size among the of *R. bataticola* isolates. Sclerotia size ranged from 103.3 to 117.2 x 90.1- 106.5 µm to 72.7-87.5 x 57.1-73.5 µm. They also reported significant variation of sclerotia size within the isolates. On the basis of microscopic observations Varma and Pathe (2013) found the size of sclerotia varied from 72.2-117.2 x 57.1-106.5 µm. Different workers had given wide range of dimensions of sclerotia size in herbaceous plants (50-150µm), roots of woody plants (80-100 µm) and in culture (50-200µm) (Small, 1924), 120µm or small, 120-200 µm or medium and 200µm or large (Haigh, 1930), 60-165 x 57 -114 µm and 36-99 x 36-81 µm (Hildebran *et al*., 1945); 150-200 µm by Philip *et al.* (1969); biggest sclerotia 101.51 µm while smallest sclerotia 66.88 µm reported by Byadgi and Hegde (1985); Sclerotia from root isolates ranged from 122- 188 x77- 127 µm, and stem isolates 84-100 x 60-71 µm was observed by Dhingra and Sinclair (1977). Jharia and Khare (1985) found sclerotia in the root isolate (84x93 µm), soil isolate (90 x75 µm) and seed isolate (85x70 µm). Monga and Sheo (1995) and Mandal *et al.* (1998) recorded the sclerotia size 58.83 to 126.63 µm and 66.14 to 128.25 µm respectively.

4.4.6 Sclerotial shape

On the basis of microscopic observations of sclerotial shape the isolates were classified in two groups that is round and oval. Round shape sclerotia recorded in isolates MP2, MP4, MP5, MP8, MP9, MP10, MP12 and MP14 whereas, oval shape sclerotia were found in MP1, MP3, MP6, MP7, MP11, MP13 and MP15 isolates (table 4.4). Present results are in agreement with the findings of Gupta *et al.* (2012) who found that shape of sclerotia among the *R. bataticola* varied from round to oval among the isolates. Isolates collected from Chhattisgarh showed round and oval sclerotia. Sharma *et al.* (2012) also observed the difference in *R. bataticola* sclerotial shape as oblong, ellipsoid, irregular and round.

4.4.7 Number of sclerotia per microscopic field

Significant variation in number of sclerotia per microscopic field of *M. phaseolina* was also observed in all the fifteen isolates. Highest number of sclerotia per microscopic field was noted in MP4 (171.94) followed by MP15 (169.64), MP11 (155.23), MP14 (150.49), MP12 (144.96), MP1 (141.95), MP2 (140.09), MP13 (134.85), MP3 (128.14), MP10 (119.70), MP7 (117.36), MP9 (112.73), MP6 (100.15) and MP8 (87.32). Least sclerotia per microscopic field were formed in MP5 (75.33). (table 4.4 and Fig. 4.7). Present results were compared with the finding of Parmar (2013) he observed 470-500 sclerotia of *R.bataticola* per microscopic field. While, Varma and Pathe (2013) grouped the *R.bataticola* isolates according to number of sclerotia per microscopic field into three categories i.e. sparse, medium and abundant. Gupta *et al*., 2012 reported the variation in number of sclerotia from 9.7 to 22.2.

4.5 Pathogenicity test

4.5.1 Pathogenicity test performed through cut stem inoculation technique and *in vivo* sick pot method

Pathogenicity test was used to test the ability of pathogen to cause disease. To test the pathogenicity of *M. phaseolina*, cut stem inoculation technique and sick pot method used during study. Detailed procedure explained in section (3.4). Artificially inoculated soybean plant through cut stem inoculation technique showed linear necrosis (Fig. 4.9 A). Seedling emerged from sick pot showed dark spots on the cotyledons (Fig. 4.9 C). The symptoms appeared on emerging hypocotyls of infected seedlings as circular to oblong, reddish-brown, lesions that may turn dark brown to black. The infected crop showed premature yellowing of leaves. Leaves of infected plants remain smaller than normal and subsequently turn yellow prior to wilting (Fig. 4.9 B). The dead leaves of infected plant remain attached to the petiole for several days after death. Microsclerotia formed in the vascular tissues and in the pith, giving a greyish-black appearance to the sub epidermal tissues of the stem. After the death of the plant, numerous, minute, pinhead-sized microsclerotia appeared, which could be seen when the epidermal tissue of the lower stems and roots peeled from the affected parts.

Deep and irregular necrotic lesions extended toward hypocotyls and root surfaces were observed in soybean (Ammon et. al., 1975). Bristow and Wyllie (1986) observed the aboveground symptoms to appear between 1 and 4 weeks before normal maturity. The pathogen causes lesions on the roots, stems, pods and seeds. From ground level upwards, superficial lesions, light brown to grey in colour, infrequently appear on the stem. A twin stem abnormality is usually observed in greenhouse infections. Foliar symptoms progress from top of the plant

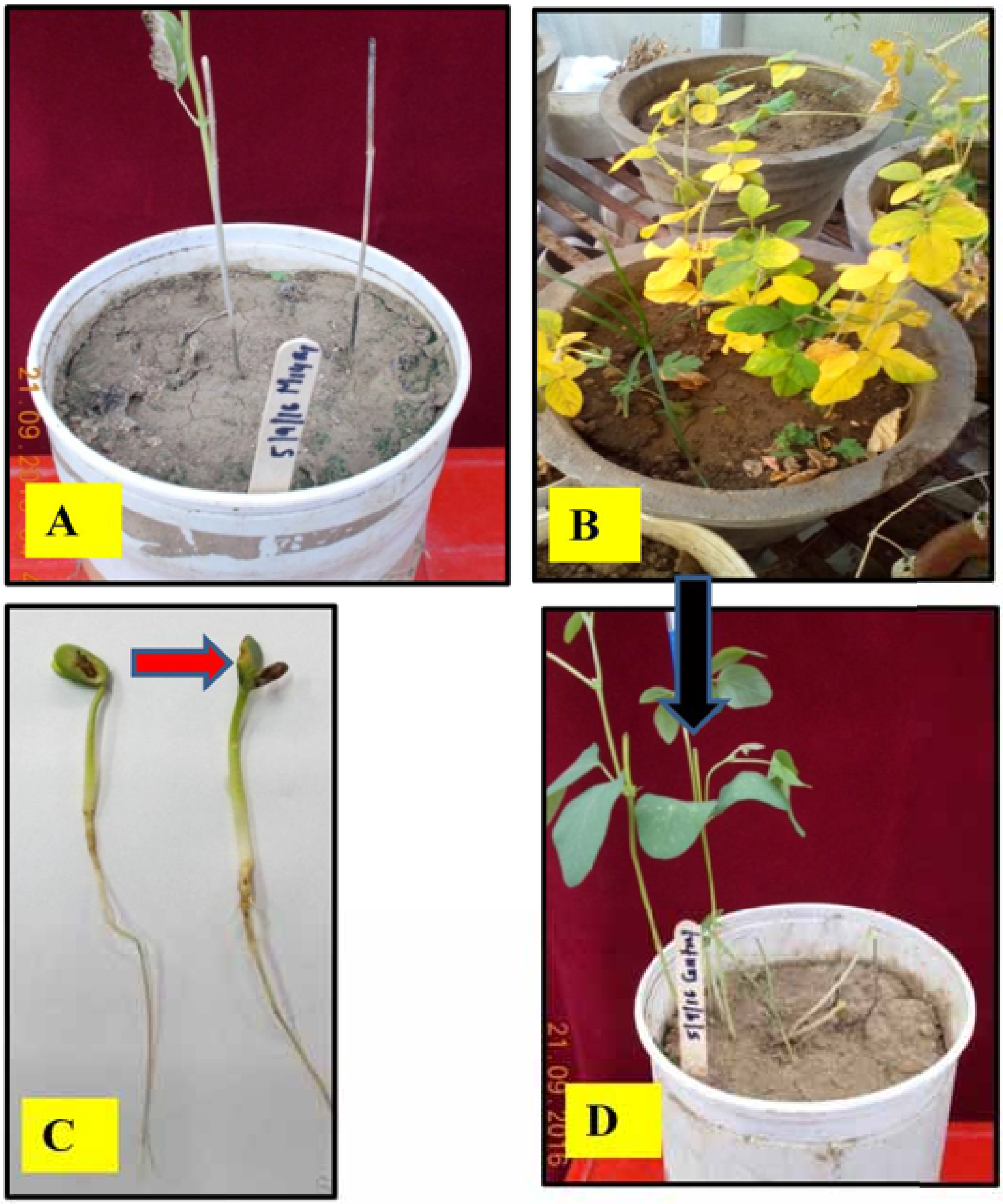

Fig. 4.9: Testing of pathogenicity on soybean plant by cut stem inoculation technique and sick pot method

A. Soybean plant showing necrotic symptom
B. Sick pot showing yellowing of soybean leaf
C. Necrotic symptom observed on the cotyledon
D. Healthy plant without showing necrosis

downwards. Leaves of infected plants remain smaller than normal and subsequently turn yellow prior to wilting (Gupta and Chauhan 2005).

4.6 Pathogenic variability

4.6.1 Aggressiveness of *M. phaseolina* isolates

4.6.1.1 *In vitro* blotter paper technique

The data presented in (table 4.5, Fig. 4.10 a-c and 4.12) revealed that the isolates were variable in their aggressiveness. The disease reaction of all the fifteen isolates on cultivar CG Soya-1, JS 97-52, JS 93-05, JS 335 and RSC 10-46 varied between 5-9 grades. Isolates showed varying degree of disease rating after inoculation on the particular variety. All the isolates of *M. phaseolina* categorized into aggressive and highly aggressive on the basis of reaction shown on varieties.

It is evident from the table 4.5 that individual disease response of all the *M. phaseolina* isolates on soybean variety CG Soya-1. Aggressiveness pattern of different *M. phaseolina* isolates behaved differently. Based on their aggressiveness the isolates were classified in two group MP1, MP2, MP5, MP7, MP8, MP9, MP10, MP12 and MP13 produced clear but small lesion on roots with new roots free from infection. Second group comprised of MP3, MP4, MP6, MP11, MP14 and MP15with many lesions on roots, completely discoloured and damaged. While, control plants without inoculation scored grade 1 with no infection on roots.

Individual disease response of all the *M. phaseolina* isolates was observed on soybean variety JS 97-52. Aggressiveness pattern of different *M. phaseolina* isolates behaved differently. Based on their aggressiveness the isolates were classified in two groups MP2, MP3, MP5, MP7, MP8, MP9, MP11 and MP12 and MP13 produced clear but small lesion on roots with new roots free from infection. Second group comprised of MP1, MP4, MP6, MP10, MP14 and MP15 with many lesions on roots, completely discoloured and destroyed. While, control plants without inoculation scored grade 1 with no infection on roots.

Individual disease response of all the *M. phaseolina* isolates was observed on soybean variety JS 93-05. Aggressiveness pattern of different *M. phaseolina*

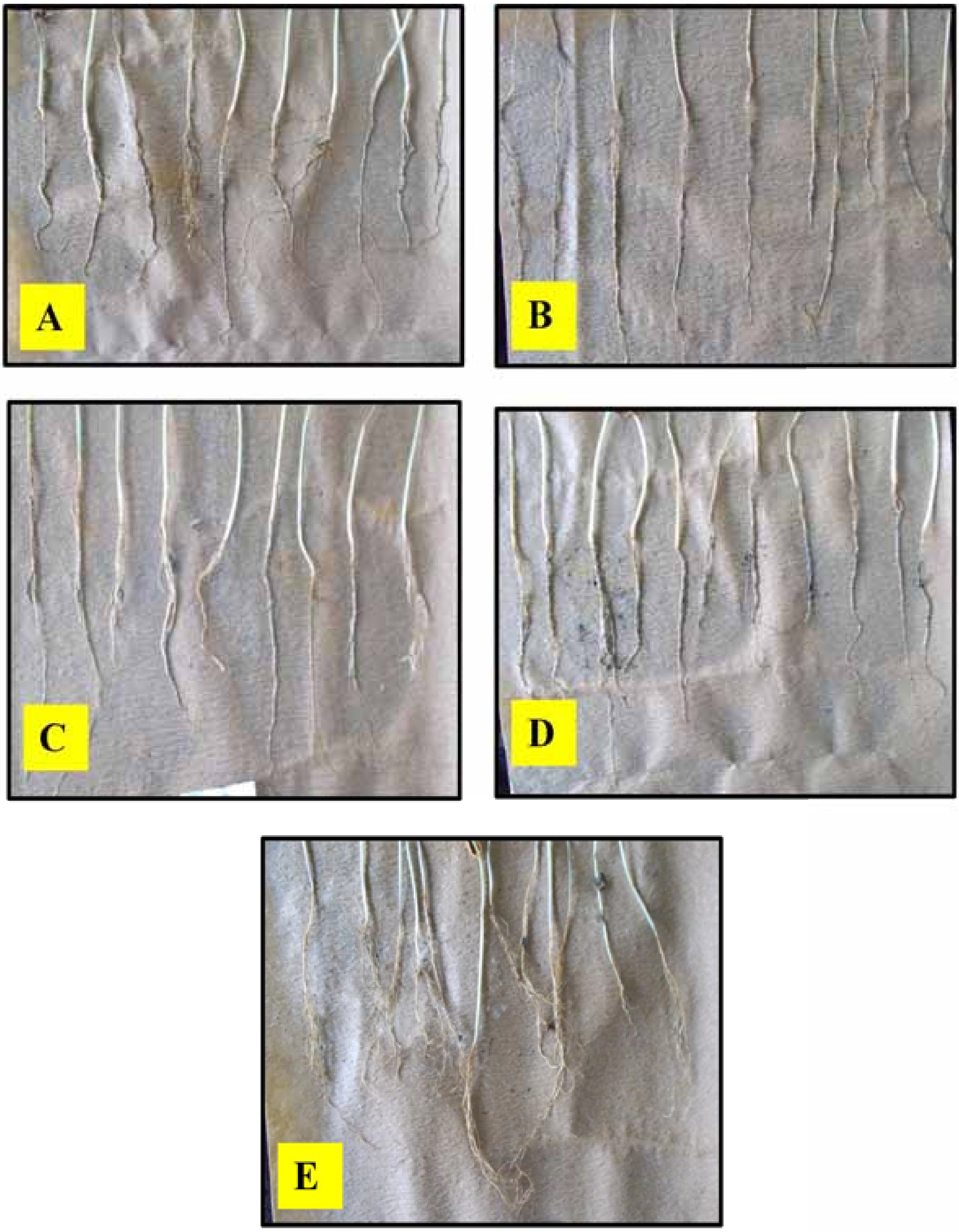

Fig. 4.10a. Extent of root damage in *in vitro* aggressiveness test of *M. phaseolina* isolate MP14 through blotter paper on soybean varities , (A.) CG Soya-1, (B) JS 97-52, (C) JS 93-05, (D) JS 335 and (E) RSC-10-46

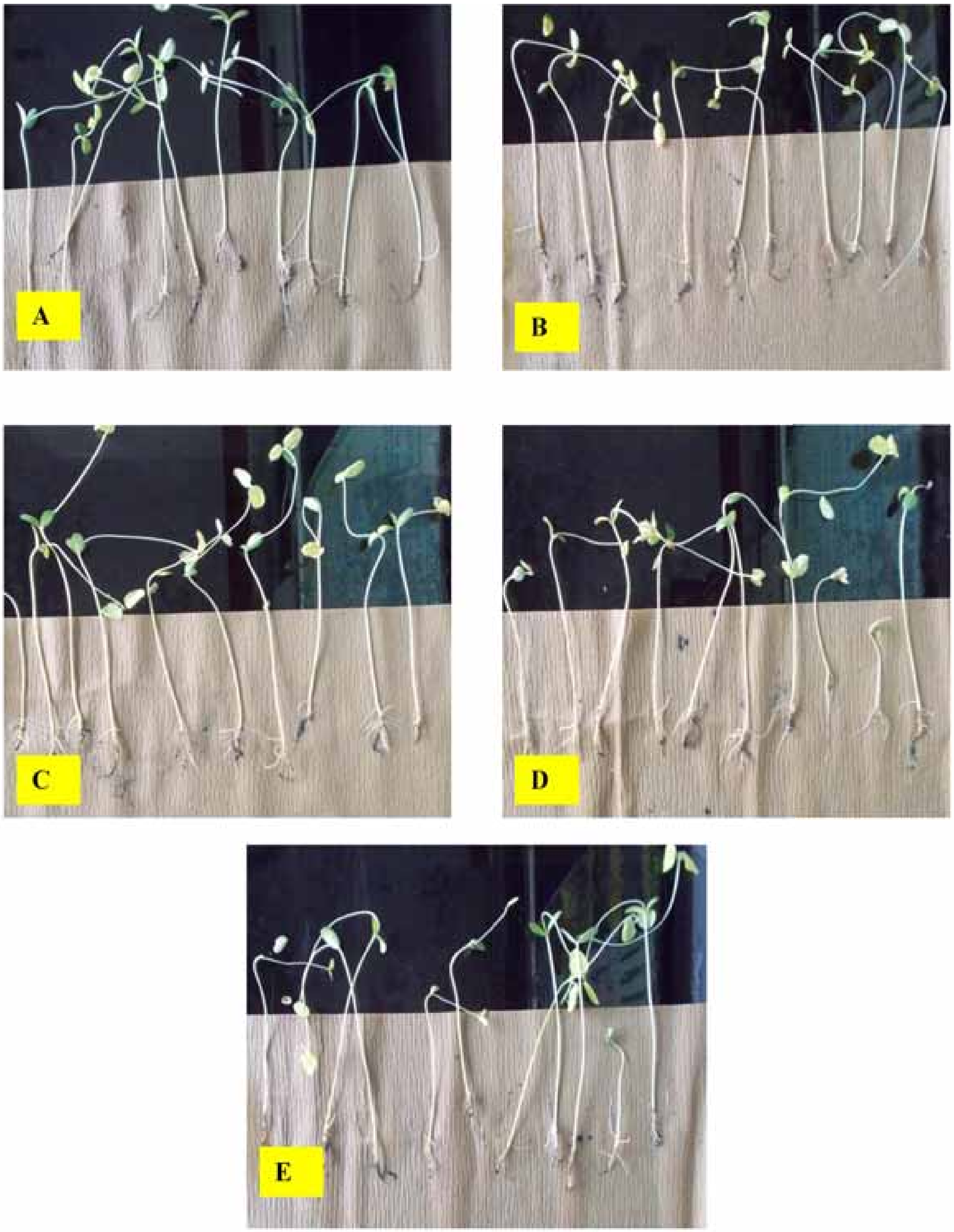

Fig. 4.10b. Extent of root damage in *in vitro* aggressiveness test of *M. phaseolina* isolate MP6 through blotter paper technIiue on different soybean varities , (A) CG Soya-1, (B) JS 97-52, (C) JS 93-05, (D) JS 335 and (E) RSC-10-46

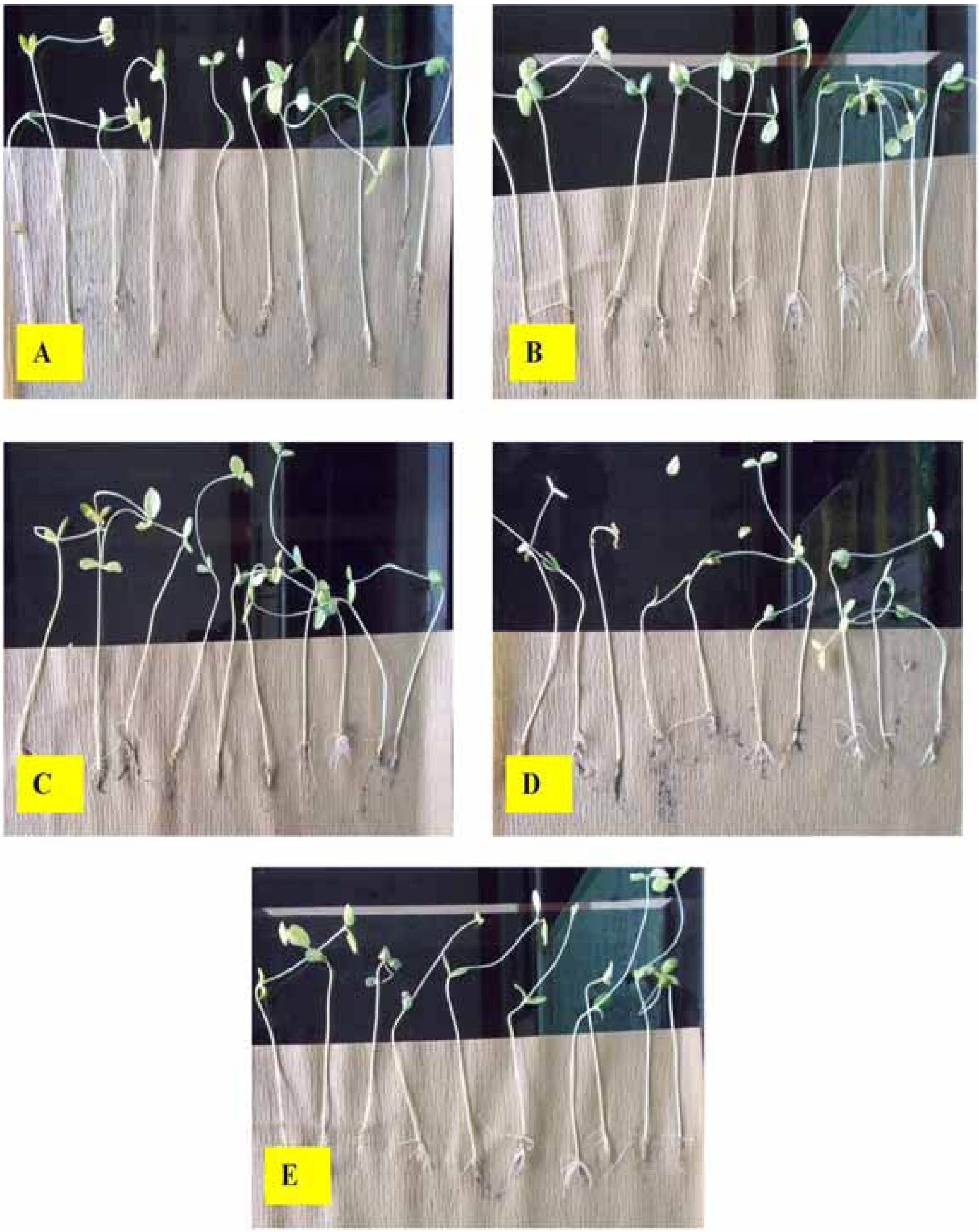

Fig. 4.10c. Extent of root damage in *in vitro* aggressiveness test of *M. phaseolina* isolate MP9 through blotter paper technIiue on different soybean varities , (A) CG Soya-1, (B) JS 97-52, (C) JS 93-05, (D) JS 335, and (E) RSC-10-46

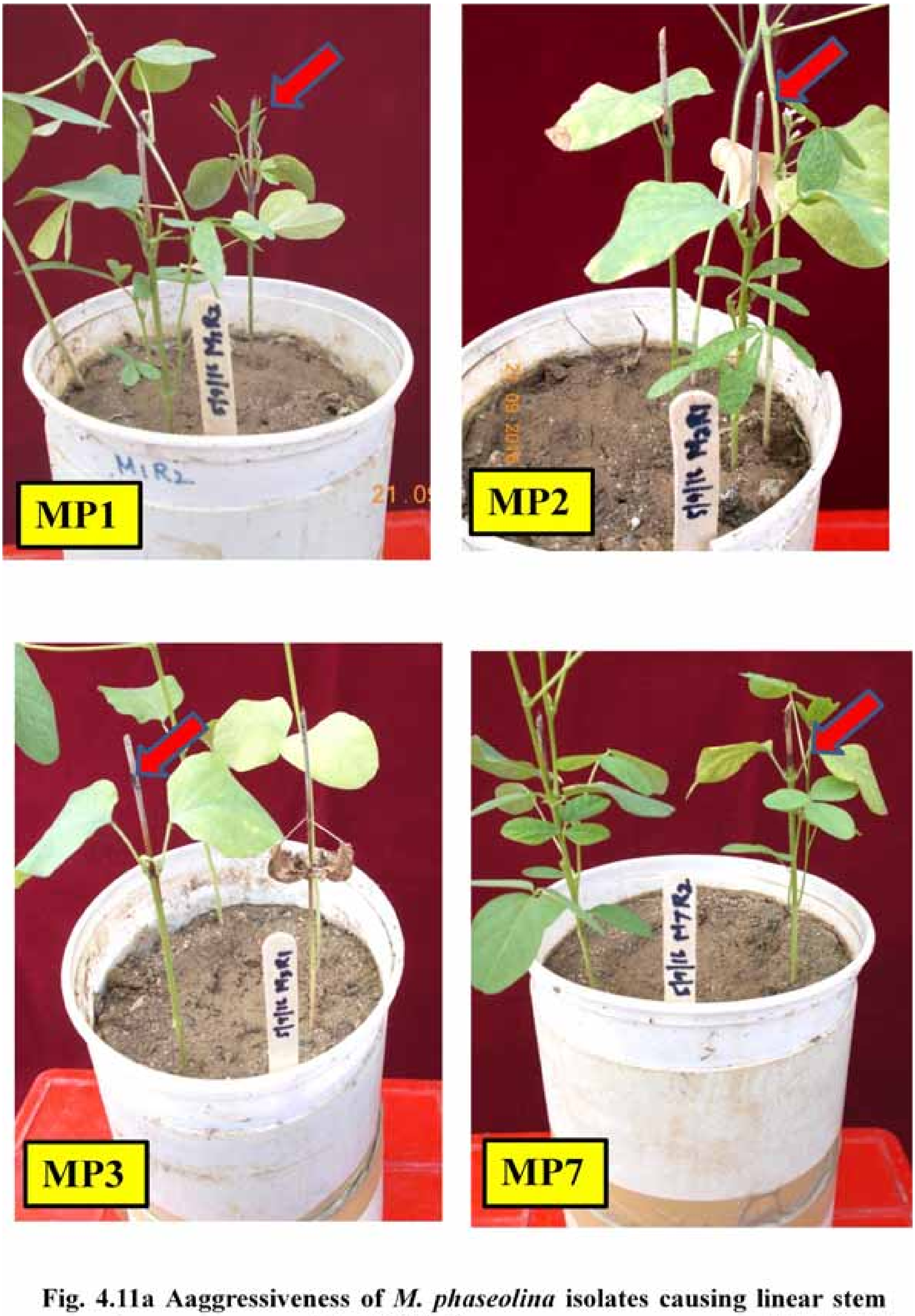

Fig. 4.11a Aaggressiveness of *M. phaseolina* isolates causing linear stem necrosis on soybean variety JS 97 -52

Fig. 4.11b Aaggressiveness of *M. phaseolina* isolates causing linear stem necrosis on soybean variety JS 97 -52

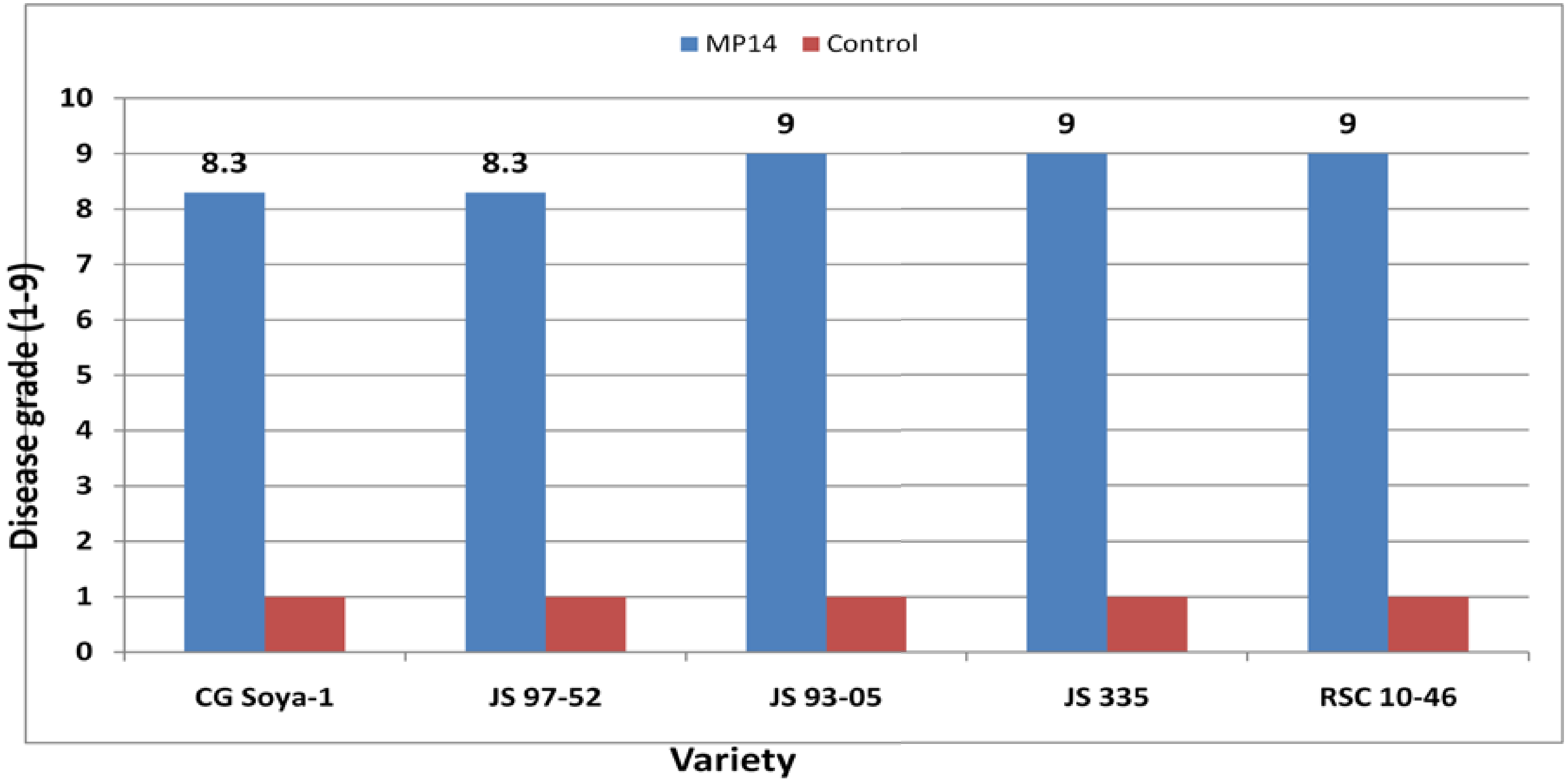

Fig. 4.12 Disease reaction performed by highly virulent isolates of *M. phaseolina* (MP14) by blotter paper technique on soybean varieties

Table 4.5: Aggressiveness of different isolates of *M. phaseolina* against different varieties of soybean under *in vitro* condition by blotter paper method

S.No	Isolate / Variety	MP1	MP2	MP3	MP4	MP5	MP6	MP7	MP8	MP9	MP10	MP11	MP12	MP13	MP14	MP15	Control	Mean
1	**CG Soya-1**	7.0	6.3	7.6	7.6	5.0	7.6	5.6	6.3	5.6	5.6	7.6	7.0	7.0	8.3	7.6	1.0	6.82
2	**JS 97-52**	7.6	7.0	5.6	8.3	5.6	8.3	6.3	7.0	6.3	7.6	5.6	5.6	5.6	8.3	7.6	1.0	6.87
3	**JS 93-05**	5.6	7.0	7.0	7.0	6.3	5.6	5.0	5.6	5.0	5.6	6.3	7.0	7.0	9.0	8.3	1.0	6.51
4	**JS 335**	8.3	7.6	7.0	7.6	5.6	5.0	7.6	7.0	7.0	7.6	6.3	8.3	6.3	9.0	8.3	1.0	7.27
5	**RSC 10-46**	7.0	7.6	5.6	7.0	6.3	8.3	6.3	6.3	5.6	7.0	5.6	7.6	5.0	9.0	7.0	1.0	6.78
	Mean	7.13	7.13	6.60	7.53	5.80	7.00	6.20	6.47	5.93	6.73	6.33	7.13	6.20	8.73	7.80	1.0	7.13

isolates behaved differently. Based on their aggressiveness the isolates were classified in two group MP1, MP2, MP3, MP4, MP5, MP6, MP7, MP8, MP9, MP10, MP11, MP12 and MP13 produced clear but small lesion on roots with new roots free from infection. Second group comprised of MP14 and MP15 with many lesions on roots, completely discoloured and destroyed. While, control plants without inoculation scored grade 1 with no infection on roots

Individual disease response of all the *M. phaseolina* isolates was observed on soybean variety JS 335. Aggressiveness pattern of different *M. phaseolina* isolates behaved differently. Based on their aggressiveness the isolates were classified in two groups MP3, MP5, MP6, MP8, MP9, MP11 and MP13 produced clear but small lesion on roots with new roots free from infection. Second group comprised of MP1, MP2, MP4, MP7, MP10, MP12, MP14 and MP15 with many lesion on roots, completely discoloured and destroyed. While, control plants without inoculation scored grade 1 with no infection on roots.

Individual disease response of all the *M. phaseolina* isolates was observed on soybean variety RSC 10-46. Aggressiveness pattern of different *M. phaseolina* isolates behaved differently. Based on their aggressiveness the isolates were classified in two groups MP1, MP3, MP4, MP5, MP7, MP8, MP9, MP10, MP11, MP13 and MP15 produced clear but small lesion on roots with new roots free from infection. Second group comprised of MP2, MP6, MP12 and MP14 with many lesions on roots, completely discoloured and destroyed. While, control plants without inoculation scored grade 1 with no infection on roots.

Average performance of all the isolates on soybean variety revealed that MP14 found to be highly aggressive showing disease reaction (8.73) and less aggressive, isolate MP5 disease grade (5.80), Table 4.5. A positive correlation between the mycelium growth and aggressiveness of MP14 and MP5 is reported during present investigation. Gupta *et al.* (2012) also used blotter paper to screen chick pea against pathogen *R. bataticola* on the basis of disease reaction shown on plant. Further they also reported that fast growing isolates are highly virulent compare to slow growing isolates. According to Gupta *et al.* (2006) the varying degree of virulence among fourty chickpea isolates were observed, through blotter paper technique. The disease reaction of the isolates varied from 6.6 – 9.0 on a scale of 1-9.

4.6.1.2 Cut stem inoculation technique

The data presented in table 4.6 and Fig. 4.13-4.15 revealed that the isolates were variable in their aggressiveness. The disease reaction of all the fifteen isolates on cultivar CG Soya-1, JS 335 and JS 97-52 in the form of linear stem necrosis (4cm to 15cm) from the cut stem portion of soybean plant (Fig. 4.11 a&b). All the isolates of *M. phaseolina* categorized into less aggressive (4.0-6.9cm), aggressive (7.0-9.9cm) and highly aggressive (10.0- 14.0cm) on the basis of reaction shown on varieties.

The information found in the table 4.6 shows that disease response of all the *M. phaseolina* isolates on soybean variety CG Soya-1 revealed that isolates MP1, MP2, MP3, MP4, MP5, MP7, MP8, MP10, MP11 and MP15 had shown lesser disease incidence and kept in the category of less aggressive. Isolates MP9, MP12 and MP13 categorized into group two i.e. aggressive. Remaining two isolates MP6 and MP15 were placed in the third group highly aggressive.

Soybean variety JS 335 showed variable level of disease reaction after inoculation with all the fifteen isolates. Nine isolates MP1, MP2, MP3, MP4, MP5, MP7, MP8, MP10 and MP15 were categorized as less aggressive. Isolate MP3, MP9, MP11, MP12 and MP13 behaved aggressive. MP6 and MP14 found to be highly aggressive and caused higher disease incidence.

Disease reaction of all the isolates on the variety JS 97-52 classified into three groups. Disease reaction revealed that isolate MP1, MP2, MP3, MP4, MP5, MP7, MP10 and MP15 were categorized as less aggressive. Isolate MP8, MP9, MP11, MP12 and MP13 fall in the category of aggressive. Similar to earlier isolate MP6 and MP14 behaved highly aggressive.

Comparative study of all the isolates on three different variety revealed that isolate MP1, MP2, MP3, MP4, MP5, MP7, MP10 and MP15 showed les

Table 4.6: Aggressiveness of different isolates of *M. phaseolina* against different varieties of soybean under *in vitro* condition by cut stem inoculation method

Variety	CG Soya-1 Disease progress in (cm)			JS 335 Disease progress in (cm)			JS 97-52 Disease progress in (cm)		
Isolates	7^{th} day	9^{th} day	14^{th} day	7^{th} day	9^{th} day	14^{th} day	7^{th} day	9^{th} day	14^{th} day
MP1	4.10	4.5	4.9	4.8	5.5	6.1	5.5	5.5	6.4
MP2	4.6	4.9	5.2	4.9	4.9	5.0	5.0	5.2	5.2
MP3	4.3	5.0	5.4	3.7	4.7	8.1	4.5	5.0	5.7
MP4	4.7	5.2	6.7	4.9	5.6	5.6	4.8	5.3	5.3
MP5	4.4	5.2	5.2	4.9	5.4	5.4	4.9	5.4	5.4
MP6	6.3	7.3	11.0	6.6	7.6	11.3	6.3	7.8	10.0
MP7	4.7	5.2	5.8	4.8	5.6	5.6	4.9	5.4	6.0
MP8	4.2	5.2	5.9	4.8	5.4	6.6	4.9	5.3	7.2
MP9	4.9	7.0	9.0	5.0	6.4	8.5	4.9	6.8	8.8
MP10	5.4	6.1	6.3	4.9	5.8	6.7	5.0	5.6	6.3
MP11	4.6	5.1	6.9	4.6	6.0	7.7	4.9	6.5	7.6
MP12	5.7	6.7	7.8	5.6	7.0	8.1	5.0	6.1	7.3
MP13	6.7	7.9	8.5	6.3	7.5	7.8	5.4	6.5	7.3
MP14	8.3	12.8	15	7.3	12.2	14.5	7.1	9.8	14.3
MP15	3.8	4.7	5.5	4.0	4.7	5.9	4.2	5.0	5.9

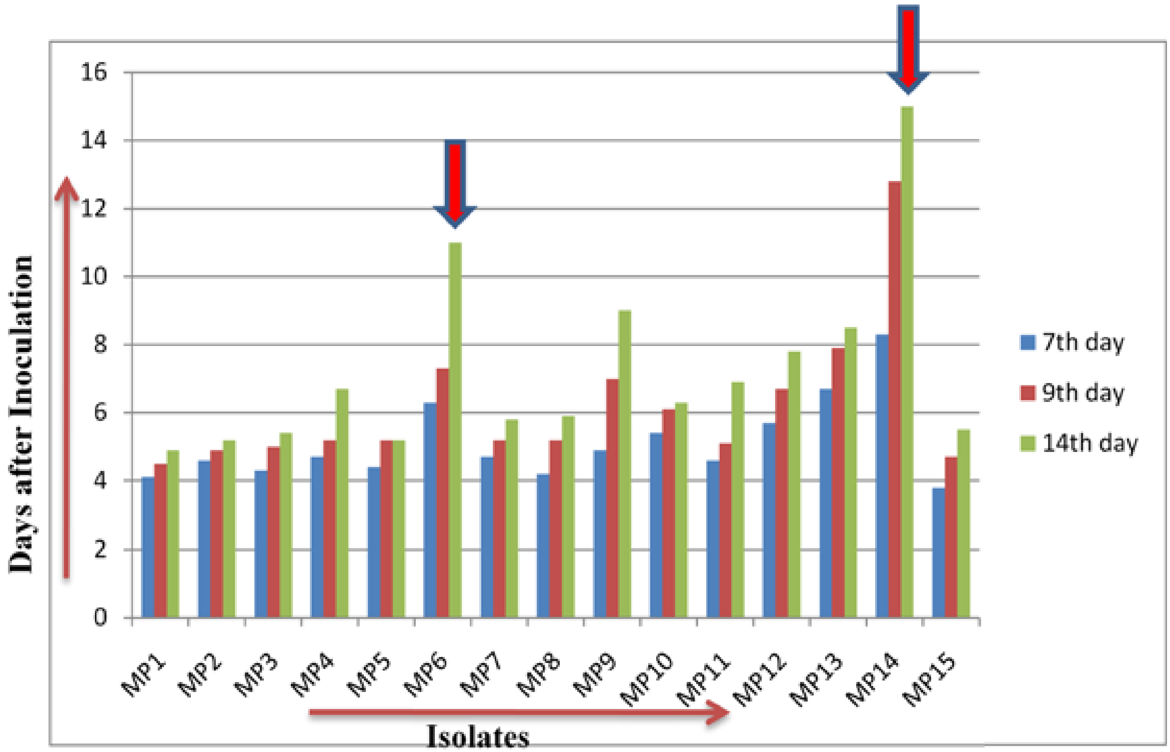

Fig 4.13 Isolates of *M. phaseolin*a showing progress of stem necrosis on soybean var. CG Soya -1

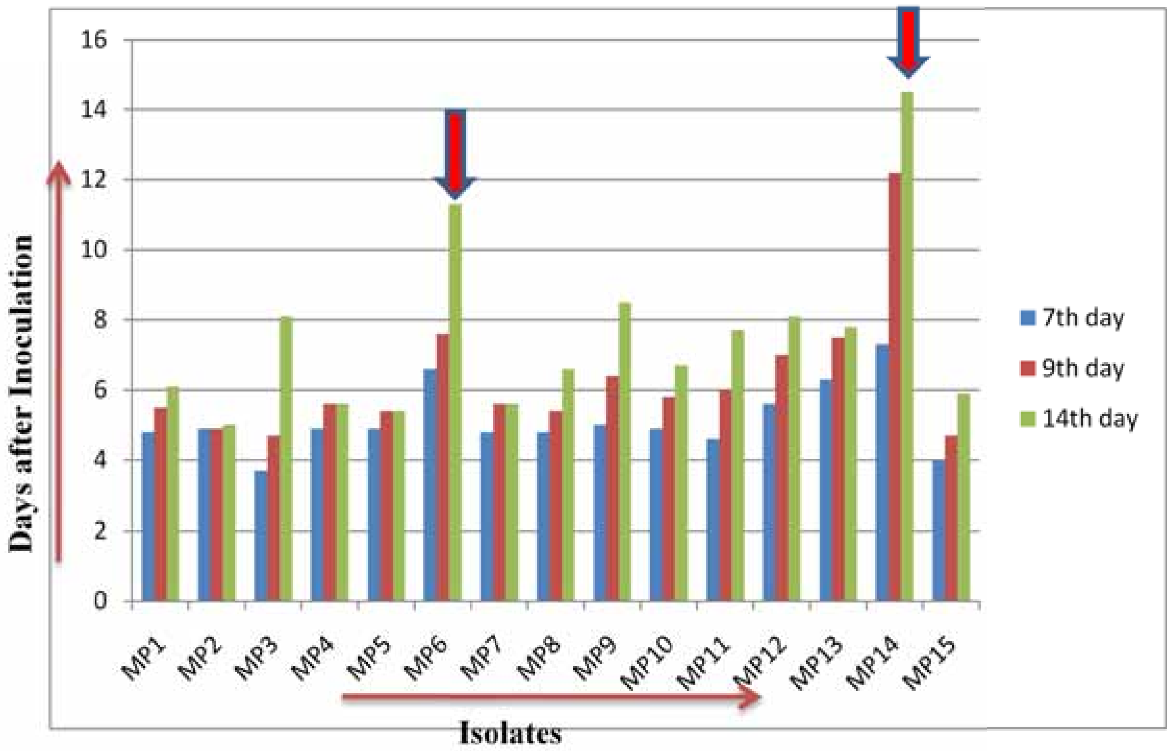

Fig 4.14 Isolates of *M. phaseolin*a showing progress of stem necrosis on soybean var. JS 335

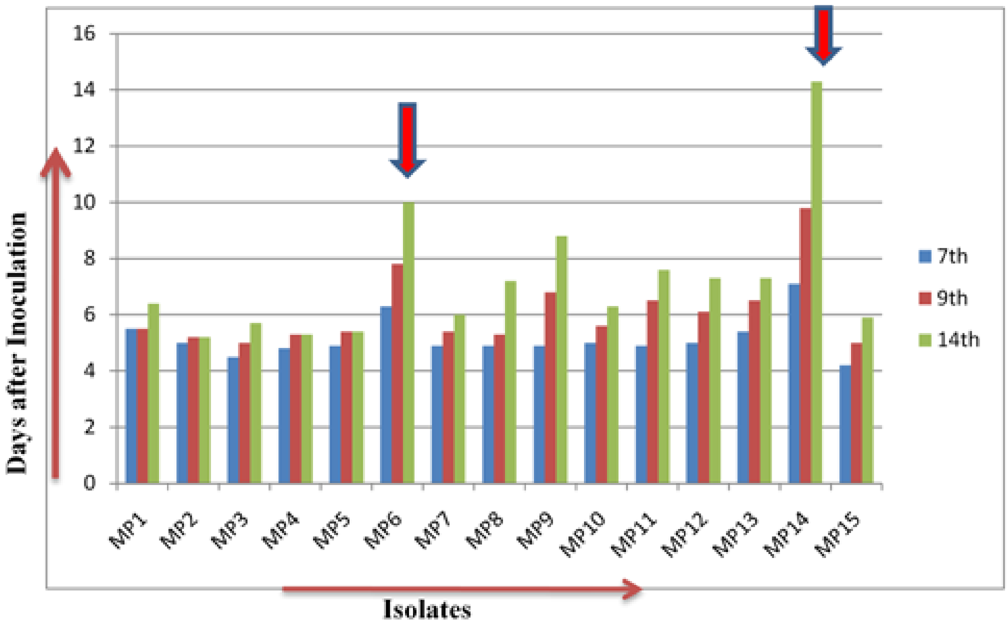

Fig 4.15 Isolates of *M. phaseolin*a showing progress of stem necrosis on soybean var. JS 97-52

aggressive reaction on all the soybean varieties. Isolate MP9, MP12 and MP13 showed aggressive reaction and MP6 and MP14 showed highly aggressive reaction. The entire fact is in agreement with the findings of Twizeyimana *et al.* (2012) who reported that the soybean genotypes had different levels of resistance to *M. phaseolina* infection and isolates had different level of aggressiveness on the basis of relative amount of necrosis caused by each isolates.

4.7 Diagnosis

Five healthy looking 60 days old soybean plants were collected from the farmer's field and each plant is considered as sample for the diagnosis experiment. Processing of plant sample for diagnosis experiment is given in section 3.6. Microscopic observation revealed that some of the healthy looking plants showed no sign of wilting symptom, but showed sclerotia of *M. phaseolina* during the diagnosis experiment. Data of microscopic observation are presented in table 4.7.

First sample and fifth sample showed presence of sclerotia in primary root, secondary root and stem portion, first 15 cm from the base of stem (Fig. 4.16 a-c). In the sample second and fourth microscopic sclerotia were located in the primary root and secondary root. Other plant tissue was not having sign of sclerotia and discoloration. Sample third was free from infection and showed no sign of micosclerotia and discolouration considered as healthy plant free from infection (Fig. 4.16c). Results of plant sample 1, 2, 4 and 5 indicated in table 4.7 showed that infection of *Macrophomina phaseolina* takes place at early stage of plant growth i.e. seedling stage but not showing any symptom of charcoal rot like wilting and yellowing of leaves. The results indicated that pathogen found in the hidden in plant parts become active under the influence of climatic condition that favours the disease development such as temperature in the range of 30-35 °C and low rainfall or no rainfall. The data presented in table also indicated different level of colonization of fungal mycelium in the plant parts.

Gangopadhyay *et al.* (1970) recovered the fungus from the radicles of a few seedlings. The fungus produced microsclerotia in 4% of symptomatic seeds after 2 day of incubation at 25 ± 2°C on acidified (pH 4.5) PDA. It was also observed that the microsclerotia developed near or adjacent to the seed coat, endosperm and hypodermis. The fungus, apparently, could penetrate and colonize soybean seeds without producing symptoms, but subsequently formed microsclerotia in asymptomatic seeds when conditions were favorable for seed germination. Gangopadhyay *et al.* (1973) observed *M. phaseolina* in cotyledons and seed coats from artificially infected seeds, which were plated separately on PDA following surface sterilization. Kunwar *et al.* (1986) observed that microsclerotia were formed in the cotyledons of asymptomatic seeds after 3–4 day of incubation and in the hypocotyl radical axis, after 4–5 day. Histological examination of symptomatic seeds showed that hyphae and microsclerotia were ecto- and endophytic, while hyphae were inter- and intracellular in tissues of the seed coat, endosperm and embryo. During germination of the asymptomatic seeds, the inoculum could invade the cotyledons and embryo within 3–5 day, and microsclerotia developed on such seeds.

4.8 Screening

4.8.1 Field screening of soybean germplasm

To find out the source of resistance, soybean entries were evaluated for their reaction against *M. phaseolina* under natural condition. These were evaluated as per standard evaluation system mentioned in section (3.7). The reactions of the entries are depicted in table 4.8 and 4.9. During the *kharif* 2015- 2016 total 237 germplasm lines were evaluated against the charcoal rot of soybean under natural field condition (Fig.4.17). Twenty four germplasm lines were found to be moderately resistant with disease incidence 1.1 to 10%. Eighty six germplasm were found to be moderately susceptible with disease incidence 10.1-25%. Maximum number of germplasm, 104 fall under the category of susceptible (25.1-50%). Twenty three germplasm is highly susceptible and more than 50 percent charcoal rot infected plant found under natural condition (table 4.8). The location severity index of charcoal rot of soybean was 6.06.

Screening of 237 entries screened earlier season were also screened during *kharif* season 2016-17. The data showed that entries fall under the two broad categories, moderately resistant and moderately susceptible. No entries were as

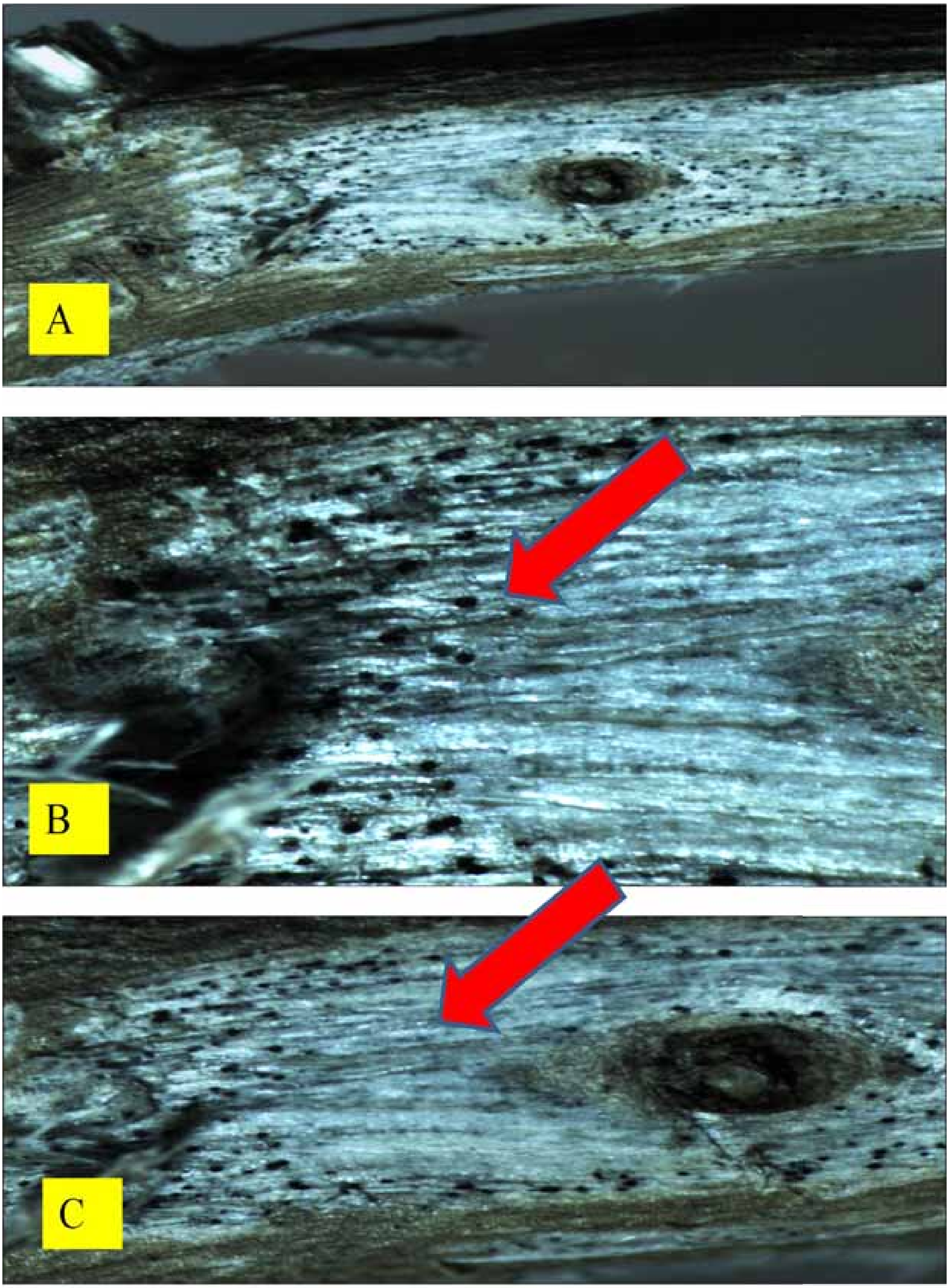

Fig. 4.16a: Microscopic observation of primary root ,sample showing minute black microsclerotia and blackening (A, B and C)

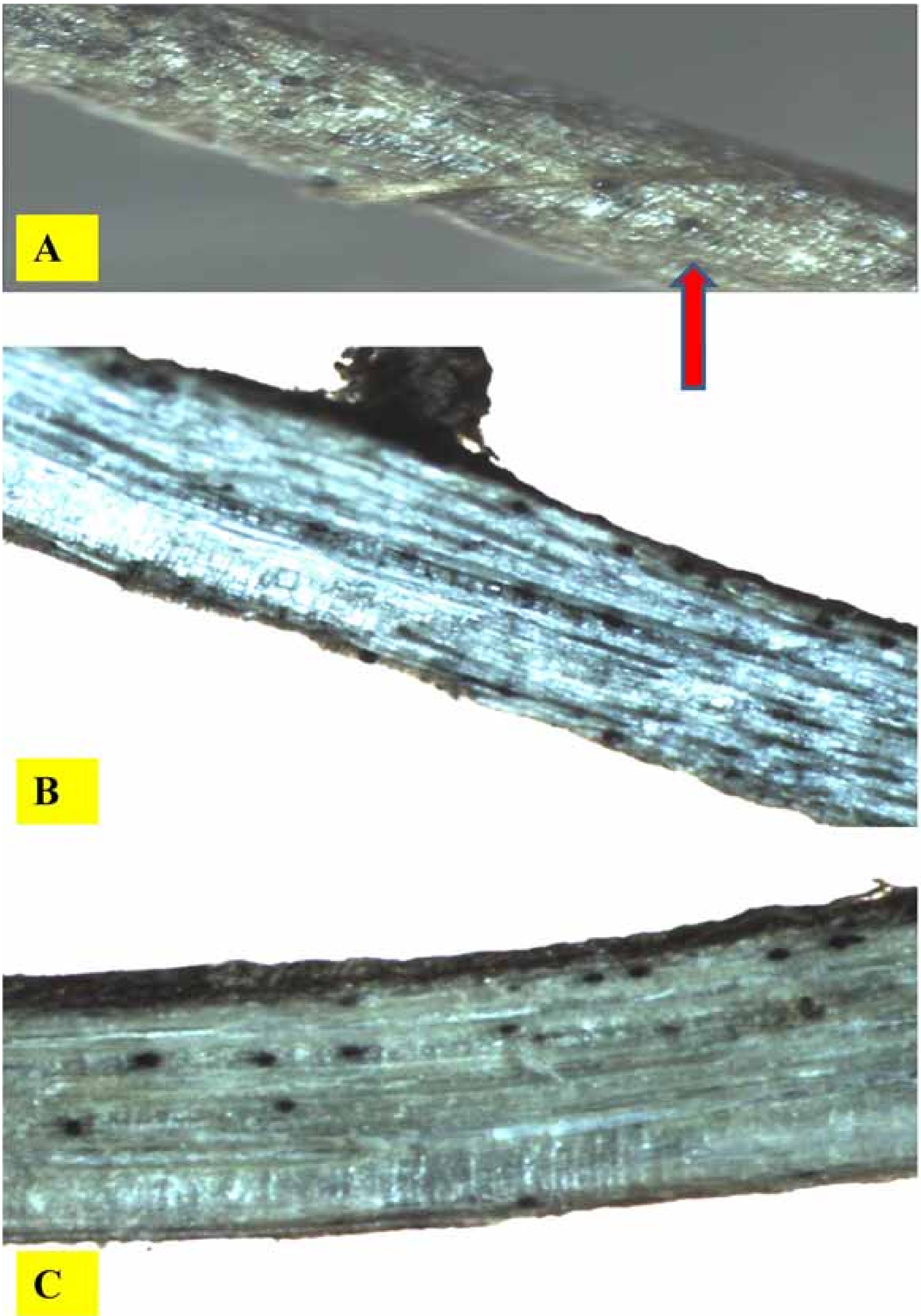

Fig. 4.16b : Microscopic observation of secondary root, sample showing minute black sclerotia on the surface(A) and after the sectioned root sample (B and C)

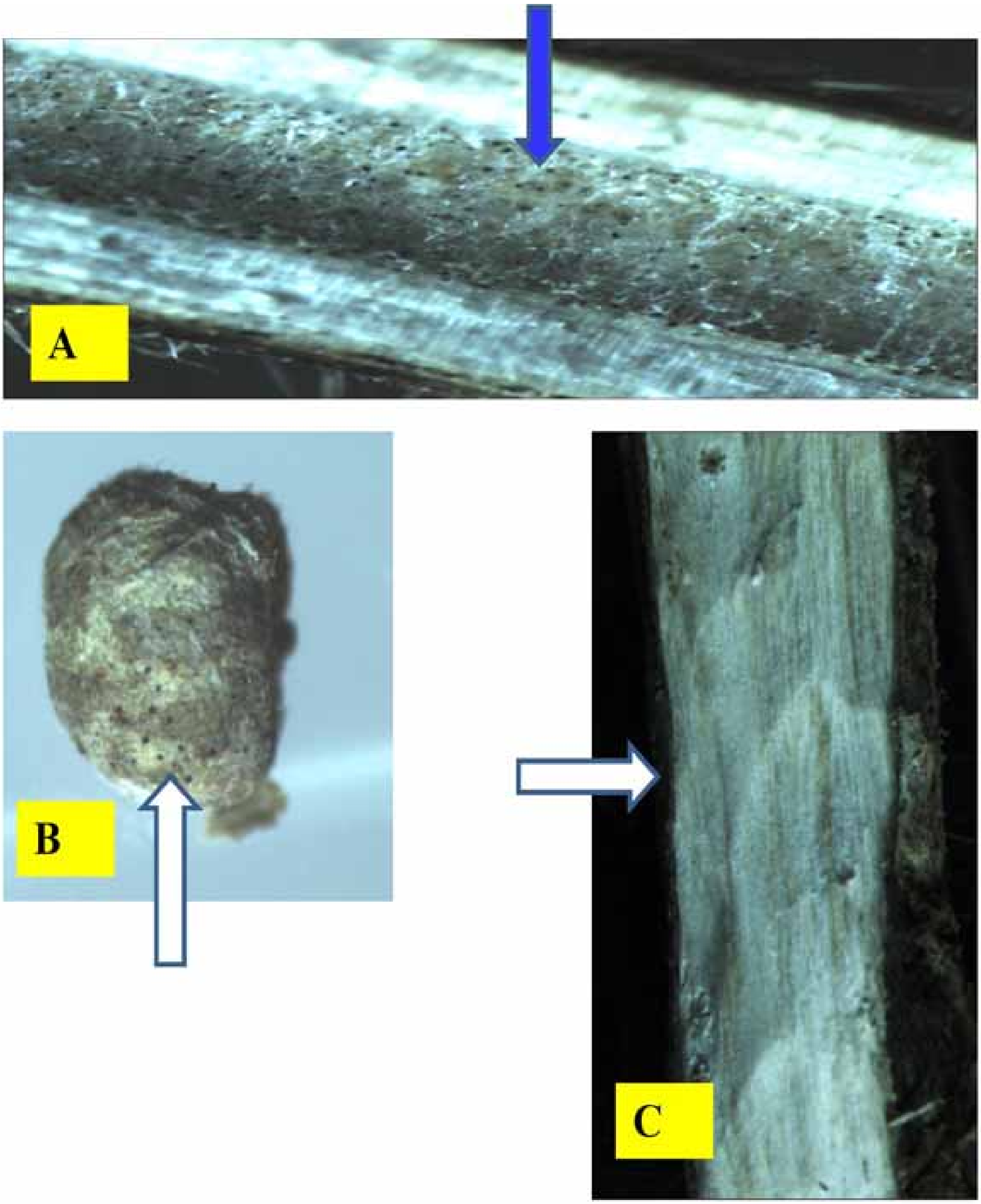

Fig. 4.16c : Microphotograph of microsclerotia found within the stem portion (A), on the surface of nitrogen fixing nodule(B) and healthy root sample showing no symptom of blackening and microsclerotia (C)

Table 4.7: Diagnosis of healthy looking soybean plants collected from farmers field and the localization of microsclerotia in the plant tissue

Sample .No.	Root portion		Stem portion		
	Primary root	Secondary root	First 15cm from base of stem	Second 15cm from base of stem	Third 15cm from base of stem
1	+	+	+	-	-
2	+	+	-	-	-
3	-	-	-	-	-
4	+	+	-	-	-
5	+	+	+	-	-

(+) Sclerotia found

(-) Sclerotia not foud

Table 4.8: Reaction of soybean genotypes to *M. phaseolina* under natural conditions during *kharif* 2015

S.No	Grade	Disease reaction	Name of the genotypes
1	**0**	**Absolute Resistant**	Nil
2	**1**	**Highly Resistant**	Nil
3	**3**	**Moderately Resistant**	PK-10-24, JS-15-14, GP-448, JS-80-21, MACS-1336, PK-10-29, SL-599, JS-15-14, PS-564, Seelajit, EC-34117, MAUS-71, MAUS-145, NRC-2008-G-1-12, NRC-2007-A-3-1, NRC-2007-12-7-2, NRC-96-02-02, NRC-95-08-01, AMS-148, EC-125788, EC-232019, EC-391336, Himso-175, JS-20-74
4	**5**	**Moderately Susceptible**	JS 335, BRAGG, BIRSA SOYA, MACS-58, NRC-20, PK-472, NRC-2, JS-97, JS 415, JS-79-263, JS-80-5417, JS- 92-1418, SL-328, MAUS-47, PB-1, PK-12-41, PK-515, JS-128-5, JS-148, SL-518, NRC-57, MACS-756, JS(SH)96-31, PK-317, H6P20, NRC-2006-4-13, NRC-2006-F-2-2, NRC-2006-A-23, NRC-2007-B-1-19, NRC-2007-1-3, NRC-2011-E-2-1-7, NRC-2012-J- 2-2-1, NRC-2011-A-3-7, Delhi-15, Delhi-16, Delhi-17, NRC-2011-H-4-10, NRC-2011- F-1-15, NRC-2011-G-3-13, NRC-2011-E-2-1-9-1, NRC-2011-E-4-11-1-1, JSM-117-4, NRC-2011-C-4- 12, NRC-2012-M-127-1, NRC-2012-M-127-3, NRC- 2012-F-1-18-3, NRC-20-G-1-2-2-5, JS-18-13,

			HIMSO-15-21, JS-82-180, NRC-37, MAUS-144, MAUS-754, MAUS-61-2, JS-93-05, JS-97-52, JS-20-21, JS-20-25, JS- 20-27, JS-20-30, JS-20-79, JS-20-87, H5P8, NRC-95-05-03, H5P4, NRC-95- 03-03, NRC-95-06-03, NRC-95-03-02, NRC-95-12- 01, NRC-95-03-01, NRC-96-05-03, H6P21, H3P23, Delhi-1, Delhi-2, EC-685243, JS-20-42, JS-20-55, JS-20-71, NRC-2012- A-3-2-1-1, NRC-2012-E-2-6-4-1, PRAB-1, PI-283327, MAUS-14-2, JS-20-76, RVS-2008-8
5	**7**	**Susceptible**	EC-389179, JS-99-76, PS-10-92, PK-416, KB-165, NRC-56, DS- 98-14, PK-13-14, PKS-7, MACS-124, JS/SH/94-21, PK-262, JS-16-40, B-S-97-12, EC- 389392, , NRC-2006-C-7, NRC-2007-G-1-15, NRC-2OO8-G-1-12, VS-495, VS- 2004-18, VS-2005-19, Delhi-14, AMS-60-2-34, Cat-2502, Cat-3299, EC-2581, EC-34078, EC-39491, EC-100027, EC-118443, MACS-798, DS-228, MACS-693, MACS-694, JS-98-21, MMSS-36, EC-391181, JS-90-41, NRC-2007-L-1-5, NRC-2007-J-3, NRC-2006-M-6, NRC-2007-B-2-4, NRC-2007-4-1-36, NRC-2007-C-1-5, VS-2004-9, VS-2004-114, VS-2004-18, VS-2004-13, VS-2173, VLS-47,

			VS-2005-12, VS-2005-21, VS-2005-22, Delhi-3, Delhi-4, Delhi-5, Delhi-7, Delhi-8, Delhi-9, Delhi-10, Delhi-11, Delhi-12, Delhi-13, Delhi-18, Delhi-19, Delhi-20, Delhi-21, Delhi-22, Delhi-23, Delhi-24, Delhi-25, Delhi-26, AMS-50-B, AMS-MB-5-18, Cat-2722, EC-389148, EC-457161, EC-685250, EC-685255, JS-20-35, JS-20-59, JS-20-72, NRC-96-03-02, NRC-2006-A-4-12, NRC-2006-4-1-2, NRC-2006-I-1, NRC-2006-J-7, NRC-2006-C-7, NRC-2008-B-3-17, NRC- 2007-A-2-3, NRC-2008-G-2-6, NRC-2008-F-6, NRC- 2008-J-8-1-1, NRC-2011-C-5-5, NRC-2011-B-1-8-1- 43,NRC-2011-F-1-23, NRC-2012-B-6-3-1-4-3, NRC-2012-M-127-2-3, NRC-2012-I-1-6, NRC-2012-12-1-9,NRC-2011-C-N-11, VS-2157, VS- 2002-9, VS-2004-19
6	**9**	**Highly Susceptible**	VS-2005-28, JS-20-78, JSM-224, MAUS-703, NRC-2012-G-3-14-1, NRC-95-10-03, Delhi-6, Delhi-11, Delhi-26, VLS-2, Cat-2388, PK-12-25, SL-517, NRC-95-02-03, EC-391167, H3P12, JSM-258, PK-327, B-458, RAUS-5, H5P3, GP-393, EC-15966

Fig. 4.17: Screening of soybean genotypes against charcoal rot , under natural field condition

Table 4.9: Reaction of soybean genotypes to *M. phaseolina* under natural conditions during 2016-17

S.No	Grade	Disease reaction	Name of the genotypes
1	0	**Absolute Resistant**	NIL
2	1	**Highly Resistant**	NIL
3	3	**Moderately Resistant**	PK-10-24, JS-15-14, GP-448, JS-80-21, MACS-1336, PK-10-29, SL-599, JS-15-14, PS-564, Seelajit, EC-34117, MAUS-71, MAUS-145, NRC-2008-G-1-12, NRC-2007-A-3-1, NRC-2007-12-7-2, NRC-96-02-02, NRC-95-08-01, AMS-148, EC-125788, EC-232019, EC-391336, Himso-175, JS-20-74, MAUS-47, PB-1, SL-518, H6P20, NRC-2006-F-2-2, Delhi-17, NRC-2012-M-127-3, NRC- 2012-F-1-18-3, JS-97-52, JS-20-21, H5P4, NRC-95-03-01, NRC-2012-E-2-6-4-1, JS-99-76, MACS-124, NRC-2007-G-1-15, AMS-60-2-34, EC-100027, EC-391181, NRC-2007-C-1-5, Cat-2722 and VS-2004-19
4	5	**Moderately Susceptible**	JS-335, BRAGG, BIRSA SOYA, MACS-58, NRC-20, PK-472, NRC- 2, JS-97, JS 415, JS-79-263, JS-80-5417, JS- 92-1418, SL-328, PK-12-41, PK-515, JS-128-5, JS-148, NRC-57, MACS-756, JS(SH)96-31, PK-317, NRC-2006-4-13, NRC-2006-A-23, NRC-2007-B-1-19, NRC-2007-1-3, NRC-2011-E-2-1-7, NRC-2012-J- 2-2-1, NRC-2011-A-3-7, Delhi-15, Delhi-16, NRC-2011-H-4-10, NRC-2011- F-

1-15, NRC-2011-G-3-13, NRC-2011-E-2-1-9-1, NRC-2011-E-4-11-1-1, JSM-117-4, NRC-2011-C-4- 12, NRC-2012-M-127-1, NRC-20-G-1-2-2-5, JS-18-13, HIMSO-15-21, JS-82-180, NRC-37, MAUS-144, MAUS-754, MAUS-61-2, JS-93-05, JS-20-25, JS- 20-27, JS-20-30, JS-20-79, JS-20-87, H5P8, NRC-95-05-03, NRC-95- 03-03, NRC-95-06-03, NRC-95-03-02, NRC-95-12- 01, NRC-96-05-03, H6P21, H3P23, Delhi-1, Delhi-2, EC-685243, JS-20-42, JS-20-55, JS-20-71, NRC-2012- A-3-2-1-1, PRAB-1, PI- 283327, MAUS-14-2, JS-20-76, RVS-2008-8, EC-389179, PS-10-92, PK-416, KB-165, NRC-56, DS-98-14, PK-13-14, PKS-7, JS/SH/94-21, PK-262, JS-16-40, B-S-97-12, EC-389392, , NRC-2006-C-7, NRC-2OO8-G-1-12, VS-495, VS- 2004-18, VS-2005-19, Delhi-14, Cat-2502, Cat-3299, EC-2581, EC-34078, EC-39491, EC-118443, MACS-798, DS-228, MACS-693, MACS-694, JS-98-21, MMSS-36, JS-90-41, NRC-2007-L-1-5, NRC-2007-J-3, NRC-2006-M-6, NRC-2007-B-2-4, NRC-2007-4-1-36, VS-2004-9, VS-2004-114, VS-2004-18, VS-2004-13, VS-2173, VLS-47, VS-2005-12, VS-2005-21, VS-2005-22, Delhi-3, Delhi-4, Delhi-5, Delhi-7, Delhi-8, Delhi-9, Delhi-10, Delhi- 11, Delhi-12, Delhi-13,

			Delhi-18, Delhi-19, Delhi-20, Delhi-21, Delhi-22, Delhi-23, Delhi-24, Delhi-25, Delhi-26, AMS-50-B, AMS-MB-5-18, EC-389148, EC-457161, EC-685250, EC-685255, JS-20-35, JS-20-59, JS-20-72, NRC-96-03-02, NRC-2006-A-4-12, NRC-2006-4-1-2, NRC-2006-I-1, NRC-2006-J-7, NRC-2006-C-7, NRC-2008-B-3-17, NRC- 2007-A-2-3, NRC-2008-G-2-6, NRC-2008-F-6, NRC- 2008-J-8-1-1, NRC-2011-C-5-5, NRC-2011-B-1-8-143, NRC-2011-F-1-23, NRC-2012-B-6-3-1-4-3, NRC-2012-M-127-2-3, NRC-2012-I-1-6, NRC-2012-12-1-9, NRC-2011-C-N-11, VS-2157, VS-2002-9, VS-2005-28, JS-20-78, JSM-224, MAUS-703, NRC-2012-G-3-14-1, NRC-95-10-03, Delhi-6, Delhi-11, Delhi-26, VLS-2, Cat-2388, PK-12-25, SL-517, NRC-95-02-03, EC-391167, H3P12, JSM-258, PK-327, B-458, RAUS-5, H5P3, GP-393, EC-15966
5	**7**	**Susceptible**	NIL
6	**9**	**Highly Susceptible**	NIL

Table 4.10: Soybean genotypes found moderately resistant during both the *kharif* saeasons 2015 and 2016

S.No.	Grade	Disease reaction	Name of the genotypes
1	3	Moderately Resistant	PK-10-24, JS-15-14, GP-448, JS-80-21, MACS-1336, PK-10-29, SL-599, JS-15-14, PS-564, Seelajit, EC-34117, MAUS-71, MAUS-145, NRC-2008-G-1-12, NRC-2007-A-3-1, NRC-2007-12-7-2, NRC-96-02-02, NRC-95-08-01, AMS-148, EC-125788, EC-232019, EC-391336, Himso-175, JS-20-74

Table 4.11: Reaction of soybean entries to *M. phaseolina* under natural conditions during *kharif* 2015

S.No	Grade	Disease reaction	Name of the genotypes
1	0	Absolute Resistant	Nil
2	1	Highly Resistant	Nil
3	3	Moderately Resistant	MACS 58, NRC 123, RVS 2010-1, JS 20-94, PS 1570, JS 71-05
4	5	Moderately Susceptible	JS 75-46, PK 262, PK 472, JS 93-05, Punjab1, Bragg, KHSB 2, VLS 58, Shivalik, PS 1569, RSC 10-13, MACS 1491, NRC 117, AMS 115, KDS 975, NRC 119, DSb 29, DS 3104, MAUS 710, TS 72, DS 3103, NRC 118, PS 1572, Himso 1686, KDS 775, VLS 90, JS 72-44, KDS754, NRS 122, KBS 24-2014, SL 1074, NRC 120, TS 69, MACS 1488, NRC 121
5	7	Susceptible	JS 72-280, Monetta, DSb 30-2, VLS 91, MACS 1480, AMS100-1, JS 335, RVS 2010-2, RSC 10-29, MAUS 740, JS 20-116, BAUS 72, VLB 202, NRC 7, RSC 10-29, AMS 1001
6	9	Highly Susceptible	Nil

absolute resistant, highly resistant, susceptible and highly susceptible. Out of 237 entries, 46 soybean entries behaved as moderately resistant (1.1 to 10% mortality) and majority 191 germplasm fall in the category of moderately susceptible (10.1 to 25% mortality) table 4.9. The location severity index of charcoal rot was 4.61.

Two year germplasm screening data revealed that significant variation was found in the soybean entries. Twenty four germplasm lines found were moderately resistant against charcoal rot in *kharif* 2015 and *kharif* 2016 (table 4.10). This may be due to the weather parameter such as maximum and minimum temperature, rainfall and relative humidity that influenced the disease development. During the *kharif* 2015 state recorded less rainfall and high temperature, seems favourable for the disease development. The mean average temperature recorded during grain filling to pod maturation was 33°C. Earlier report, revealed that dry conditions favour survival of microsclerotia in the soil, but mycelial growth and infection require moist conditions and are favoured by a temperature above 27°C (Hagedorn 1991). A high level of root infection can occur before reproductive development if there is a preponderance of hot and dry weather early in the growing season (Olaya and Abawi 1993). It was also observed that the population density of *M. phaseolina* increased slowly from the V5 to R6 growth stages and then rapidly from the R6 to R7 growth stages (Mengistu *et al.* .2011). Those 24 entries which were moderately resistant during the period of *kharif* 2015 could be used for further screening and testing for identification of resistant source against charcoal rot of soybean.

4.8.2 Field screening of varietal entries

The data of varietal performance of fifty seven varieties presented in table 4.11 indicated that during *kharif* 2015-16 six entries *viz.,* MACS 58, NRC 123, RVS 2010-1, JS 20-94, PS 1570, JS 71-05 were found to be moderately resistant. Thirty five entries namely, JS 75-46, PK 262, PK 472, JS 93-05, Punjab1, Bragg, KHSB 2, VLS 58, Shivalik, PS 1569, RSC 10-13, MACS 1491, NRC 117, AMS 115, KDS 975, NRC 119, DSb 29, DS 3104, MAUS 710, TS 72, DS 3103, NRC 118, PS 1572, Himso 1686, KDS 775, VLS 90, JS 72-44, KDS754, NRS 122, KBS 24-2014, SL 1074, NRC 120, TS 69, MACS 1488 and NRC 121 were found

Table 4.12: Reaction of soybean entries to *M. phaseolina* under natural conditions during kharif 2016

S.No	Grade	Disease reaction	Name of the genotypes
1	**0**	**Absolute Resistant**	Nil
2	**1**	**Highly Resistant**	Nil
3	**3**	**Moderately Resistant**	MACS 58, NRC 123, RVS 2010-1, JS 20-94, PS 1570, JS 71-05, JS 75-46, PK 262, PK 472, JS 93-05 , Punjab1, Bragg, KHSB 2, VLS 58, Shivalik, PS 1569, RSC 10-13, MACS 1491, AMS 115, KDS 975, NRC 119, DSb 29, DS 3104, MAUS 710, TS 72, DS 3103, NRC 118, PS 1572, Himso 1686, KDS 775, VLS 90, JS 72-44, KDS754, RSC 10-29, NRS 122 , KBS 24-2014, SL 1074, NRC 120, TS 69, MACS 1488, NRC 121
4	**5**	**Moderately Susceptible**	JS 72-280, Monetta, DSb 30-2, VLS 91, MACS 1480, AMS100-1, JS 335, RVS 2010-2, RSC 10-29, MAUS 740, JS 20-116, BAUS 72, VLB 202, PS-1572, SL-1074, PS-1569, RVS-2010-1, NRC-117, PS-1556, DS-3101, VLS-89, SL-1028
5	**7**	**Susceptible**	**Nil**
6	**9**	**Highly Susceptible**	**Nil**

Table 4.13: Soybean entries found moderately resistant during both the *kharif* 2015 and 2016

S.No.	Grade	Disease reaction	Name of the genotypes
1	3	Moderately Resistant	MACS 58, NRC 123, RVS 2010-1, JS 20-94, PS 1570, JS 71-05

to be moderately susceptible. Sixteen varities *viz.,* JS 72-280, Monetta, DSb 30-2, VLS 91, MACS 1480, AMS100-1, JS 335, RVS 2010-2, RSC 10-29, MAUS 740, JS 20-116, BAUS 72, VLB 202, NRC 7, RSC 10-29, AMS 1001 were susceptible. None of the entries found to be absolute resistant, highly resistant and highly susceptible. The location severity index of charcoal rot was 5.35.

Varietal screening of soybean during *kharif* 2016-17 found that fourty two entries namely MACS 58, NRC 123, RVS 2010-1, JS 20-94, PS 1570, JS 71-05, JS 75-46, PK 262, PK 472, JS 93-05 , Punjab1, Bragg, KHSB 2, VLS 58, Shivalik, PS 1569, RSC 10-13, MACS 1491, AMS 115, KDS 975, NRC 119, DSb 29, DS 3104, MAUS 710, TS 72, DS 3103, NRC 118, PS 1572, Himso 1686, KDS 775, VLS 90, JS 72-44, KDS 754, RSC 10-29, NRS 122, KBS 24-2014, SL 1074, NRC 120, TS 69, MACS1488 and NRC 121 were found to be moderately resistant and twenty two entries *viz.,* JS 72-280, Monetta, DSb 30-2, VLS 91, MACS 1480, AMS100-1, JS 335, RVS 2010-2, RSC 10-29, MAUS 740, JS 20-116, BAUS 72, VLB 202, PS-1572, SL- 1074, PS-1569, RVS-2010-1, NRC-117, PS-1556, DS-3101, VLS-89 and SL-1028 were found to be moderately susceptible. None of the variety was found to be absolute resistant, highly resistant, susceptible and highly susceptible (table 4.12). The location severity index of charcoal rot was 3.84.

Two year screening data revealed that significant variation was in the entries. Six entries was found moderately resistant against charcoal rot in *kharif* 2015 and *kharif* 2016 (table 4.13). This may be due to the variation in the weather condition. The results showed that the period of *kharif* 2015 was more suitable for screening of soybean entries because the weather parameter such as mean low rainfall and average temperature favoured the disease development and made most entries susceptible. The disease incidence during *kharif* 2016-17 was low as compared to *kharif* 2015-16. The weather parameters such as high rainfall and low average temperature were less favourable for disease development. That may be reason that more number of entries fall in the categories of moderately resistant and less number in the categories of moderately susceptible.

Smith, (1997) conducted field screening of twenty four commercial and experimental soybean cultivars from early group III to late group IV for their reaction to *Macrophomina phaseolina* from 1992 to 1994 for identification of resistance and susceptibility to *Macrophomina phaseolina.* Cultivars were planted on two dates to assess the effect of environment on yield and lower stem and taproot colonization by the fungus at growth stage R7. Based on seed yields and the levels of lower stem and taproot colonization by *M. phaseolina*, four cultivars Asgrow 4715, Delta Pineland 3478, Hamilton, and Jackson II—were rated moderately resistant to *M. phaseolina*. Seed yields and *M. phaseolina* levels in host tissues were affected by planting date, relative maturity class, *H. glycines* response, and the environmental conditions that prevailed over the 3 years of the study. Mengistu *et al.* (2011) conducted experiment to measure the seasonal progress of charcoal rot (caused by *Macrophomina phaseolina*) over two growing seasons in four separate experiments: irrigated infested, irrigated non-infested, non-irrigated infested, and non-irrigated noninfested. Yield loss due to charcoal rot ranged from 6 to 33% in irrigated environments. Yield loss due to charcoal rot was consistently measured in all paired comparisons in irrigated environments, suggesting that charcoal rot can be an important disease in irrigated environments also.

4.9 Management of charcoal rot

4.9.1 *In vitro* condition

4.9.1.1 Bioefficacy of *Trichoderma viride* against *Macrophomina phaseolina*

The antagonist *Trichoderma viride* was evaluated for its antagonistic effect against *M .phaseolina* under *in vitro* conditions by dual culture technique as explained in materials and methods (section 3.8.1.1). Inhibition zone in mm was recorded and percent inhibition was calculated and the results obtained are presented in (table 4.14, Fig. 4.18 and 4.28).

It was clear from the data that *T. viride* reduced the radial growth of all the fifteen isolates of *M. phaseolina*. The antagonist was used to check the growth of *M. phaseolina* either by overgrowing or by exhibiting inhibition zones (Fig. 4.18). Maximum radial growth inhibition (59.72%) was observed in the isolate MP5 followed by MP3 (59.40%), MP11 (57.72%), MP7 (57.50%), MP10 (57.41%), MP9 (55.67%), MP15 (56.16%), MP6 (52.21%), MP4 (51.38%), MP12 (44.72%), MP2 (42.47%,), MP13 (42.13%), MP1 (39.45%), MP14 (37.52%) and MP8 (32.55%).

Data presented in Table 4.14 indicated that MP3 (59.40%), MP11 (57.72%), MP7 (57.50%) and MP10 (57.41%) were statistically at par with MP5. The minimum percent of inhibition was observed in the isolate MP8(32.55%). Earlier Manjunatha *et al*. (2013) isolated six *T. viride* isolates and evaluated their antagonistic efficacy against *M. phaseolina*, the cause of root rot of chickpea using the dual culture technique. All the isolates were found to cause significant reduction in fungal growth when compared to the control. Kumar *et al.* (2013) also reported the inhibition of *M. phaseolina* mycelium growth (per cent) by *T. harzianum, T. viride, T. atroviride* were varied from 61.1 to 70.1, 58.6 to 66.6, 52.0 to 63.1 respectively. *T. viride* showed significant antifungal activities by inhibiting the mycelia radial growth of *M. phaseolina* by 71.42% (Doley and Jite, 2012). *T. viride* exhibited reduction in mycelial growth of *M. phaseolina* by 44.94% (Ashwini *et al*., 2014). Sharma *et al*. (2012) also reported the antagonistic effect of *T. harzianum* and *T. viride* against *M. phaseolina*. Kaushal (2008) reported that *T. harzianum* was effective in inhibiting the mycelial growth of *R. bataticola* the causal organism of chickpea dry root rot.

4.9.1.2 Evaluation of different botanicals against *M. phaseolina*

Six different botanicals at three different concentrations (5%, 10% and 15%) were tested under *in-vitro* condition against highly virulent isolate MP14 and observation on inhibition of radial growth were recorded and presented in table 4.15, Fig. 4.19a & b and 4.29. Data indicate that except ginger extract and castor oil all treatments were signicantly superior in reducing the mycelial growth in comparison to control.

The growth inhibition of *M. phaseolina* at 5 percent concentration of different botanicals ranged from 100 to 0 percent after 3 days of inoculation. The maximum inhibition of mycelial growth was recorded in the neem oil followed by garlic (58.50%), neem oil + castor oil + karanj oil (29.93%) and karanj oil

(17.03%). Ginger extract and castor oil showed no inhibition on the radial growth of mycelium and full growth recorded after 3 days of inoculation as compared to control (table 4.15).

The percent growth inhibition of *M. phaseolina* at 10 percent concentration ranged from 100 percent to 0 percent inhibition after 3 days of inoculation. Complete inhibition of mycelial growth was recorded in the neem oil followed by garlic extract (77.65%), neem oil + castor oil + karanj oil (74.81%) and karanj oil (22.96%). Ginger extract and castor oil showed no inhibition on the radial growth of mycelium and full growth recorded after 3 days of inoculation as compared to control (table 4.15).

The growth inhibition of *M. phaseolina* at 15 percent strength ranged from 100 percent to no inhibition after 3 days of inoculation. Complete inhibition of mycelium growth was recorded in the neem oil and neem oil + castor oil + karanj oil which was followed by garlic extract (83.77%) and karanj oil (30.74%). Average growth inhibition of all the treatments indicated that maximum growth inhibition was recorded in neem oil followed by garlic extract, neem oil + castor oil + karanj oil, and karanj oil. Similarly ginger extract and castor oil is not found suitable for inhibition of mycelium growth at all the concentrations (table 4.8). The present results are confirmed with the findings of Dhingani *et al*, (2013) who reported that the inhibition of *Macrophomina phaseolina* mycelium is done by botanicals. The extract of garlic cloves (*Allium sativum* L.) was excellent with maximum inhibition (73 %) of mycelia growth and sclerotial formation. Tandel *et al*. (2010) conducted the *in vitro* poison food technique experiment against *M. phaseolina* using eleven different phytoextracts. They revealed that the extract of onion bulb produced maximum inhibition (98.14%) followed by extract of acacia, ginger, neem, garlic and karanj. The sclerotial formation was also not taken place in all these phytoextracts. Contrary to this, ginger extract showed no inhibition on the growth of *M. phaseolina* and is not suitable for seed treatment of soybean.

4.9.1.3 Evaluation of different fungicides against *M. phaseolina*

Efficacy of seven fungicides viz., tebuconazole, hexaconazole, trifloxystrobin + tebuconazole, carbendazim + mancozeb, carboxin + thiram and carbendazim was tested under *in vitro* condition against virulent isolates of *Macrophomina phaseolina* (MP14) at two different concentrations *viz.*, 100 ppm and 200 ppm by employing poisoned food technique as explained under materials and methods (section 3.8.1.3). Observations on colony diameter of the fungus were recorded after three day of inoculation. The percent inhibition of *M. phaseolina* mycelium at different concentrations was calculated and presented in the table 4.16, Fig. 4.20 and 4.30. All the fungicides are found suitable for radial growth inhibition of *M. phaseolina*. There was a significant decrease in mycelial growth with an increase in concentration of fungicides. All the fungicides strongly inhibited the growth of the test fungus at 200 ppm concentration.

The complete inhibition (100%) of *M. phaseolina* mycelium at 100 ppm was observed with tebuconazole, hexaconazole, propiconazole and carbendazim+mancozeb and carbendazim. That was followed by trifloxystrobin + tebuconazole (76.85%) and carboxin + thiram (75.25%). All the fungicides completely inhibited the growth of *M. phaseolina* at 200ppm.

Data presented in table 4.16 indicated that growth inhibition level with trifloxystrobin+ tebuconazole and carboxin + thiram were statistically at par with each other at 100 ppm concentration. Similar result was also observed by Navi *et al.* (2016) while conducting the *in vitro* poison food technique using fungicides picoxystrobin, fluoxastrobin, pyraclostrobin, fluxapyroxad + pyraclostrobin, azoxystrobin, fluxapyroxad and prothioconazole + frifloxystrobin against the pathogen *M. phaseolina.* They reported that all the fungicides found very effective against the *M. phaseolina*. The fact is in agreement with the finding of Kumar *et al*. (2016) who reported that nanoform of Trifloxystrobin 25% + Tebuconazole 50% (75 WG) was found effective against the pathogen *M. phaseolina* at different concentrations. They observed that maximum inhibition of the mycelial growth was obtained with 15 ppm concentration which accounted for 99.6% reduction of mycelium growth over control. This was followed by complete inhibition of

Table 4.14: Bioefficacy of *Trichoderma viride* against *M. phaseolina in vitro*

S.N.	Isolates	Inhibition zone (mm)		% inhibition
		Control	*Macrophomina* isolates *	
1	MP1	51.58	31.23	39.45
2	MP2	35.50	20.42	42.47
3	MP3	53.33	21.65	59.40
4	MP4	48.83	23.74	51.38
5	MP5	46.83	18.86	59.72
6	MP6	49.66	23.73	52.21
7	MP7	38.83	16.50	57.50
8	MP8	52.83	35.63	32.55
9	MP9	47.83	21.20	55.67
10	MP10	41.33	17.60	57.41
11	MP11	39.50	16.70	57.72
12	MP12	51.45	28.44	44.72
13	MP13	50.50	29.22	42.13
14	MP14	58.42	36.50	37.52
15	MP15	32.46	14.23	56.16
SEm±			**0.862**	
C.D (5%)			**2.490**	

Table 4.15: Effect of different botanicals on inhibiton of radial growth of *M. phaseolina in vitro*

S.No	Botanicals	Radial growth (mm) (concentration %)			Inhibition %			Average Inhibition (%)
		5	10	15	5	10	15	
1	Garlic extract	28.33	20.1	14.67	58.50	77.65	83.77	73.30
2	Ginger extract	90.00	90.00	90.00	0.00	0.00	0.00	0.00
3	Neem oil	0.00	0.00	0.00	100.00	100.00	100.00	100.00
4	Castor oil	90.00	90.00	90.00	0.00	0.001	0.00	0.00
5	Karanz oil	71.67	69.33	62.33	17.03	22.96	30.74	18.64
6	Neem oil+Castor oil+Karanz oil	57.00	16.66	0.00	29.93	74.81	100	68.25
7	Control	90.00						
	SEm±	**0.991**	**0.839**	**0.694**	**1.807**	**1.381**	**0.772**	**9.070**
	CD(5%)	**3.053**	**2.585**	**2.138**	**5.568**	**4.254**	**2.378**	**27.948**

Table 4.16: Effect of different fungicides on radial growth of *Macrophomina phaseolina*

S.No	Fungicides	Radial growth (mm) Concentration (ppm)		Inhibition %		Average Inhibition (%)
		100	200	100 ppm	200 ppm	
1	Tebuconazole	0.00	0.00	100.00	100.0	100.00
2	Hexaconazole	0.00	0.00	100.00	100.0	100.00
3	Propiconazole	0.00	0.00	100.00	100.0	100.00
4	Trifloxystrobin + Tebuconazole	21.00	0.00	76.66	100.00	88.33
5	Carbendazim + Mancozeb	0.00	0.00	100.00	100.00	100.00
6	Carboxin + Thiram	22.27	0.00	75.25	100.00	87.62
7	Carbendazim	0.00	0.00	100.00	100.00	100.00
8	Control	90				
	SEm±	**0.335**		**0.265**		
	CD (5%)	**1.018**		**0.804**		

Table 4.17: Effect of highly aggressive isolate of *M. phaseolina*, MP14 on survival of different soybean varieties

Variety	CG Soya-1	JS 97-52	JS 93-05	JS 335	RSC 10-46	Average
Seedlings survival percentage	53.33	46.67	50.00	50.00	53.33	50.66
Control survival percentage	80.00	70.00	70.00	80.00	80.00	76.00
SEm±	0.447					
CD (5%)	NS					

Table 4.18: Efficacy of different biocntrol agents against *M. phaseolina in vivo* sick pot condition

S.No	Treatment	% Disease incidence (PDI)	% Disease reduction over control	Shoot length (cm)	Root length (cm)
1	*T. viride*	18.33	65.62	27.43	11.63
2	*P. fluorescens*	23.33	56.25	26.97	8.5
3	*B. japonicum*	36.67	31.23	26.87	11.90
4	Control	53.33	0.00	19.00	8.00
	SE(m)	**3.005**		**1.717**	**1.020**
	CD (5%)	**9.799**		**NS**	**2.222**

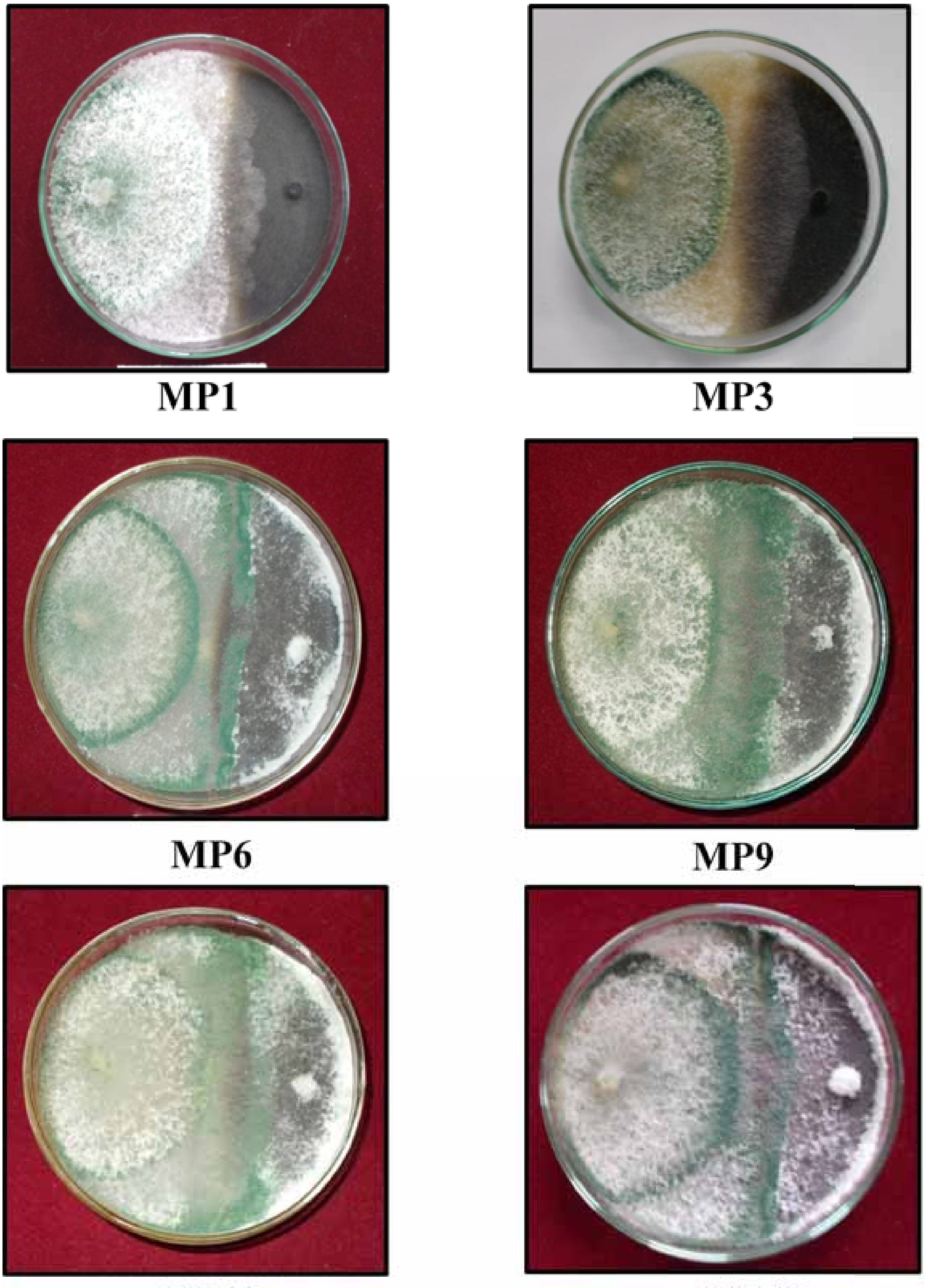

Fig. 4.18: Bioefficacy of *T. viride* against *M. phaseolina isolates in vitro* condition

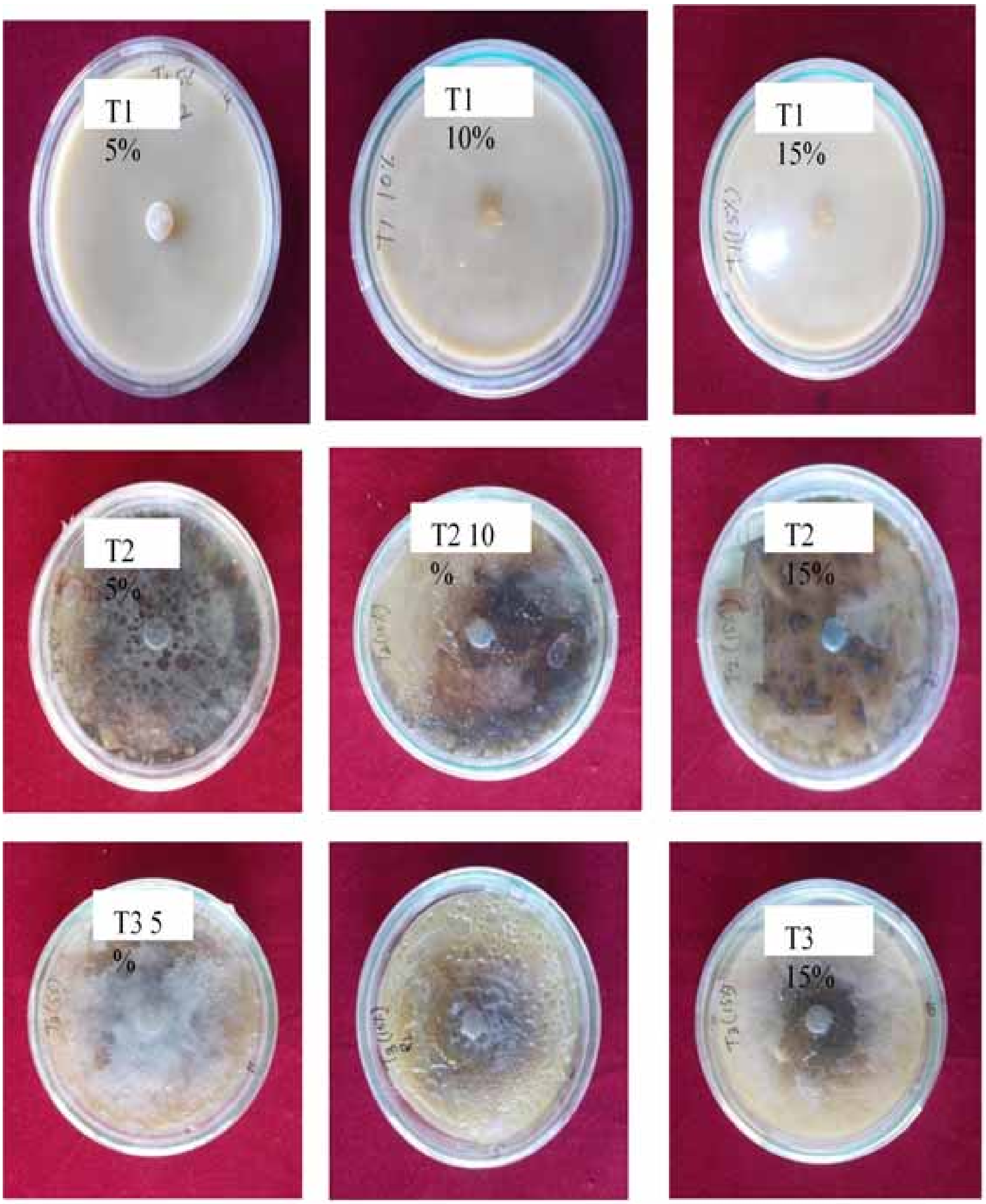

Fig. 4.19a *In vitro* evaluation of different botanicals at different concentration against *M. phaseolina*

T1- Neem oil,
T2- Castor oil
T3- Karanj oil

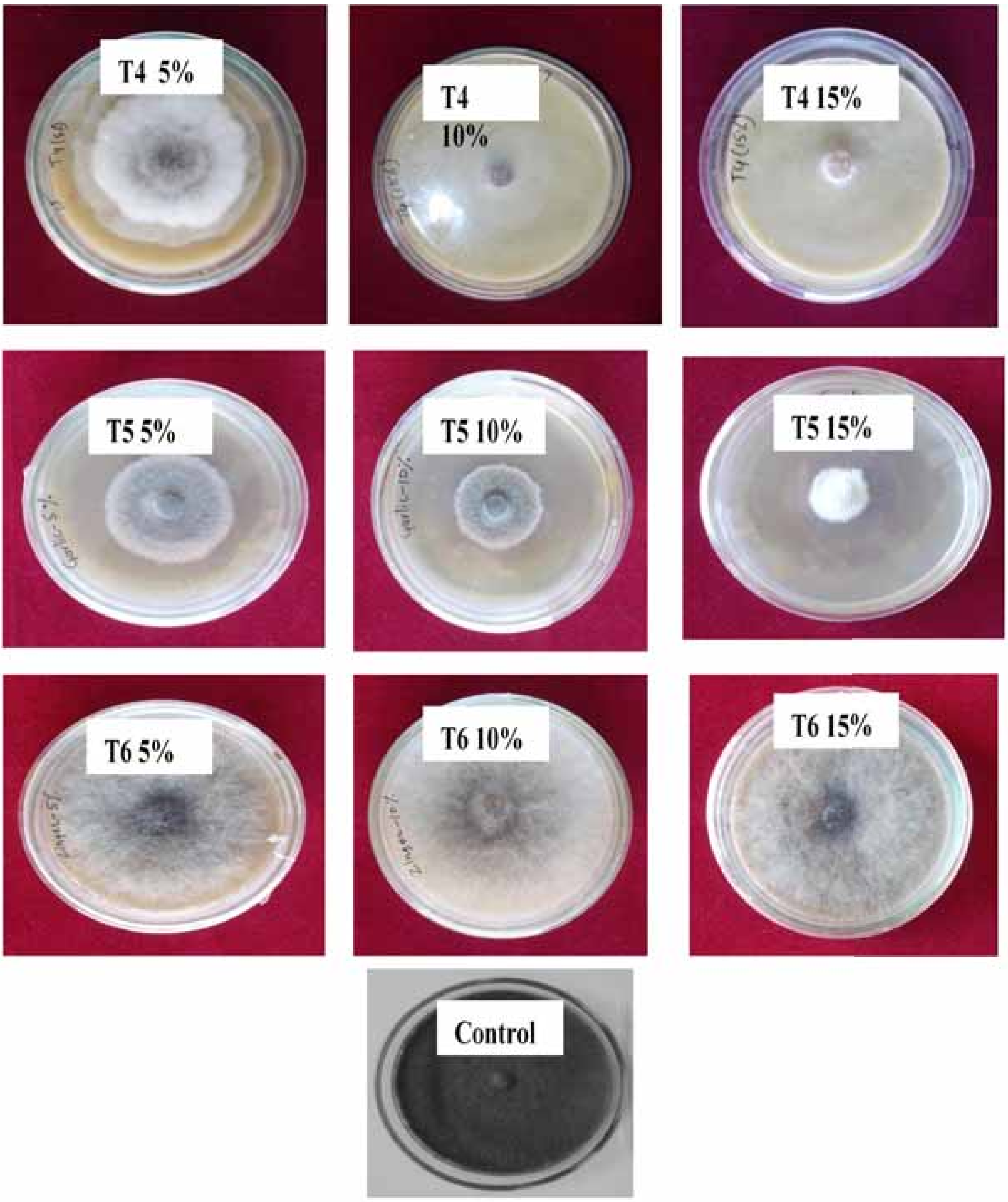

Fig. 4.19b ***In vitro*** **evaluation of different botanicals at different concentration against *M. phaseolina***

T4- Neem oil+ castor oil + Karanj,oil T5- Garlic extract, T6- Zinger extract, and control

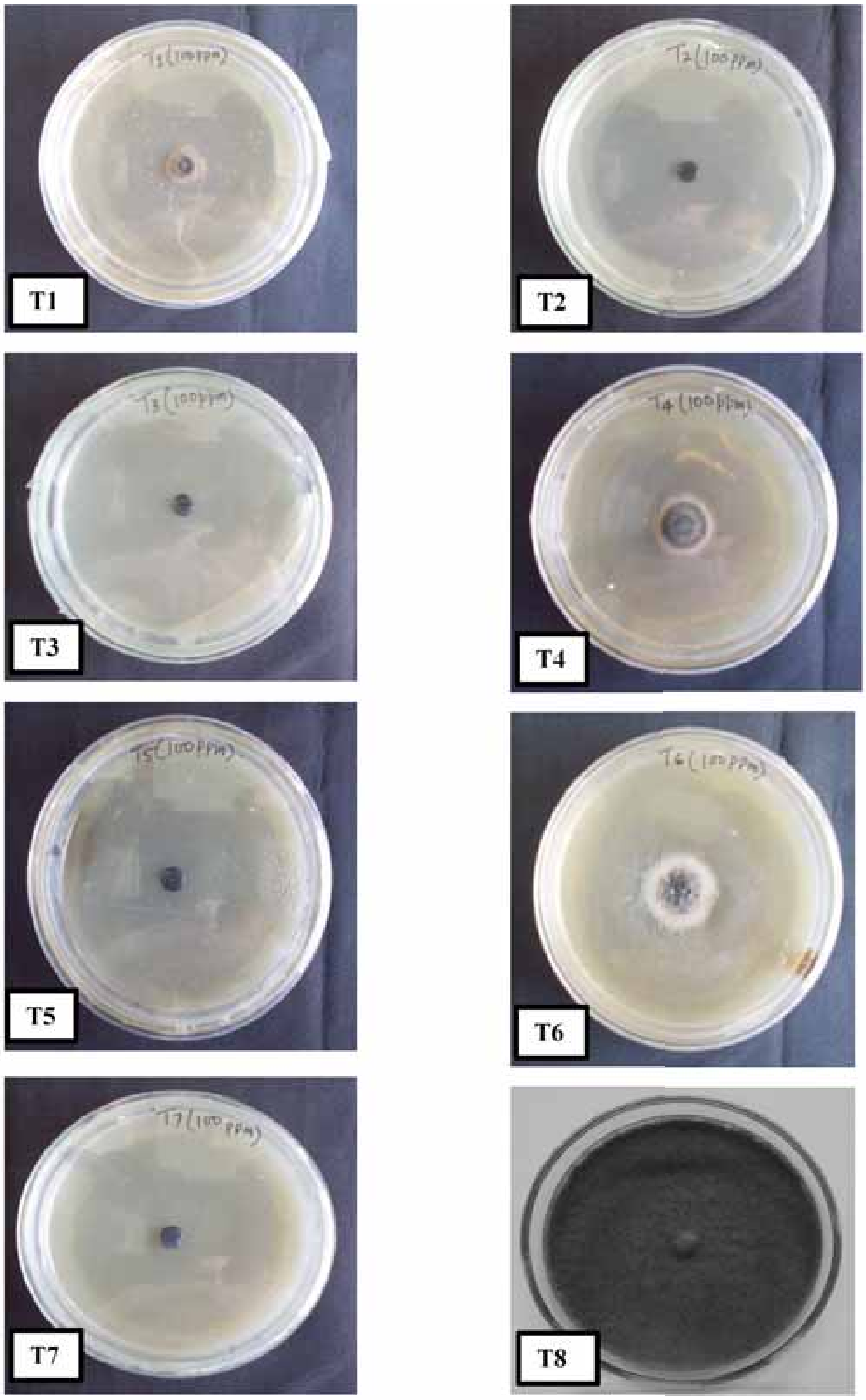

Fig. 4.20: Effect of different fungicides on radial growth of *M. phaseolina* (100ppm) T1-Tebuconazole, T2-Hexaconazole, T3-Propiconazole, T4-Trifloxystrobin + Tebuconazole, T5-Carbendazim + Mancozeb, T6-Carboxin+ Thiram, T7-Carbendazim and T8- Control

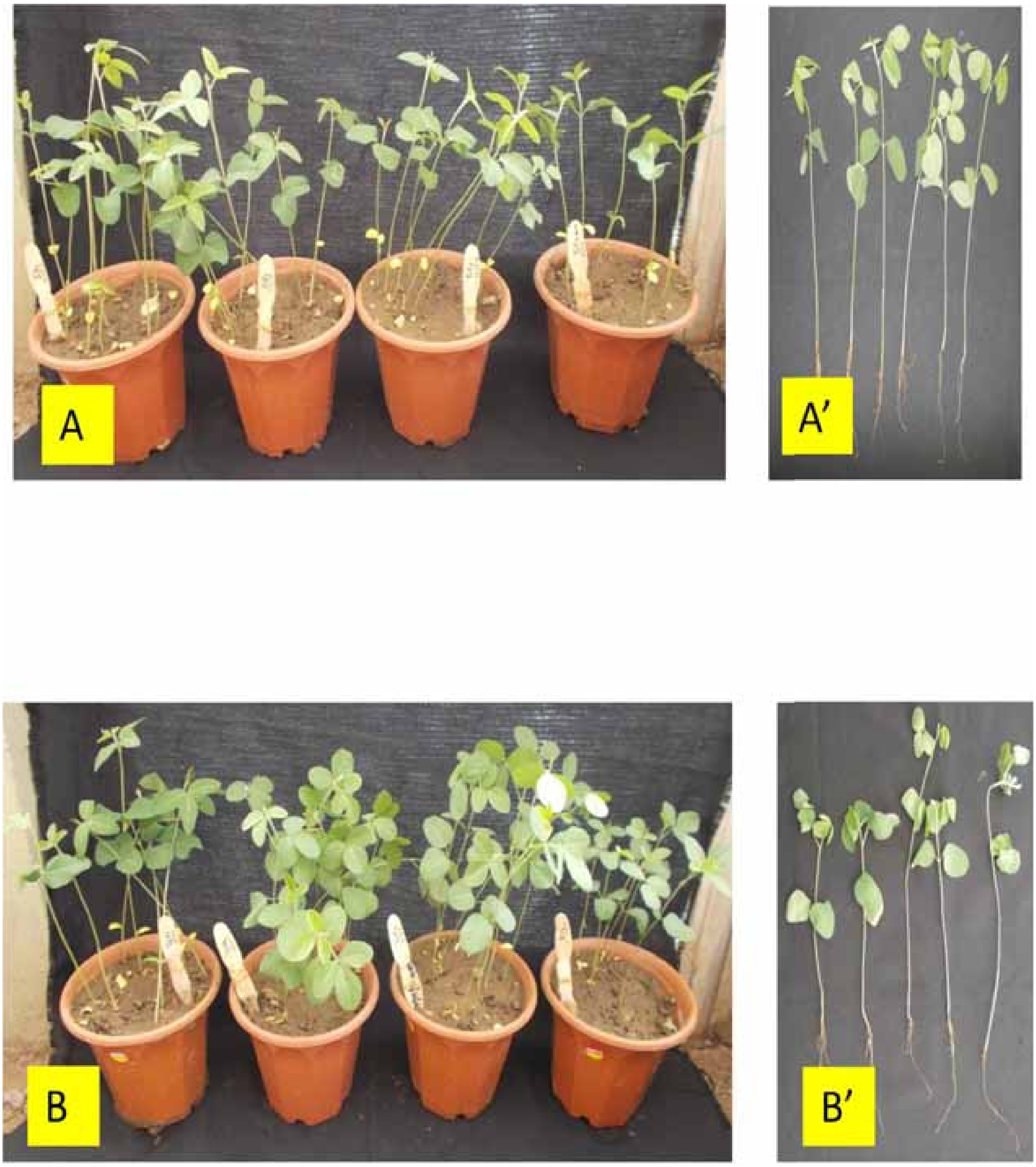

Fig. 4.21a: Effect of biological agents on the survival of soybean plant; their root and shoot length under *in vivo* sick pot condition
A& A' : Effect of *T.viride* on the soybean plant
B& B' : Effect of P. *fluorescens* on soybean plant

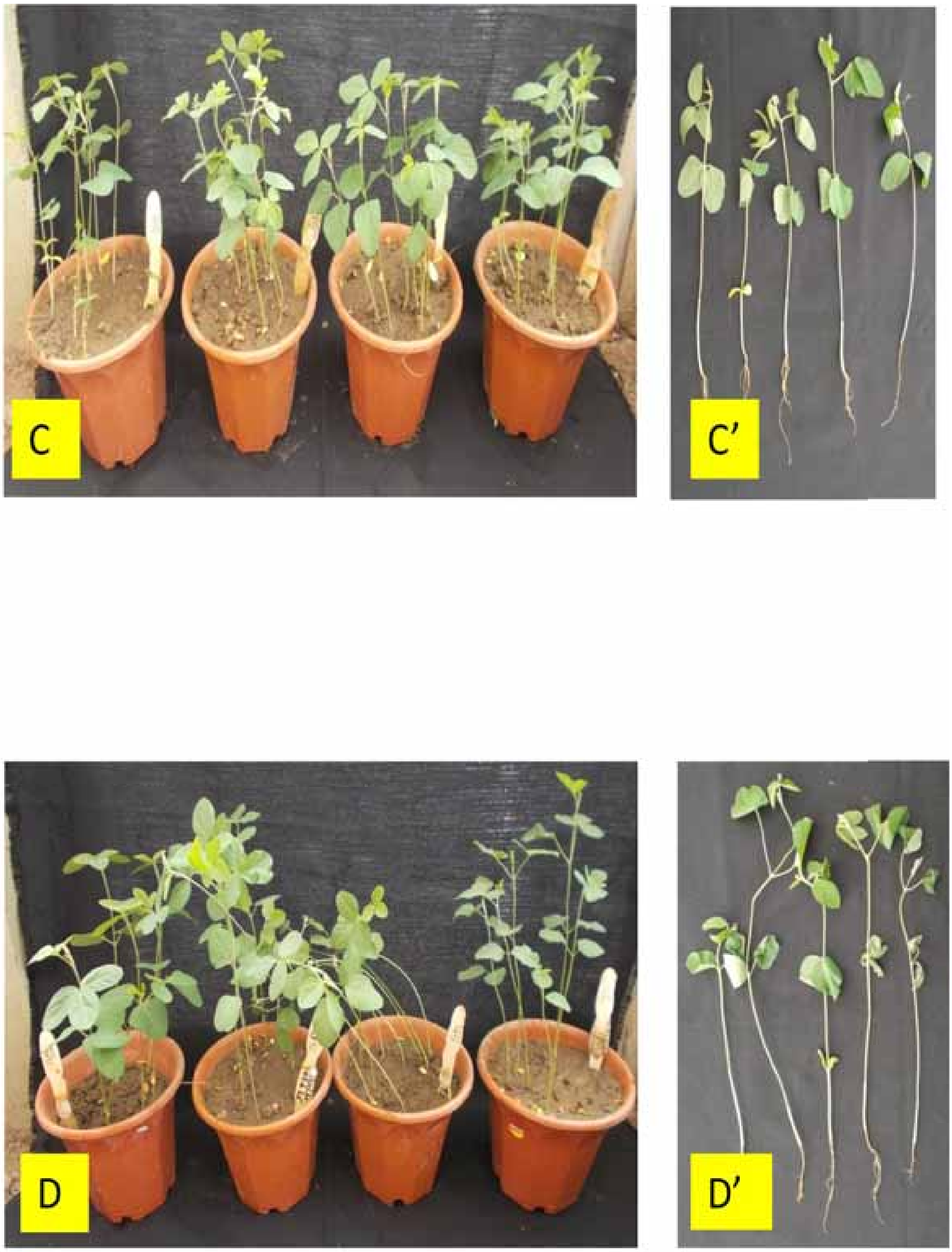

Fig. 4.21b Effect of biological agents on the survival of soybean plant; root and shoot length under *in vivo* sick pot condition
C&C' Effect of *B. japonicum* on the soybean plant
D&D' Control

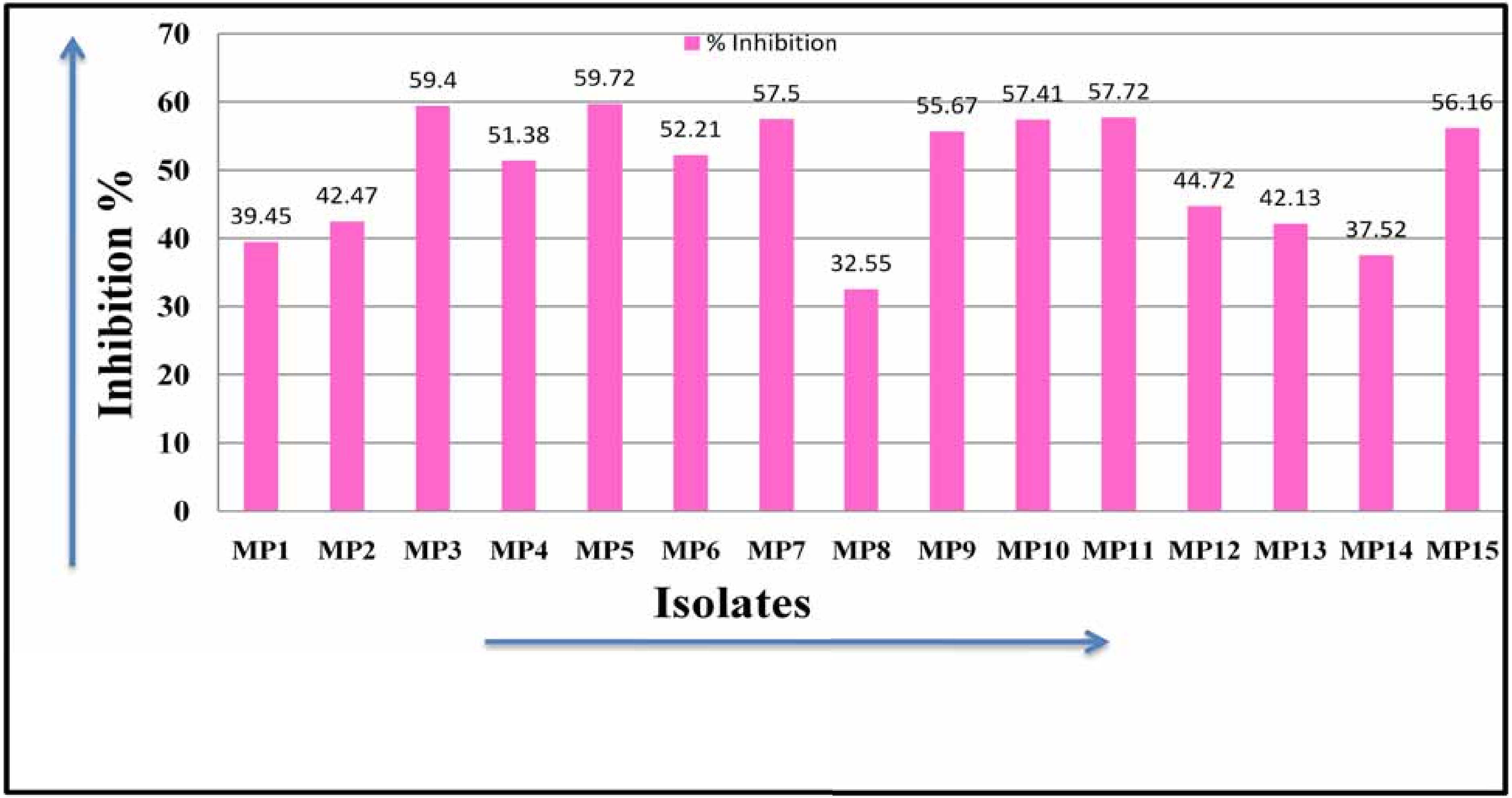

Fig 4.28: Bioefficacy of *Trichoderma viride* against *Macrophomina phaseolina in vitro* condition

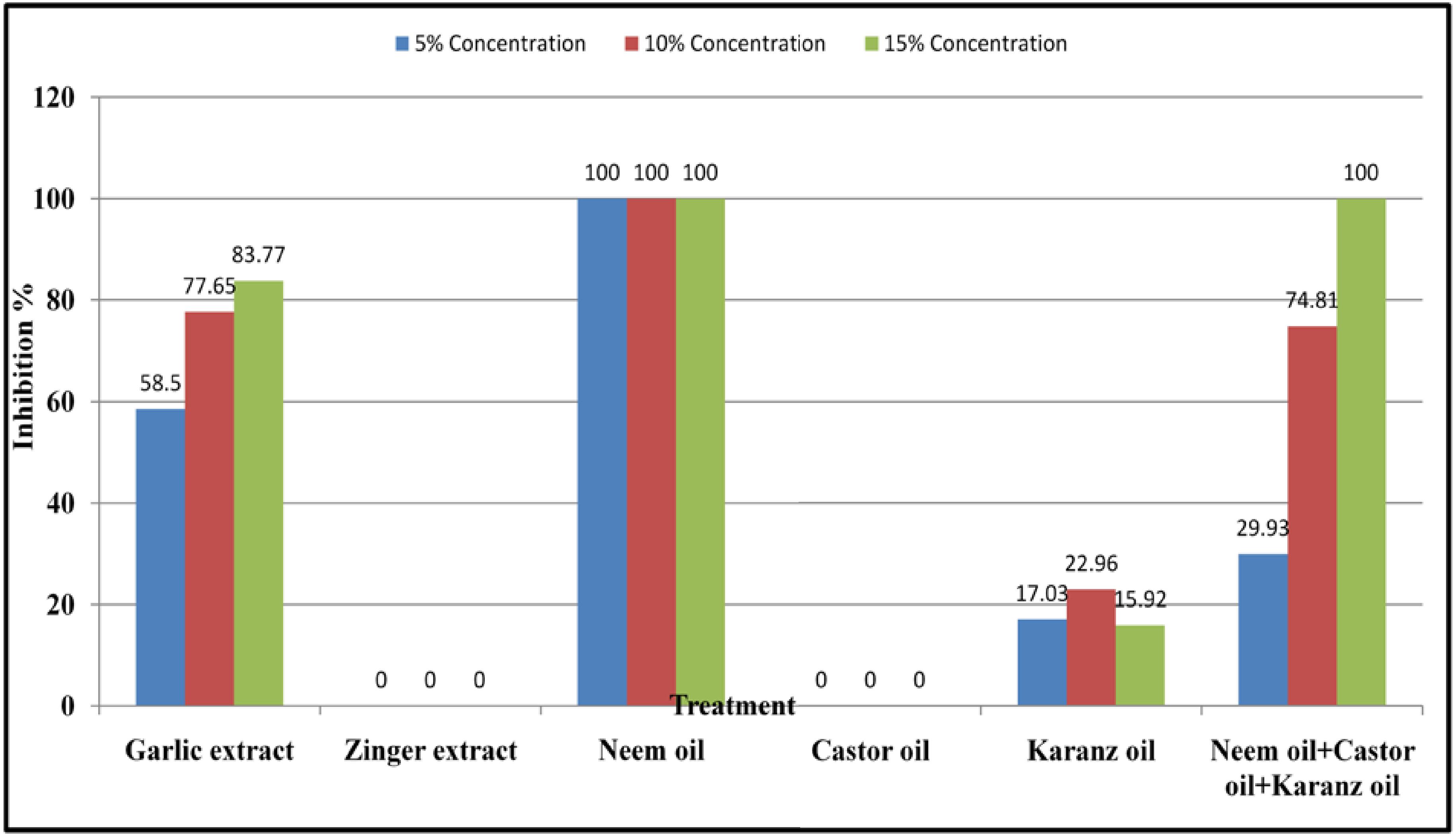

Fig 4.29 ***In-vitro*** **efficacy of different botanicals on inhibition of radial growth of** ***M. phaseolina***

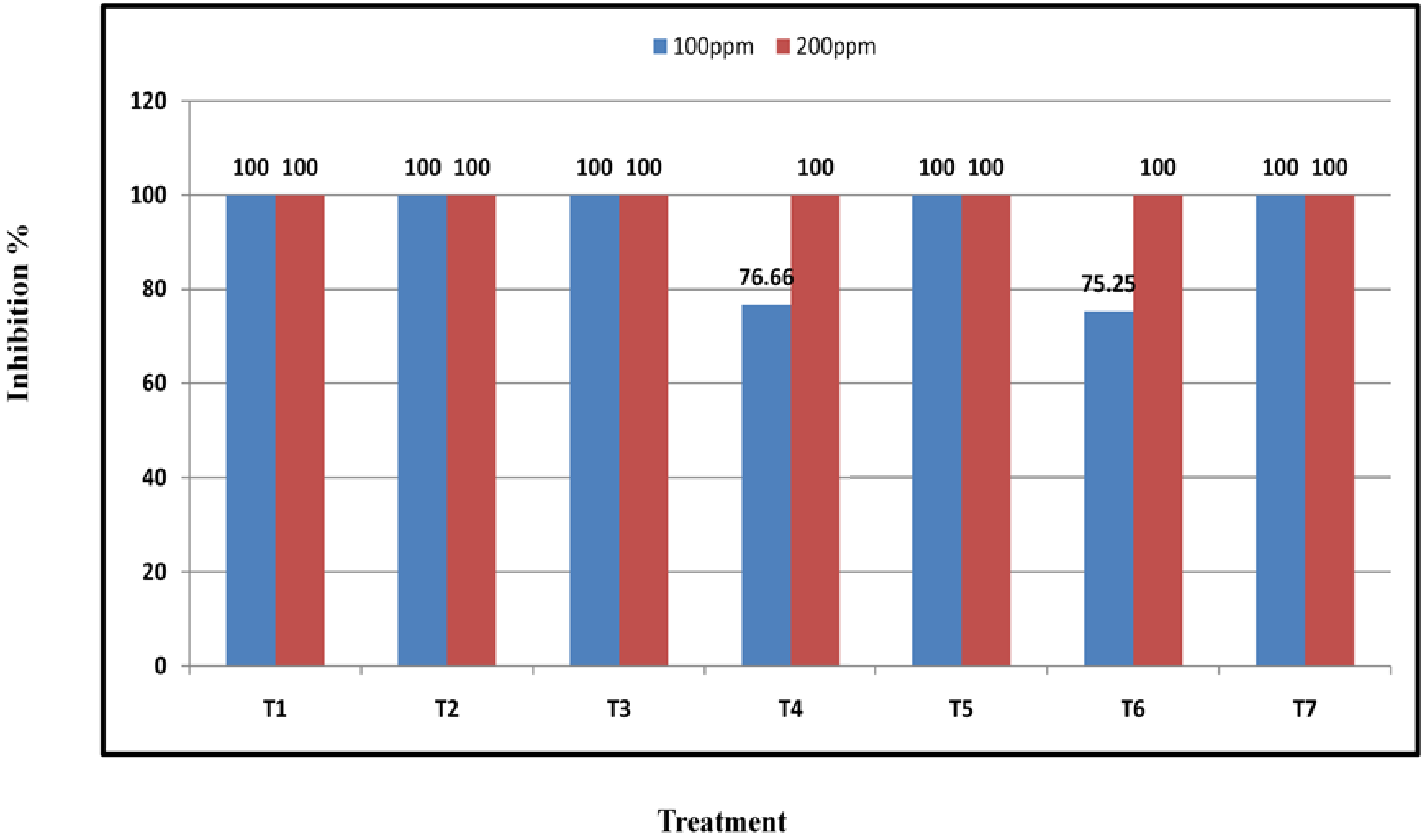

Fig 4.30 *In-vitro* efficacy of systemic fungicides on inhibition of radial growth of *M. phaseolina*

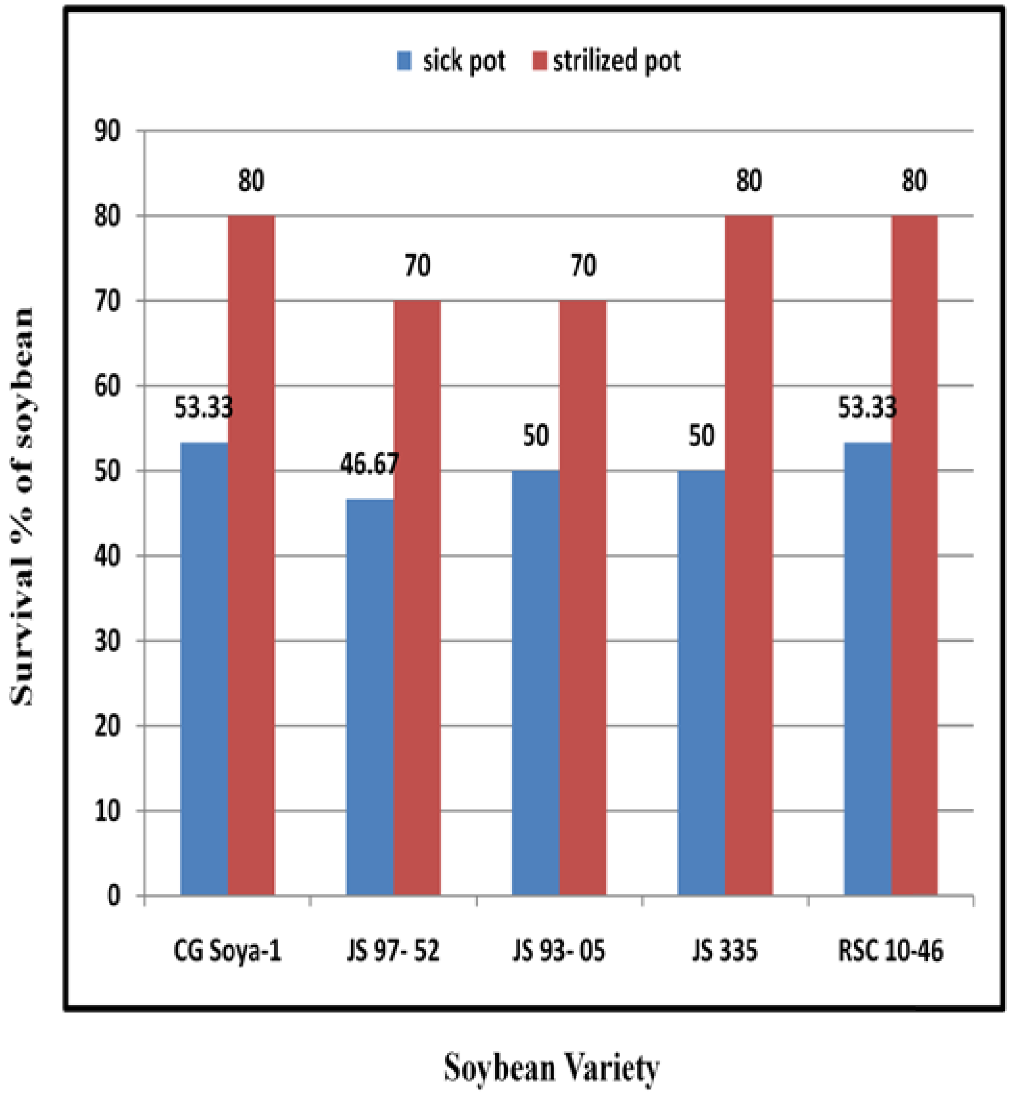

Fig 4.31: Survival percentage of soybean varieties against highly aggressive isolates of *M. phaseolina*, MP14 under *in vivo* glass house condition

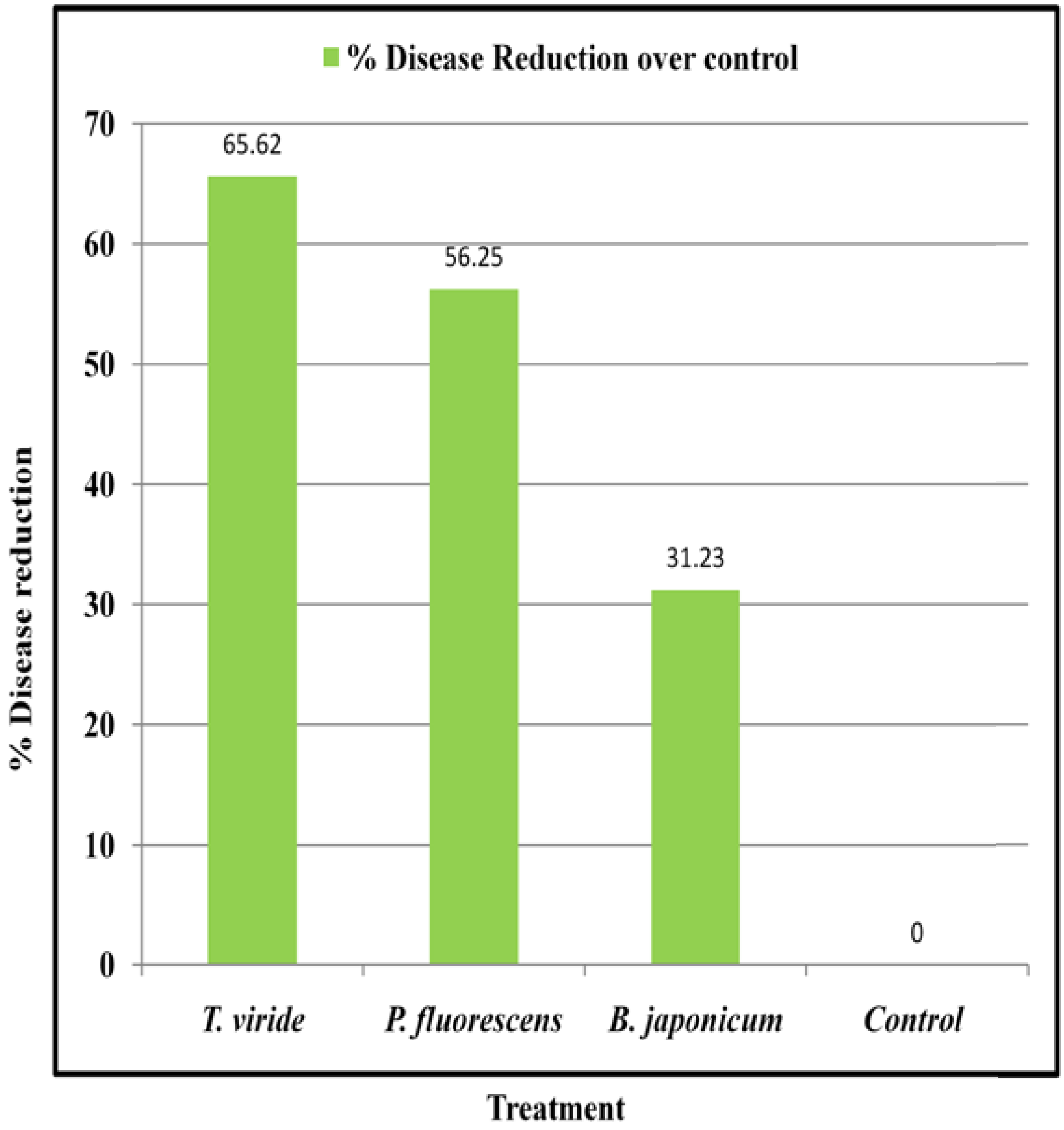

Fig 4.32: Performance of biological agents against highly aggressive isolates of *M. phaseolina* (MP14) under sick pot condition

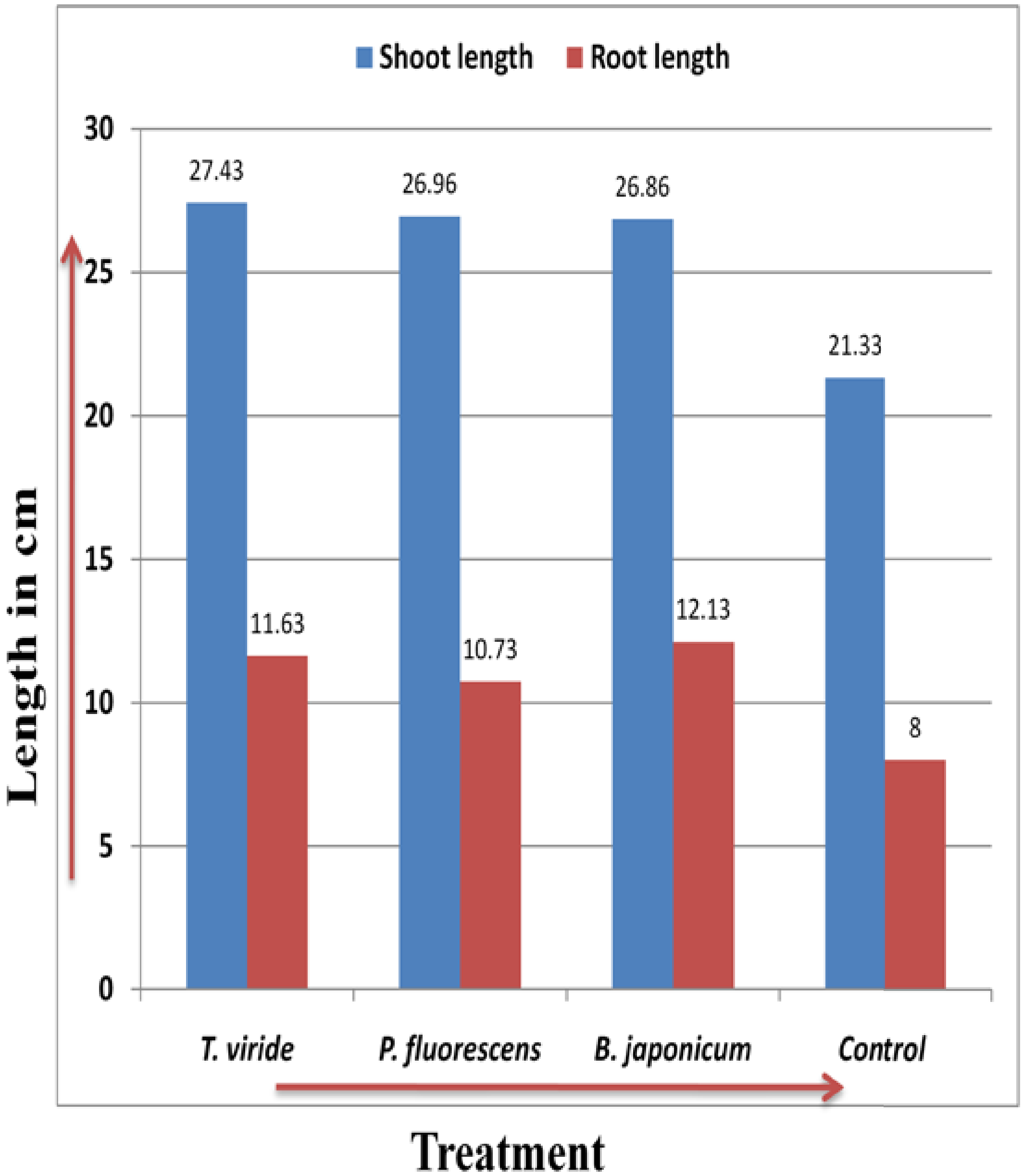

Fig 4.33: Effects of biological agents on the sobean shoot length(cm) and root length(cm) under sick pot condition against *M. phaseolina* isolate (MP14)

mycelium growth at 25 ppm which accounted for 100% reduction of mycelium growth over control.

4.9.2 *In vivo* studies under glass house condition

4.9.2.1 *In vivo* sick pot method to test the performance of highly aggressive isolate (MP14)

The highly aggressive isolate MP14 was used for testing the performance of five soybean varieties, using sick pot method under glass house condition. The observation on the survival per cent of soybean plants after 30 days revealed that disease incidence vary from 50 percent to 53.33 percent in all the varieties under sick pot condition compared to control 70.00 percent to 80.00 percent (table 4.17, Fig. 4.21a & b and 4.31). The survival percent of variety CG Soya-1 and RSC 10-46 was high among all the varieties 53.33 percent and in controlled condition the survival rate of CG Soya-1 and RSC 10-46 was 80 percent. Soybean varieties JS 93-05 and JS 335 showed 50 percent survival percentage and in controlled condition the survival rate was 70 percent and 80 percent. Least survival percent 46.67 was in variety JS 97-52 and in control 70 percent Fig. 4.9.14. Table 15 indicate that all the varieties are susceptible to the isolates under sick pot. CG Soya-1, JS 93-05, JS 335 and RSC 10-46 behaved as susceptible.

Reznikov *et al.* (2016) used *Macrophomina phaseolina* (MP) isolate number 55, mass multipled and applied in the field. Inoculation reduced the number of plant emergence in the field. Mohan and Balabaskar (2012) revealed varied levels of pathogenicity with different isolates of *M. phaseolina on* groundnut. The isolate from Vengatakuppam (MP18) was found to be the most virulent with highest incidence of 67.34 per cent and the isolate MP5 collected from Pattampakkam was the least virulent with the lowest disease incidence of 35.60 per cent under *in vivo* condition. Subramanian *et al.* (2011) found great variation in aggressiveness of 65 isolates *M. phaseolina* against three cultivar of mungbean. Kanchan and Biswas (2009) observed the potency of infection of different isolates of *R. bataticola* which varied on four different cultivars of pigeon pea *viz*. Prabhat, Bahar, T-7 and T-21. The *R. bataticola* isolates T-4 and T-5 showed maximum disease severity. Similarly, variations in pathogenicity were observed by Beas *et al*. (2006) among *M. phaseolina* isolates taken from different hosts as well as from different parts of the same host. Ratnoo *et al*. (1997) found pathogenic variation in the isolates of *M. phaseolina* from different cowpea growing areas of Udaipur. Sobti and Sharma (1992) observed varying virulence level which ranged 13 to 63 per cent of disease incidence on groundnut causing rot root.

4.9.2.2 *In vivo* sick pot method to test the efficacy of different biocontrol agents

The biological agents *T. viride*, *P. fluorescens* and *B. japonicum* are used under sick pot condition against highly virulent isolate MP14. It indicated varying percent of disease incidence, percent disease reduction over control, shoot length and root length (table 4.18, Fig.4.32 and 4.33). Under sick pot condition occurrence of percent disease incidence was 18.33 percent under seed treated with *T. viride* followed by *P. fluorescense* 23.33 percent and *B. japonicum* 36.67 percent. Sick pot having untreated seed showed 53.33 percent disease incidence. Compared to control *T.viride* showed 65.62 percent disease reduction, followed by *P. fluorescense* 56.25 percent and *B. japonicum* 31.23 percent (table 4.18 and Fig. 4.32).

Shoot length of soybean plant under different treatments is given in table 4.11 & Fig. 4.33. The soybean seed treated with *T. viride* showed maximum length (27.43cm) followed by *P. fluorescense* (26.97cm), *B. japonicum* (26.87) with non significant difference. In control it was lowest (19.00cm) Fig. 4.33.

Data presented in table 4.18 showed significant effect of biological agent on root length of soybean. Seed treatment with *B. japonicum* showed maximum root length (11.90cm) followed by *T. viride* (11.63cm), *P. fluorescense* (8.5cm) as compared to control (8.00cm). *T. viride*, *P. fluorescens* and *B. japonicum* were statistically at par with each other.

Adekunle *et al*. (2001) experienced that cowpea seed treated with three *Trichoderma* spp. and planted in soils amended with *M. phaseolina resulted in* significantly greater plant stand percentage than the control. Seed treated with *T.*

harzianum at sowing was effective to provide a considerable reduction of the disease caused by *M. phaseolina* in sesame (Pineda, 2001). Black gram seeds treated with *Trichoderma viride* significantly reduced the sclerotial germination of *M. phaseolina* (Rettinassababady *et al.,* 2000). The incidence of root rot in blackgram was significantly reduced by 50 % when treated with *Trichoderma* spp. alone or in combination with biofertilizer both under glass house and field conditions (Indra and Gayathri, 2002). Gupta and Chauhan (2005) also recommended the soybean (4–5 g⁄kg seed) treatment with *T. viride* or *T. harzianum* it was found to be very effective for managing soil borne pathogen *M. phaseolina.* Manjunatha and Naik (2010) evaluated several isolates of two bio-control agents, *T. viride* and *P. fluorescens*. Bio-control agents were assessed for their ability to reduce the growth of *M. phaseolina* under laboratory conditions and subsequently used for field studies. Govindappa *et al.* (2010) reported that seed treatment with bioagents, *P. fluorescens* and *T. harzianum* (10 g/kg) proved to be effective in controlling disease under laboratory, greenhouse and field conditions.

Chakraborty and Purkayastha (1984) reported the antagonistic property of *Rhizobium japonicum*. It reduced the severity of charcoal rot disease in soybean on account of the fungitoxic action of rhizobitoxine. Dubey *et al.* (2015) reported *Bradyrhizobium* sp. from *Vigna mungo* for their plant growth promoting (PGP) attributes and antifungal properties *in vitro*. All the isolates produced IAA but none of them produced HCN. Contrary to this, in our experiment *B. japonicm* was not found effective to manage the pathogen *M. phaseolina* at field level, as compared to systemic fungicide carbendazim.

The biocontrol agents not only controlled dry root rot but also promoted plant growth, and this gives them an advantage over the use of chemical fungicides against root rot in disease management prolonged use of the bio-control agents will increase their population in rhizosphere (Suriachandraselvan *et al*., 2004 and Naik and Sen, 1995) and thereby enhancing the disease suppressive characteristics of the soil (Singh and Nema, 1987).

4.9.3 Under natural field condition

4.9.3.1 Effect of organic soil amendments against charcoal rot of soybean

The present study was undertaken during *kharif* 2015-16 and *kharif* 2016-17 at Plant Pathology farm, IGKV, Raipur to test the effect of organic soil amendments for the management of disease charcoal rot of soybean under the field condition. Treatments were allocated under randomized block design (RBD) and the disease incidence recorded as described in materials and methods section (3.8.3.1)

The percent disease incidence, plant height (cm), branches/plant, pods/ plant, chaffy pods/plant, percent disease incidence, percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight (g), yield kg/ha, percent increase in yield over control were obtained with different treatments (table 4.19, Fig. 4.22 and 4.34-4.47). The result indicated that the percent disease incidence was low 10.60 percent, when the neem cake was used as organic amendment for soil application, followed by karanj cake 15.60 percent, castor cake 16.00 percent, linseed cake 18.67 percent, FYM 19.82 percent and mustard cake 21.60 percent over control 31.22 percent. Within the treatment all were significant and performed better than control. Karanj cake and castor cake, FYM and mustard cake and linseed and FYM were at par with each other (Fig. 4.34).

Up to 66.34 percent disease incidence was reduced by soil application of neem cake, followed by mustard cake 50.87 percent, castor cake 50.68 percent, karanj cake 49.17 percent, linseed cake 39.22 percent and FYM 38.17 percent as compared to control (Fig. 4.35).

Data about the chaffy pods/plant indicated that application of neem cake significantly lower the number of chaffy pods (3.63) followed by castor cake (4.10), karanj cake (4.75), mustard cake and linseed cake (5.17) and FYM (5.27). The maximum chaffy pods reported in control (9.0). Application of neem cake showed 54.90 percent chaffy pod reduction and lowest were found in mustard cake i.e. 35.93.

Table 4.19: Effect of organic soil amendments on incidence of charcoal rot of soybean (Pooled data of two years)

Treatment	Plant height (cm)	Branches/plant (No)	Pods/ plant (No)	Chaffy pods/ plant (No)	%Disease incidence PDI	%Disease reduction over control	% reduction in chaffy pods over control	100 seed weight (g)	Yield Kg/ha	% Increase in yield over control
T1 (FYM)	73.58	3.74	61.00	5.27	19.82 *(26.39)	38.17 (38.05)	39.84 (39.01)	9.23	1797	24.85
T2 (Neem cake)	73.28	4.27	82.13	3.63	10.60 (18.98)	66.34 (54.51)	54.90 (47.84)	9.91	2166	47.75
T3 (Mustard cake)	68.76	4.12	67.17	5.17	21.60 (23.27)	50.87 (45.47)	35.93 (36.71)	9.22	1893	30.47
T4 (Karanj cake)	67.13	4.07	66.10	4.75	15.60 (23.23)	49.17 (44.49)	35.94 (36.76)	9.24	1937	32.41
T5 (Linseed cake)	69.91	4.50	60.90	5.17	18.67 (25.57)	39.22 (38.60)	38.19 (38.01)	9.23	1883	29.70
T6 (Castor cake)	72.86	4.72	71.03	4.10	16.00 (23.53)	50.68 (45.37)	47.75 (43.68)	9.38	1956	37.64
T7 (Control)	67.81	3.98	52.12	9.00	31.22 (33.94)	0.00 (0.00)	0.00 (0.00)	7.69	1518	0.00
SEm±		**0.321**	**6.103**	**0.483**	**0.728**	**2.249**	**1.996**	**0.191**	**21.940**	
CD (5%)	**NS**	**NS**	**NS**	**1.488**	**2.268**	**7.007**	**6.217**	**0.590**	**67.605**	

**Figures in parenthesis arc sine transformed values*

Table 4.20: Effect of Seed treatments with botanicals against incidence of charcoal rot of soybean

Treatment	Plant height (cm)	Branch /plant (No)	Pods/ plant (No)	Chaffy pods/ plant (No)	%Disease incidence PDI	%Disease reduction over control	% reduction in chaffy pods over control	100 seed weight (g)	Yield Kg/ha	% Increase in yield over control
(T1) Neem oil	75.51	3.61	86.33	3.67	10.67 *(19.03)	53.83 *(41.18)	56.00	9.51	2165	17.3
(T2) Castor oil	73.54	3.50	65.00	6.00	18.33 (25.34)	20.67 (26.97)	27.3	8.92	1846	0.05
(T3) Karanj oil	71.77	3.20	67.33	4.33	16.00 (23.56)	30.71 (33.58)	48.13	9.34	2044	10.8
(T4) Garlic Extract	71.05	3.26	80.67	4.33	14.00 (21.95)	39.53 (38.93)	47.66	9.67	2152	16.63
(T5) Ginger Extract	71.02	3.43	61.67	9.00	21.13 (27.35)	8.51 (13.95)	-8.79	8.67	1789	-3.03
(T6) Neem oil+ Castor oil+ Karanz oil	74.60	3.56	83.33	4.67	13.13 (21.23)	43.25 (41.10)	43.93	9.70	2145	16.2
(T7) Control	71.50	3.66	62.67	8.33	23.13 (28.79)	0.00 (0.00)	0.00	8.70	1845	0.00
SEm±	**1.588**	**0.226**	**1.25**	**0.43**	**0.450**	**2.912**	**5.467**	**0.090**	**24.877**	
CD(5%)	**NS**	**NS**	**3.85**	**1.32**	**1.450**	**9.073**	**17.033**	**0.278**	**76.653**	

**Figures in parenthesis are arc sine transformed values*

Effect of treatment on pods/plant and chaffy pods/plant revealed that neem cake had 9.91g seed index and lowest in control i.e. 7.69g.

With regards to yield, the highest yield (2166kg/ha) was recorded in the case of neem cake followed by castor cake (1956 kg/ha), karanj cake (1937 kg/ha), mustard cake (1893kg/ha) linseed cake (1883 kg/ha) and 1797kg/ha in FYM (Fig. 4.36). Soil application of neem cake showed 42.68% yield increase over control and lowest in soil application of FYM as compared to control (Fig. 4.37).

The findings are in agreement with Mohanbabu *et al.* (2002) who also conducted greenhouse experiment to assess the effect of neem cake in different problems soils *viz.,* sandy, black hard pan, saline, sodic, alkaline, red crusted soil, acid soil and normal soil in suppressing the growth of blackgram root rot pathogen *Macrophomina phaseolina* (Tassi.) Goid and they observed that the percent disease incidence reduced up to 7.15percent.

According to Meena *et al.* (2014) oil cakes showed significant reduction of *Macrophomina phaseolina* (Tassi) causing stem rot disease of jute, *Corchorus* spp. They use the oil seed cake extract for poison food technique, maximum mycelial growth inhibition (52.40%) was recorded with neem cake (*Azadirachta indica*) at the concentrations of 20% followed by 42.61 percent and 29.60 percent with concentration of 15 percent and 10 percent, respectively. However, maximum mycelial growth inhibition (19.42%) was recorded with mustard cake (*Brassica juncea*) at the concentration of 20% followed by 16.64% and 12.20% at the concentration of 15% and 10% respectively. In general mycelial growth inhibition was dose dependent and it was maximum in case of neem cake than mustard cake. Similarly Dhingani *et al*. (2013) also studied the efficacy of various oil cakes against *Macrophomina phaseolina* (Tassi.) Goid causing dry root rot of chickpea. The four organic extracts were tested against *M. phaseolina* by poisoned food technique *in vitro*. The mycelium inhibition was recorded in extracts of neem cake (59.40 %) followed by farm yard manure (42.56 %), castor cake (13.31%) and mustard cake (4.90%).

Fig. 4.22: Field view of experimental trial on organic soil amendments

Fig. 4.23: Field view of experimental trial on botanicals as seed treatment

Fig. 4.24: Field view of experimental trial on seed treatment with systemic fungicides

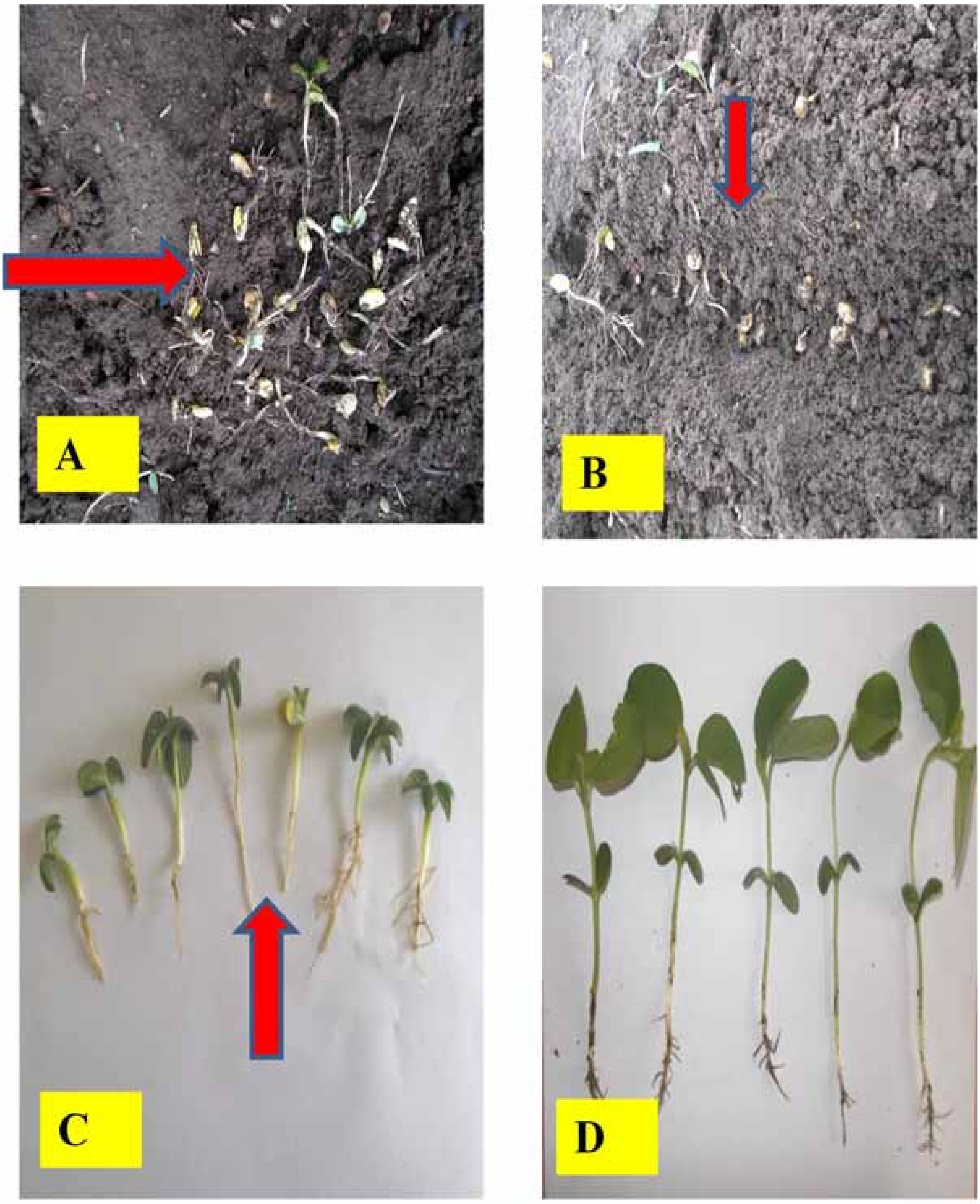

Fig. 4.25: Soybean seedling showings fungicide toxicity at the field level (A-C); healthy seedlings (D)

Fig. 4.26: Field view of experimental trial on seed treatment with biocontrol agents

Fig. 4.27: Field view of experimental trial on folia spray of systemic fungicides

4.9.3.2 Effect of seed treatment with botanicals against charcoal rot of soybean

The present study was undertaken during *kharif* 2016 at Plant Pathology farm, IGKV, Raipur to test the effect of botanicals for the management of disease charcoal rot of soybean under the field condition. Seven treatments were evaluated under randomized block design (RBD) and the disease incidence recorded as described in materials and methods section (3.8.3.2)

The percent disease incidence, plant height (cm), branches/plant, pods/ plant, chaffy pods/plant, percent disease incidence , percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight (g), yield kg/ha, percent increase in yield over control were obtained with different treatments (table 4.20, Fig. 4.23 and 4.38- 4.41). The result indicated that the percent disease incidence was low 10.67 percent, when the neem oil was used as seed treatment for disease management followed by neem oil + castor oil + karanj oil 13.13 percent, garlic extract 14.00 percent, karanj oil 16.00 percent, castor oil 18.33 percent and ginger extract 21.13 percent over control 23.13 percent. Neem oil + castor oil + karanj oil and garlic extract were statistically at par with each other in both percent disease incidence as well as percent disease reduction over control (Fig. 4.38).

Up to 53.83 percent disease incidence can be reduced by seed treatment with neem oil, followed by neem + castor + karanj oil 43.25 percent, garlic extract 39.53 percent, karanj oil 30.71 percent, castor oil 20.67 percent and least in ginger extract 8.51% as compared to control. Neem oil + castor oil + karanj oil and garlic extract were statistically at par with each other in both percent disease incidence as well as percent disease reduction over control (Fig. 4.39).

Data showed that there were no significant difference among plant height and number of branches per plant; however significant effect of treatments were recorded on pods per/plant, chaffy pods/plant and 100 seed weight (table 4.20). Maximum number of pods/plant were observed when seed were treated with neem oil (86.33), which was followed by neem oil + castor oil + karanj oil (83.33), garlic extract (80.67) and karanj oil (67.33). Minimum number of pods/plant was found in ginger extract. Castor oil and karanj oil statistically at par with each other in case of number of pods.

Seed treatment with neem oil was significantly superior in reducing the number of chaffy pods among all the treatments. It reduced the chaffy pods (3.67) followed by karanj oil and garlic extract (4.33), neem + castor + karanj oil (4.67). The chaffy pods reported in control were 8.33 which is less than the seed treatment with ginger. Application of neem oil showed 56.00 percent reduction in chaffy and lowest effect in ginger extract (-8.79%) i.e. instead of reduction ginger increased the percent of chaffy pods.

Data showed that the effect of seed treatment on seed index, seed treatment with neem oil had 9.51g seed index and lowest in ginger extract i.e. 8.67g. Treatment showed that seed treatment with ginger extract had seed index less than the control i.e. 8.70g.

With regard to yield, the highest yield (2165kg/ha) was recorded in the case of neem oil followed by garlic extract (2152 kg/ha) significantly at par with each other , neem + castor + karanj (2145 kg/ha), karanj oil (2044 kg/ha), castor oil (1846 kg/ha) and ginger extract (1789kg/ha) were next in rank. Untreated seed showed yield 1845kg/ha comparatively more than the seed treatment with ginger extract (1789kg/ha) (Fig. 4.40).

As compared to control seed treated with neem oil showed 16.63 percent increased yield over control and seed treatment with ginger extract showed 3.03 % decrease in yield over control Fig. 4.41.

Maximum number of pods/plant were observed when seeds were treated with neem oil (86.33). It was followed by neem oil + castor oil + karanj oil, garlic extract (80.67) and karanj oil (67.33). Minimum no of pods/plant were found in ginger extract. Castor oil and karanj oil were statistically at par with each other in case of number of pods per plant. Bharadwaj *et al*. (2013) studied the effect of botanicals *viz.,* mustard oil, neem oil, karanj oil, cedar oil, apricot oil and olive oil used as seed treatment on seed mycoflora of pea, *Pisum sativum* L against the incidence of *Fusarium* sp. They found that the incidence of *Fusarium* sp. was nil in all tested concentrations of 5percent, 3 percent and 1 per cent each of Neem oil, Karanj oil and mustard oil , cedar oil and olive oil at 5 and 3 per cent each and apricot at 5 per cent. All the tested vegetable oils showed superiority over control. Neem leaf extract, Marigold leaf extract and Garlic bulb extract at 5 % as seed treatment significantly reduced the charcoal rot incidence and increased yield in lentil (Sinha and Sinha, 2004). Seed infection of soybean caused by *M. phaseolina* was completely inhibited by dipping the seeds for 5 min in ginger, garlic and neem extracts (Hossain *et al*., 1999). Maheshbabu and Hunje (2008) conducted experiment on effect of seed treatment with botanicals on storability of soybean. Seed treatment with botanicals T1: Sweet flag rhizome powder @ 10 g/kg of seed, T2: Neem leaf powder @ 20 g/kg of seed , T3 : Neem oil @ 10 ml/kg of seed, T4 : Castor oil @ 10 ml/kg of seed, T5 : Turmeric powder @ 10 g/kg of seed, T6: Deltamethrin @ 40 mg/kg of seed and T7: Control and laid out in randomized complete block design with factorial concept. All the quality parameters such as germination (%) root length (cm) shoot length (cm) seedling vigour index showed significant differences due to seed treatment with botanicals. Above findings are in agreement with the seed treatment with botanicals in present study and holds goods for the management of *M. phaseolina*. Rana *et al.* (2014) conducted the in vitro poison food technique against *Macrophomina phaseolina* by using plant extracts. In vitro studies indicated that plant leaf extracts of *Adenocalymma alliaceum* (77.20%) and *Allium* sp. (74.71%) demonstrated the highest inhibition of mycelia growth of *M. phaseolina* at 10% concentration.

4.9.3.3 Effect of seed treatment with fungicides against charcoal rot of soybean

The present study was undertaken during the *kharif* 2016 at Plant Pathology farm, IGKV, Raipur to test the effect of seed treatment with systemic fungicides for the management of disease charcoal rot of soybean under the field condition. Treatments were allocated under randomized block design (RBD) and the disease incidence (%), plant height (cm), branches/plant, pods/plant, chaffy pods/plant, percent disease incidence, percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight (g), yield kg/ha, percent increase in yield over control were obtained with different treatments recorded as described in materials and methods section (3.8.3.3). The results obtained during the experiment are presented in (table 4.21, Fig. 4.24 and 4.42-4.45).

Results indicated that seed treatment with fungicides carbendazim + mancozeb (T5), carboxin + Thiram (T6) and carbendazim (T7) resulted in significant reduction in the disease incidence 7.33 percent, 8.66 percent and 9.00 percent respectively as compared to control 22.33 percent and they are statistically at par with each other (Fig. 4.42). Seed treatment with tebuconazole (T1), hexaconazole (T2), propiconazole (T3) and trifloxystrobin and tebuconazole (T4) did not show significant percent disease reduction over control and instead of decrease the percent disease increased over control *i.e.,* 69.40, 65.69, 91.71 and 76.25 percent respectively (Fig. 4.43). However the seed treated with fungicides such as tebuconazole, hexaconazole, propiconazole and trifloxystrobin and tebuconazole showed higher disease incidence as compared to control i.e. 37.67 percent, 37.00 percent, 42.67 percent and 39.33 percent respectively.

Data presented in (table 4.21) indicated that treatments T6 (carboxin + thiram) and carbendazim (T7) were at par with carbendazim + mancozeb (T5). However the seed treatment with tebuconazole (T1), hexaconazole (T2), propiconazole (T3) and trifloxystrobin and tebuconazole (T4) did not found suitable for management of this disease.

There were significant differences observed between plant height and number of branches per plant. Seed treatment with (T7) carbendazim, carboxin + thiram (T6) and carbendazim +thiram (T5), showed increased plant height over control. Carboxin + thiram (T6) and carbendazim +thiram (T5) were at par with (T7) carbendazim. Other treatments; tebuconazole, hexaconazole, propiconazole and trifloxystrobin and tebuconazole showed reduced plant height among all the treatments and also with control.

Effect of treatment on pods/plant and chaffy pods/plant revealed that maximum number of pods/plant were obtained when seeds were treated with carboxin + thiram, carbendazim + mancozeb and carbendazim showed 77.67, 78.0 and 76.67 respectively. Carboxin + thiram (T6) was statistically at par with carbendazim + mancozeb (T5). Seed treatment with tebuconazole, hexaconazole, propiconazole and trifloxystrobin and tebuconazole showed negative effect on the pods/plant and reduced their number 41.33, 48.0, 50.67 and 50.33 respectively, less than the control.

More number of chaffy pods/plant was indication of diseased plant and directly reduced the yield. Seed treatment with carbendazim + mancozeb (T5), carboxin + thiram (T6) and carbendazim (T6) significantly reduced the number of chaffy pods over control *i.e.* 2.67, 3.00 and 3.33 respectively. In other treatment T1, T2, T3 and T4 showed increase number of chaffy pod/plant over control indicated that treatment was not suitable for the management of charcoal rot of soybean. The reduction in yield was mainly due to more number of unfilled pods and seed index which occurred due to the disease charcoal rot of soybean.

Weight of 100 seed is directly responsible for plot yield, showed non significant among the treatments. Highest seed index (10.57g) recorded in the seed treatment with the fungicide carbendazim + mancozeb, carboxin + thiram (10.26g) followed by carbendazim+ mancozeb (9.82g) more than the control 8.28g. Treatment with tebuconazole, hexaconazole, propiconazole and trifloxystrobin and tebuconazole did not show significant increase in seed index indeed, less than the control (table 4.21)

With regards to yield, the highest yield (2552 kg/ha) was recorded in seed treatment with carbendazim + mancozeb followed by treatment with carboxin + thiram (2421 kg/ha) and carbendazim (2373 kg/ha). Treatment T1, T2, T3 and T4 did not show increase in yield over control (1563, 1677, 1464 and 1685 kg/ha respectively) Fig. 4.44. This may be due to the fungicides toxicity to the soybean plant and reduced germination percentage and poor plant population (Fig. 4.25a&b). The percent increase in yield over control (-10.5%, -4.0%, -16.2% and -3.5% respectively indicated that yield of these plots decreased compared to the control plot (1747kg/ha) Fig. 4.45. Thus the seed treatment with systemic fungicide carboxin+ thiram, carbendazim+ mancozeb and carbendazim was found suitable for management of charcoal rot and significant increase in yield of soybean crop. Anitha and Jahagirdar (2016) did priming of soybean with fungicides under laboratory condition, they found that carboxin 37.5 + thiram 37.5 WS @ 0.2% recorded significantly higher seed germination and root length in soybean. The increase in seed yield was attributed to increased number of pods per plant, pod weight, seed weight in primed treatments as compared to control. Increase in yields indicated that carboxin + thiram was suitable for seed treatment.

Chemical seed treatments have been shown to be effective to control *M. phaseolina* on various crops. For cotton, several fungicides were tested as seed treatment to determine their efficacy in controlling *M. phaseolina* infection. Reznikov *et al*, (1996) reported that seed treatments with fungicides were effective in counteracting the plant emergence reduction caused by the pathogen in both seasons and cultivars. Seed treatment with tebuconazole resulted in minimum collar rot (9.1%) and stem rot (22.6%) incidence followed by hexaconazole + captan (10.1 and 26.1%) and carboxin + thiram (13.5 and 24.8%) compared to other treatments. The treatments, hexaconazole + captan and tebuconazole recorded significantly more pod yield (737 and 707 kg/ha-1) and haulm yield (1340 and 1299 kg/ha-1). However, seed treatment with tebuconazole resulted in highest cost benefit ratio of 1:7.5 followed by hexaconazole + captan with 1:5.2. Contrary to this, the tebuconazole and hexaconazole is not found suitable for seed treatment in this case. It may be the reason that groundnut is not sensitive to azole group of fungicides like soybean.

Jadon (2015) also evaluated ten systemic seed dressing fungicides and their combinations for management of major soil borne diseases of groundnut. The fungicides viz., hexaconazole, tebuconazole, propiconazole, difenconazole, vitavax, carbendazim along with captan and mancozeb and various combinations were applied as seed treatment at recommended doses. The results indicated that tebuconazole 2 DS @ 1.5 g/kg seed, mancozeb 75% WP @ 3 g/kg seed, carbendazim 12% + mancozeb 63% WP @ 3 g/kg seed, were very effective in the management of soil borne diseases viz., stem rot, collar rot and aflaroot of

Table 4.21: Effect of seed treatment with fungicides against incidence of charcoal rot of soybean

Treatment	Plant height (cm)	Branch /plant	Pods/ plant	Chaffy pods/ plant	%Disease incidence (PDI)	%Disease reduction over control	% reduction in chaffy pods over control	100 seed weight (g)	Yield Kg/ha	% Increase in yield over control
(T1) Tebuconazole	48.44	3.34	41.33	14.33	37.67 *(37.83)	-69.40	-118.31	7.73	1563.33	-10.5
(T2) Hexaconazole	51.63	3.50	48.00	12.33	37.00 (37.44)	-65.69	-87.94	7.96	1677.67	-4.0
(T3) Propiconazole	55.30	3.20	50.67	13.67	42.67 (40.76)	-91.71	-115.87	7.81	1464.33	-16.2
(T4) Trifloxystrobin + Tebuconazole	50.70	3.26	50.33	11.33	39.33 (38.82)	-76.25	-80.15	8.28	1685.33	-3.5
(T5) Carbendazim + Mancozeb	71.56	4.44	77.67	2.67	7.33 (15.65)	67.14	73.80	10.57	2552.33	46.0
(T6) Carboxin + Thiram	71.98	4.56	78.00	3.00	8.66 (17.10)	61.07	52.37	10.26	2421.00	38.6
(T7) Carbendazim	72.14	4.33	76.67	3.33	9.00 (14.43)	59.76	46.82	9.82	2373.33	35.8
(T8) Untreated control	70.85	4.36	53.33	6.33	22.33 (28.18)	0.00	0.00	8.2	1747.67	0.0
SEm±	**2.006**	**0.222**	**14.35**	**0.576**	**0.744**	**5.887**	**9.601**	**2.19**	**467.66**	
CD (5%)	**6.144**	**0.679**	**43.54**	**1.763**	**2.279**	**18.029**	**29.405**	**NS**	**NS**	

**Figures in parenthesis are arc sine transformed values.*

Table 4.22: Effect of seed treatment with biocontrol agents against incidence of charcoal rot of soybean

Treatment	Plant height (cm)	Branches/ plant (No)	Pods/ plant (No)	Chaffy pods/ plant (No)	%Disease incidence PDI	%Disease reduction over control	% reduction in chaffy pods over control	100 seed weight (g)	Yield Kg/ha	% Increase in yield over control
T. viride	71.20	3.98	75.00	3.46	7.09 *(2.84)	15.27	8.46	9.95	2274	9.50
P. fluorescens	69.60	3.70	72.40	3.56	8.70 (3.11)	7.00	5.82	9.62	2253	8.50
B. japonicum	74.00	3.70	77.20	6.00	16.40 (4.16)	-77.83	-58.73	9.10	1940	-6.60
Control	69.40	3.80	72.00	3.78	9.40 (3.21)	0.00	0.00	9.26	2077	0.00
SEm(±)	**0.859**	**0.140**	**1.682**	**0.296**	**0.075**	**9.007**	**8.644**	**0.153**	**53.40**	
CD(5%)	**2.667**	**NS**	**NS**	**0.921**	**0.233**	**28.061**	**26.929**	**0.477**	**166.38**	

**Figures in parenthesis are arc sine transformed values*

Table 4.23: Effect of foliar spray of systemic fungicides against charcoal rot of soybean

Treatment	**Plant height (cm)**	**Bran ches/ plant (No)**	**Pods/ plant (No)**	**Chaffy pods/ plant (No)**	**%Disease incidence PDI**	**%Disease reduction over control**	**% reduction in chaffy pods over control**	**100 seed weight (g)**	**Yield Kg/ha**	**% Increase in yield over control**
Tebuconazole	68.01	4.07	77.8	4.03	19.00 (25.82)	33.40 (35.21)	28.03	9.62	1925	14.60
Hexaconazole	68.61	4.00	80.2	4.27	17.33 (24.58)	36.49 (37.13)	23.75	9.45	2045	21.72
Propiconazole	68.56	3.87	69.0	3.67	20.67 (27.02)	24.16 (29.30)	34.46	9.57	1948	15.95
Trifloxystrobin + Tebuconazole	70.45	3.87	82.2	3.53	19.17 (25.95)	29.71 (33.01)	36.96	9.83	2100	25.00
Carbendazim + Mancozeb	70.66	3.67	73.77	4.20	18.33 (25.33)	32.80 (34.90)	25.00	9.70	2005	19.34
Carboxin + Thiram	70.33	3.6	71.13	4.30	14.67 (22.49)	46.20 (42.79)	23.21	9.57	1980	17.85
Control	68.33	3.6	60.27	5.60	27.30 (31.48)	0.00 (0.00)	0.00	8.63	1680	0.00
SEm(±)	**1.669**	**0.211**	**2.719**	**1.008**	**0.643**	**1.095**	**9.763**	**0.109**	**10.315**	
CD(5%)	**NS**	**NS**	**8.378**	**1.426**	**2.006**	**3.411**	**NS**	**0.337**	**31.784**	

**Figures in parenthesis are arc sine transformed values*

groundnut, when used separately, with apparent yield advantage over untreated plots without showing any phytotoxic effect on groundnut. Minimum incidence of pre emergence rot and post emergence seedling rot (13%) were recorded in treatment with carbendazim and thiophanete methyl compared to control (43 and 49 percent) respectively in cluster bean (Jaiman *et al*., 2009).

4.9.3.4 Effect of seed treatment with biocontrol agents against charcoal rot of soybean

The present study was undertaken during *kharif* 2016-17 at Plant Pathology farm, IGKV, Raipur to test the effect of organic soil amendments for the management of disease charcoal rot of soybean under the field condition. Seven treatments were allocated under randomized block design (RBD) and the disease incidence recorded as described in materials and methods section (3.8.3.4)

The percent disease incidence, plant height (cm), branches/plant, pods/ plant, chaffy pods/plant, percent disease incidence , percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight (g), yield kg/ha, percent increase in yield over control were obtained with different treatments (table 4.22, Fig. 4.26 and 4.46-4.49).

Among all the treatments minimum percent disease incidence was observed when seeds were treated with *T. viride* (7.09%) followed by *P. fluorescens* (8.70%) while maximum incidence was recorded in *B. japonicum* (16.40%) which was greater than the control (9.40%) Fig. 4.46.

Highest percent disease reduction over control was observed in *T. viride* (15.27%) and lowest was in *P. fluorescens* (7.0%) Fig. 4.47).

There was significant difference in plant height. Seed treatment with *B. japonicum* showed increased plant height (74.00 cm) over control and minimum was in *P. fluorescens* (69.60 cm).

No significant results were observed on the effect of seed treatment on number of branches per plant and pods per plant.

Seed treatment with *T. viride* significantly reduced the number of chaffy pod over control (8.46%) followed by *P. fluorescens* (5.82%). Seed treatment with *B. japonicum* increased number of chaffy pods than the control.

Highest seed index (9.95g) was recorded in seed treatment with *T. viride* followed by *P. fluorescens* (9.62g). Seed treatment with *B. japonicum* recorded comparatively lower seed index (9.10g) than the control (9.26g).

With regard to yield, highest yield (2274 kg/ha) was recorded in *T. viride* followed by *P. fluorescens* (2253kg/ha). Least yield was recorded in *B. japonicum* (1940kg/ha) which was lower than the control (2077kg/ha) Fig. 4.48.

The results are in agreement with the findings of Srivastava *et al*. (1996) regarding management strategies to control charcoal rot using biocontrol agents *T. harzianum* and *Pseudomonas fluorescens* which prevent the host infection or suppress the growth of the pathogen *M. phaseolina* and significantly reduce the germination of sclerotia by 60 % in natural field soil. Adekunle *et al*. (2001) also experienced that cowpea seed treated with three *Trichoderma* spp. and planted in soils amended with *M. phaseolina* resulted in significantly greater plant stand percentage than the control. Seed treated with *T. harzianum* at sowing was effective to provide a considerable reduction of the disease caused by *M. phaseolina* in sesame (Pineda, 2001). Black gram seeds treated with *Trichoderma viride* significantly reduced the sclerotial germination of *M. phaseolina* (Rettinassababady *et al.,* 2000). The incidence of root rot in blackgram was significantly reduced by 50 % when treated with *Trichoderma* spp. alone or in combination with biofertilizer both under glass house and field conditions (Indra and Gayathri, 2002). Gupta and Chauhan (2005) also recommended the soybean (4–5 g⁄kg seed) treatment with *T. viride* or *T. harzianum* found to be very effective for managing soil borne pathogen *M.phaseolina.* Triveni *et al.* (2015) studied the influence of bio filmed formulations composed of *Trichoderma viride* and *Anabaena torulosa* against *Macrophomina phaseolina* (Tassi) Goid on infected cotton crop, in terms of plant growth and biocontrol parameters. Scanning electron microscopy revealed significant colonisation of biofilms on the root surface, which could be correlated with lowest mortality of 5.67%, recorded using *T. viride–B. subtilis* biofilm. Manjunatha and Naik (2010) evaluated several isolates of two bio-control agents, *T. viride* and *P. fluorescens*. Bio-control agents were assessed for their ability to reduce the growth of *M. phaseolina* under laboratory conditions and subsequently used for field studies. It was found that trial combining with soil application through bioagent enriched farm-yard manure, along with seed treated with the bio-control agent showed maximum germination, least root rot incidence and highest yield, as compared to the other biological or chemical seed treatments included. Govindappa *et al.* (2010) reported that seed treatment with bioagents, *P. fluorescens* and *T. harzianum* (10 g/kg) proved to be effective in controlling disease under laboratory, greenhouse and field conditions. The efficacy of these biocontrol agents are equivalent to the standard fungicide Bavistin (carbendazim). Seed treatment with these biocontrol agents enhanced the seed germination and growth parameters against root-rot disease. Nagamani *et al*. (2014) reported that seed treatment with carbendazim and *T. viride* along with soil application of FYM (FarmYard Manure) fortified with *T. viride* as the most effective in decreasing the mortality per cent of chick pea agaist dry root rot caused by *R. bataticola*.

Choudhary (2011) characterized plant growth-promotion activities of Rhizobacteria A5F and FPT721 and *Pseudomonas* sp. strain GRP3 for their antagonistic activities against *M. phaseolina*. Contrary to this, in present experiment with *B. japonicm* it was not found effective to manage the pathogen *M. phaseolina* at field level, as compare to systemic fungicide carbendazim as control and also not effective like biocontrol agents *T. viride* and *P. fluorescens.*

4.9.3.5 Effect of foliar spray of systemic fungicides against charcoal rot of soybean

The percent disease incidence, plant height (cm), branches/plant, pods/ plant, chaffy pods/plant, percent disease incidence , percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight (g), yield kg/ha, percent increase in yield over control were obtained with different treatments (table 4.23 and Fig. 4.50- 4.53).

Results indicated that all the treatments showed significant reduction in disease incidence. Minimum incidence of charcoal rot (14.67%) was observed, when foliar spray of carboxin + thiram was done. This was followed by hexaconazole (17.33%), carbendazim + mancozeb (18.33%), tebuconazole (19.00%) and trifloxystrobin + tebuconazole (19.17%). Maximum disease incidence was observed in case of propiconazole (20.67%). Hexaconazole was statistically at par with carbendazim + mancozeb, tebuconazole and trifloxystrobin + tebuconazole (Fig. 4.50).

Maximum disease reduction over control was observed in carboxin +thiram (46.20%) followed by hexaconazole (36.49%), carbendazim+ mancozeb (32.80%), tebuconazole (30.40%) and trifloxystrobin + tebuconazole (29.71%) while lowest was in propiconazole (24.16%) Fig. 4.51.

Spray of all the systemic fungicides which was used in experiment did not show significant results with respect to plant height and number of branches and percent reduction in chaffy pods over control.

Maximum number of pods/plant (82.2%) were obtained when foliar spray of trifloxystrobin + tebuconazole was done. This was followed by hexaconazole (80.2), tebuconazole (77.80), carbendazim + mancozeb (73.77) and carboxin + thiram (71.13) while least number of pods per plant were observed in plants sprayed with propiconazole .

Minimum number of chaffy pods per plant were observed when plants were sprayed with trifloxystrobin+ tebuconazole (3.53) followed by T3propiconazole (3.67), tebuconazole (4.03), carbendazim + mancozeb (4.20) and hexaconazole (4.27). Maximum number of chaffy pods was found to be in carboxin +thiram (4.30)

Weight of 100 seed is directly responsible for plot yield showing less significant results among the treatments. Highest seed index (9.83) recorded in the plants sprayed with trifloxystrobin+ tebuconazole, followed by carbendazim +

mancozeb (9.70), tebuconazole (9.62), propiconazole and carboxin + thiram (9.57). Lowest seed index was observed in foliar spray of hexaconazole (9.45).

With regard to yield the highest yield (2100kg/ha) was recorded in case of trifloxystrobin+ tebuconazole. This was followed by hexaconazole (2045kg/ha), carbendazim + mancozeb (2005 kg/ha), carboxin + thiram (1980kg/ha) and propiconazole (1948 kg/ha). While lowest yield was recorded in tebuconazole (1925kg/ha) Fig. 4.52.

According to the report of Thirumalachar and O'Brien 6g of aureofungin solubalised in 6g of sodium lauryl sulphate and 6g of soap powder and spray solution made up to 30 gallons (40ppm) when sprayed against early blight of potato –*Alternaria solani*, it controlled the disease, and the late spray before the lifting of the tubers protected them against the getting infected by charcoal rot fungus. This was due to systemic nature of aureofungin which gets in to the tube when aerial parts are sprayed.

According to Chand *et al.* (2016) charcoal rot of blackgram and greengram causes root rot, collar rot, stem rot, leaf blight, pod and seed infection. Control measure to manage the disease including resistant varieties, planting on good soil moisture, standard agronomic practices, seed treatment with agallol, captan and thiram are effective in controlling the seed borne infection and promoting the germination of seeds. Foliar spray of carbendazim, thiophanatemethyl and benomyl gave more than 90% control of foliage blight.

Rana *et al.* (2014) developed the strategies against leaf blight of green gram incited by *Macrophomina phaseolina* by using plant extracts and fungicides under *in vitro* and *in vivo* conditions. The results revealed the significant performance by foliar spray of carbendazim (0.1%), mancozeb(0.2%) and leaf extracts of *A. alliaceum* (10%), *Allium* spp. (10%) were more effective in reducing leaf blight disease in mungbean plants under greenhouse conditions. The present study revealed the induced systemic resistance (ISR) in enhancing the disease resistance in green gram plants against leaf blight disease by the application of plant extracts and fungicides.

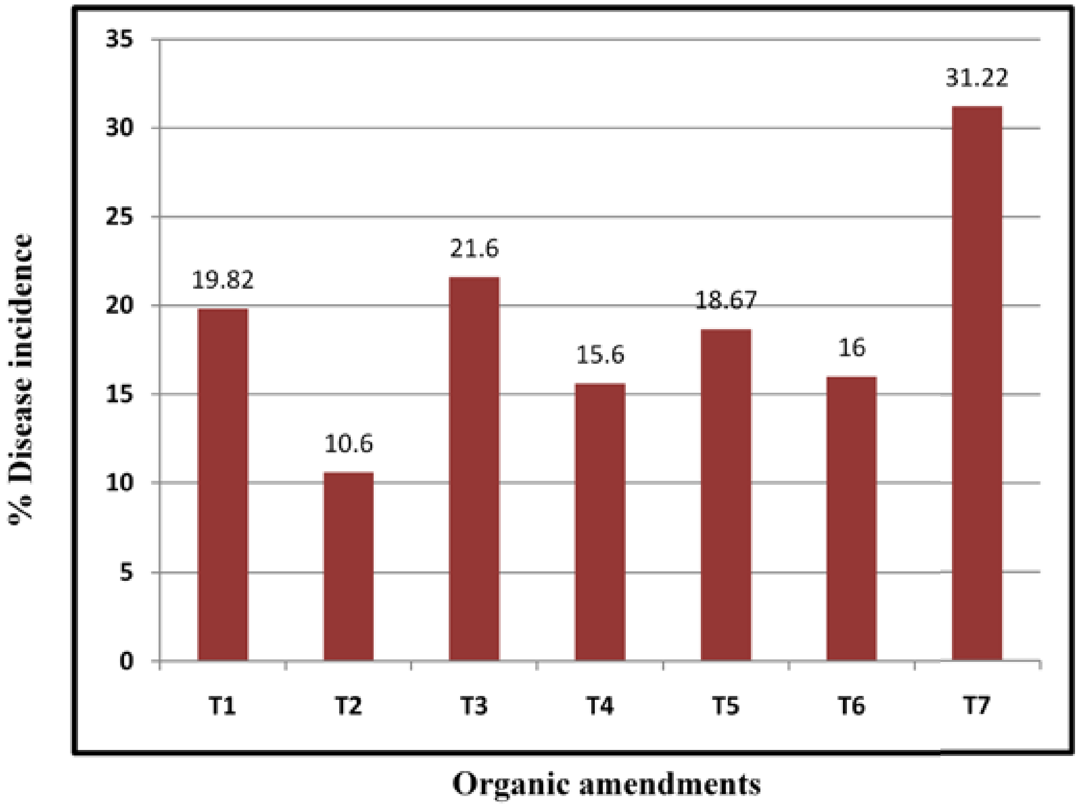

Fig 4.34: Application of organic soil amendments on the incidence of charcoal rot

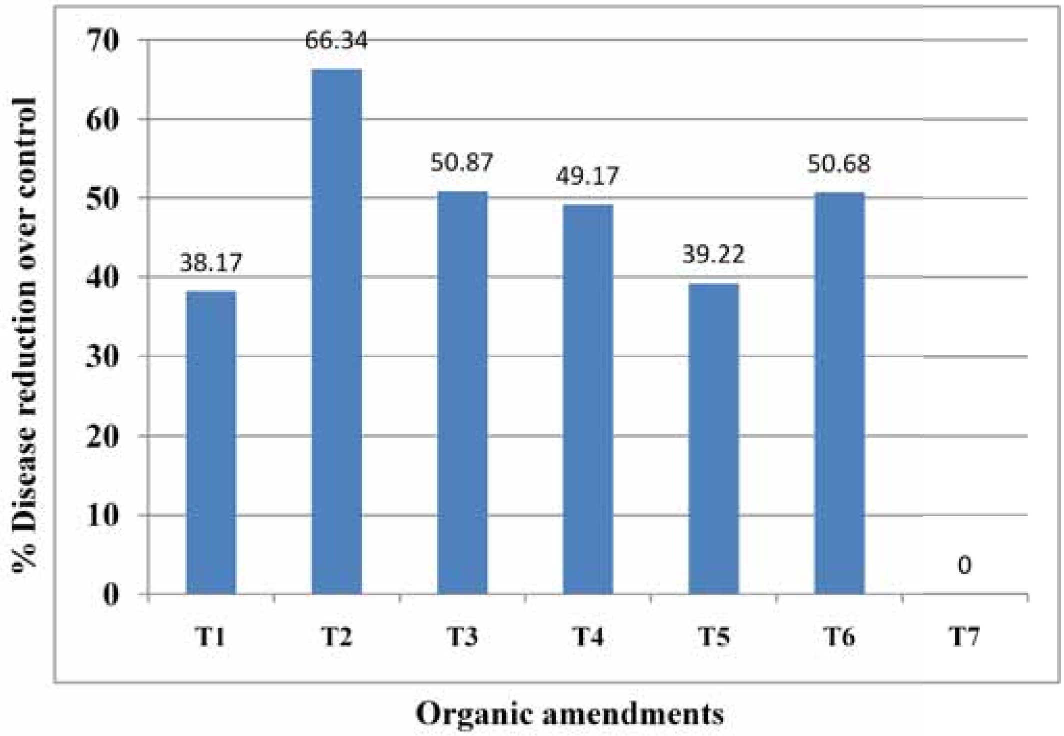

Fig.4.35: Effect of organic soil amendments on the disease reduction

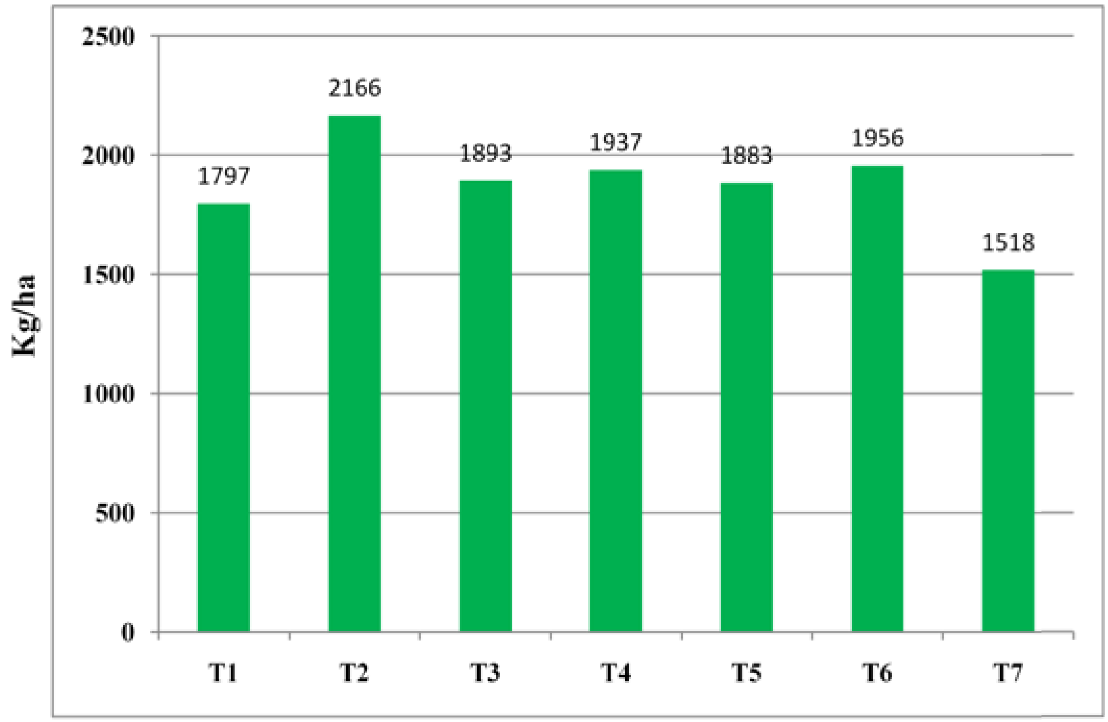

Fig 4.36: Effect of organic amendments on the soybean yield against charcoal rot

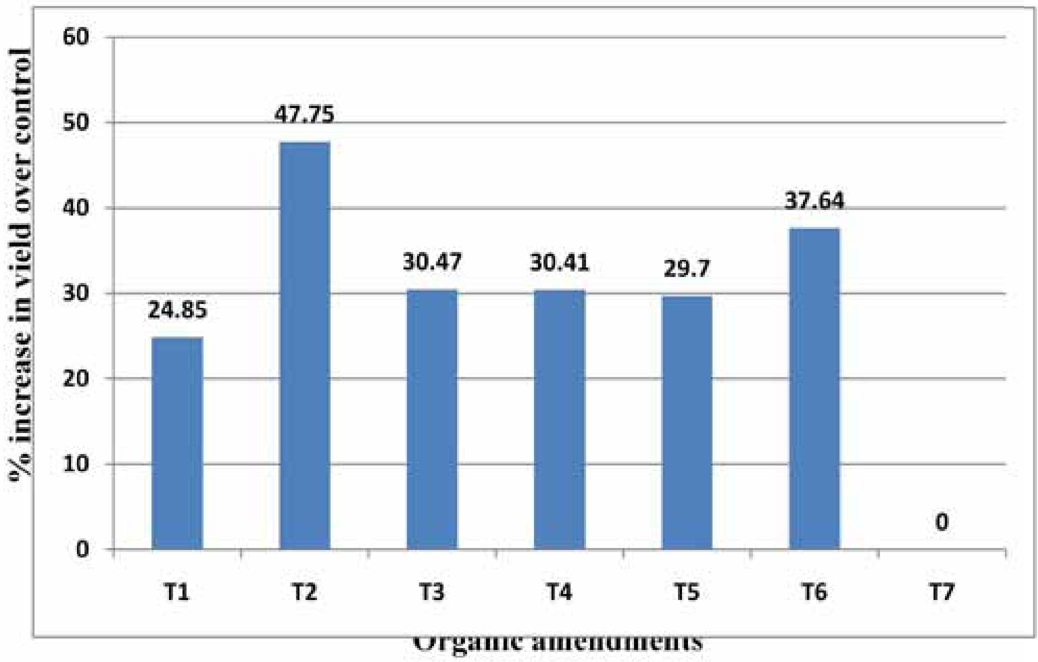

Fig 4.37: Effect of organic amendments on the percent increased in soybean yield over control

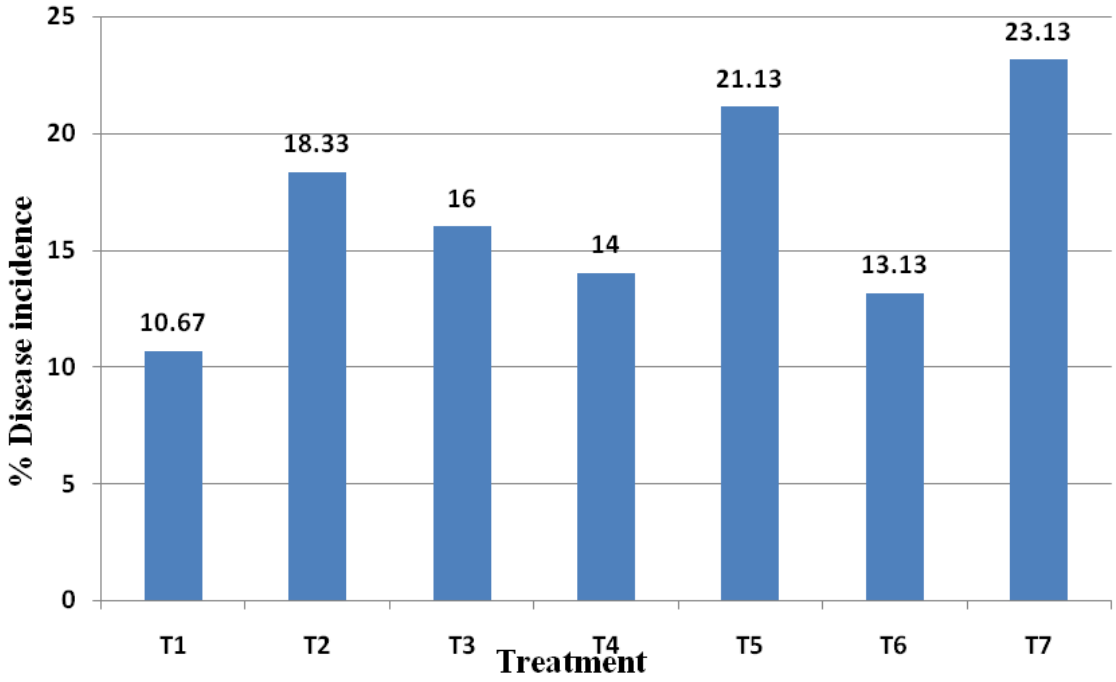

Fig. 4.38: Effect of seed treatment with botanicals on the incidence of charcoal rot

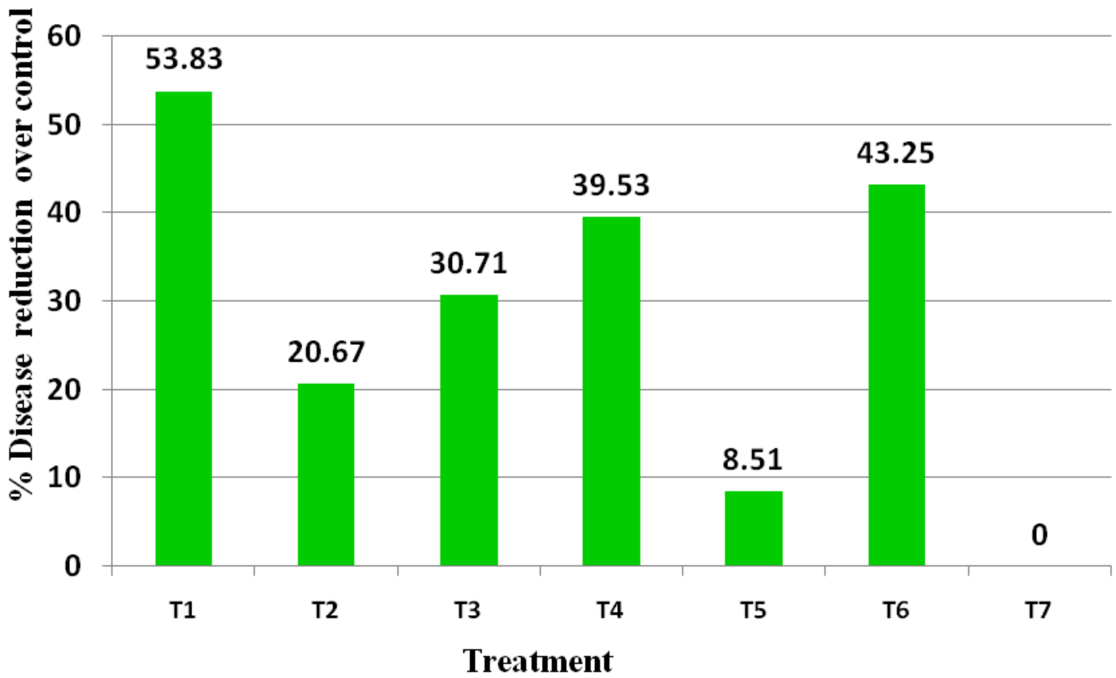

Fig 4.39: Charcoal rot disease reduction over control by seed treatment with botanicals

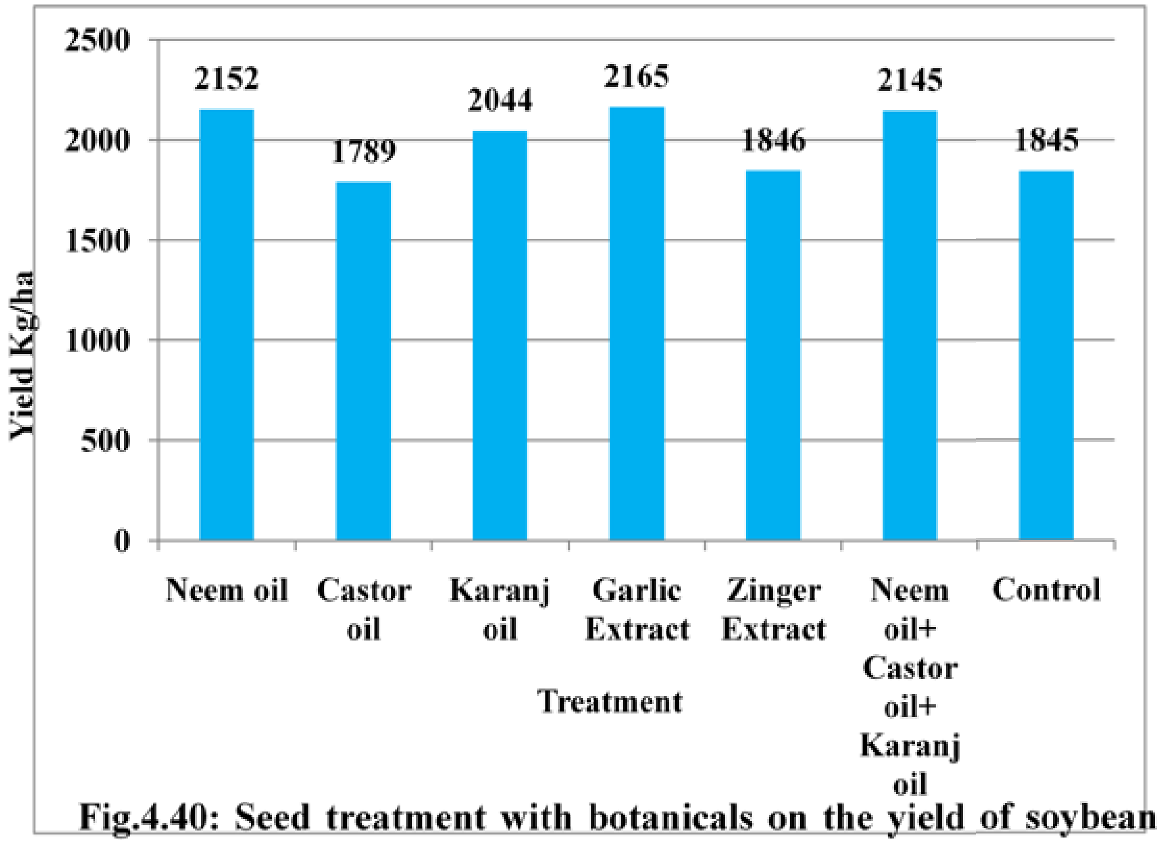

Fig.4.40: Seed treatment with botanicals on the yield of soybean against the disease

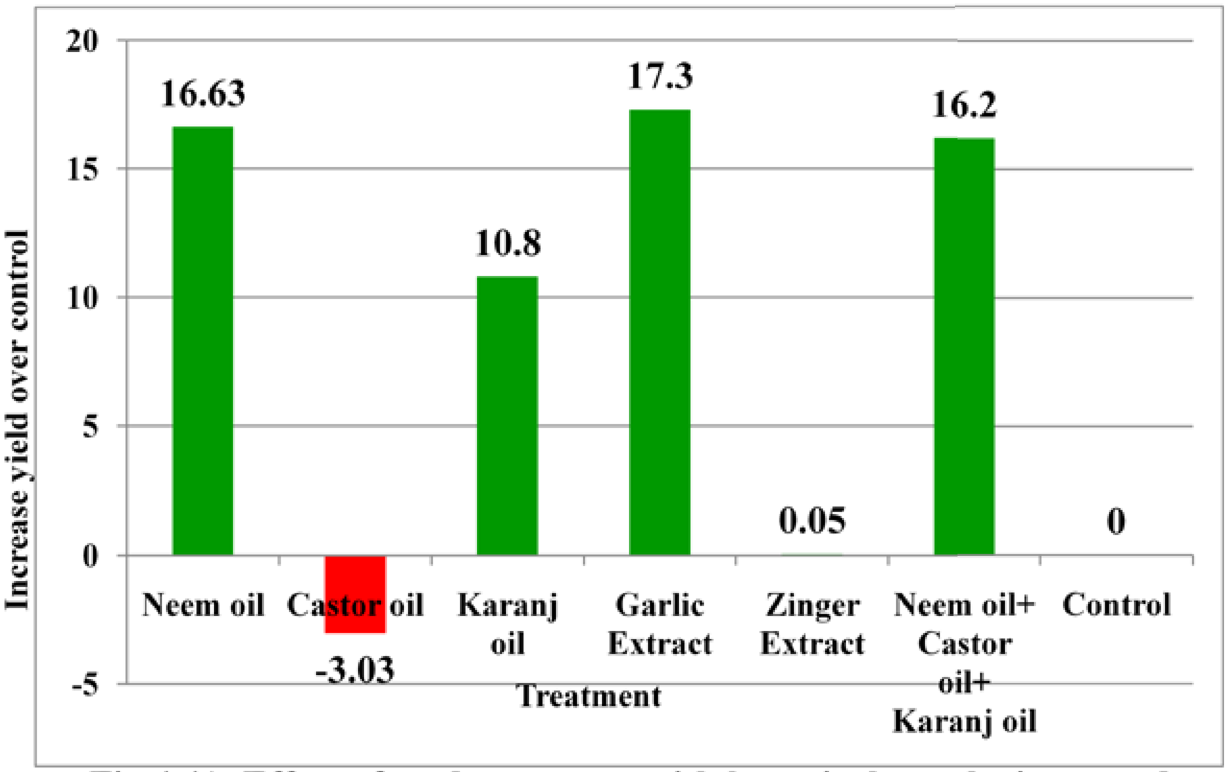

Fig.4.41: Effect of seed treatment with botanicals on the increased yield over control

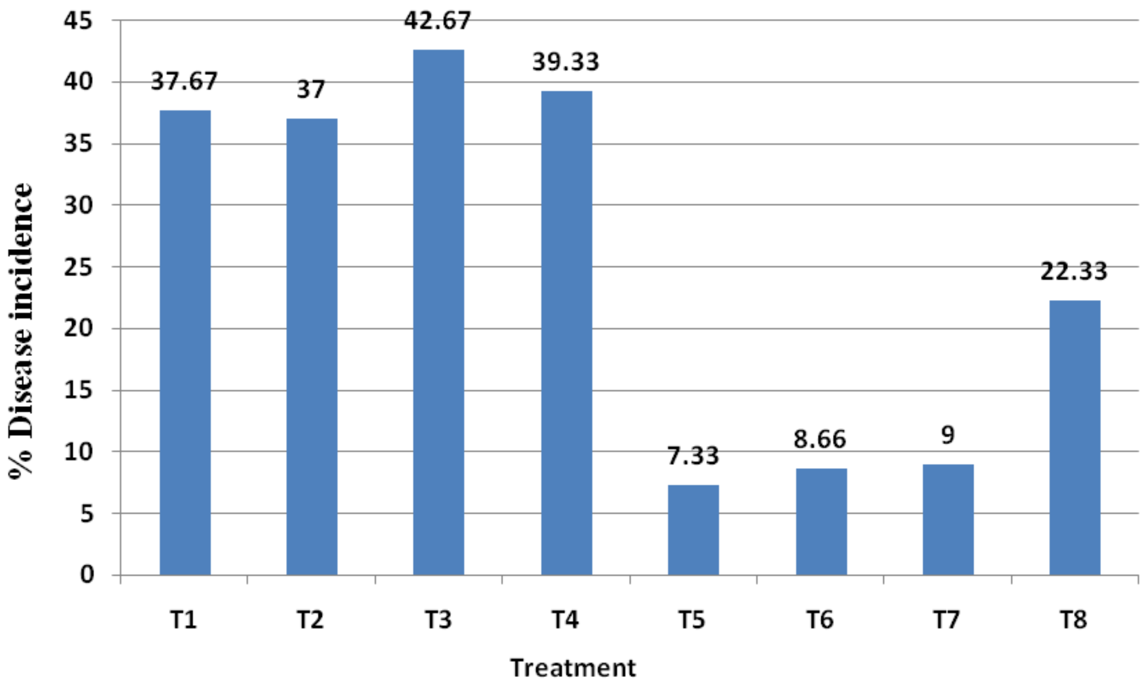

Fig.4.42: Effect of systemic fungicides on the incidence of charcoal rot

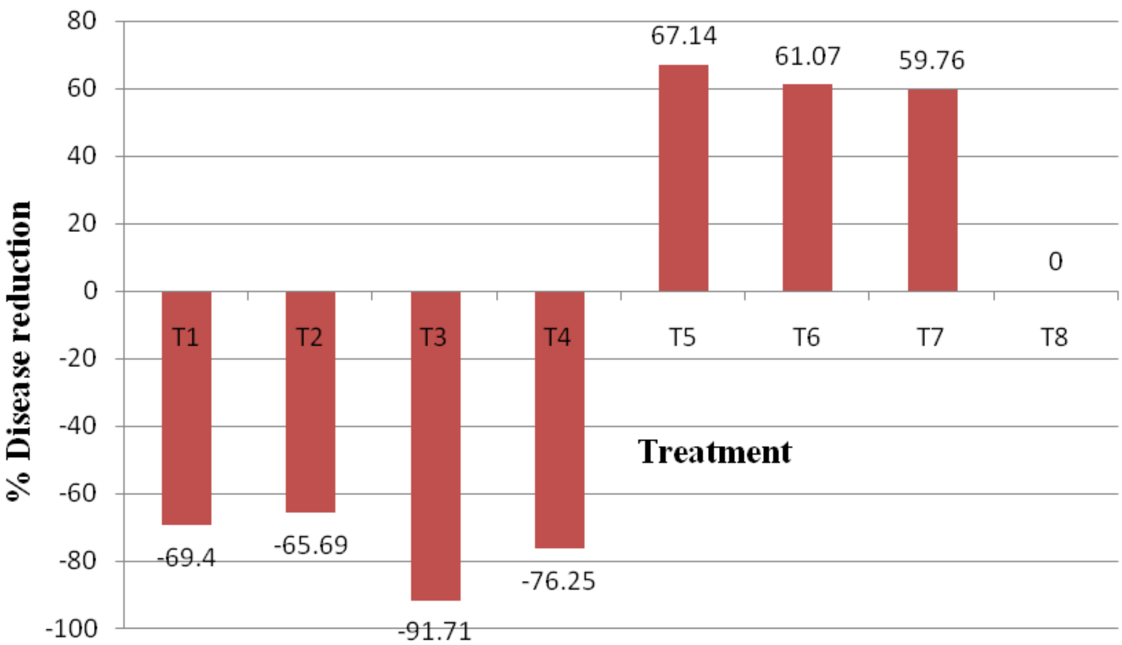

Fig. 4.43: Effect of systemic fungicides on the charcoal rot disease reduction over control

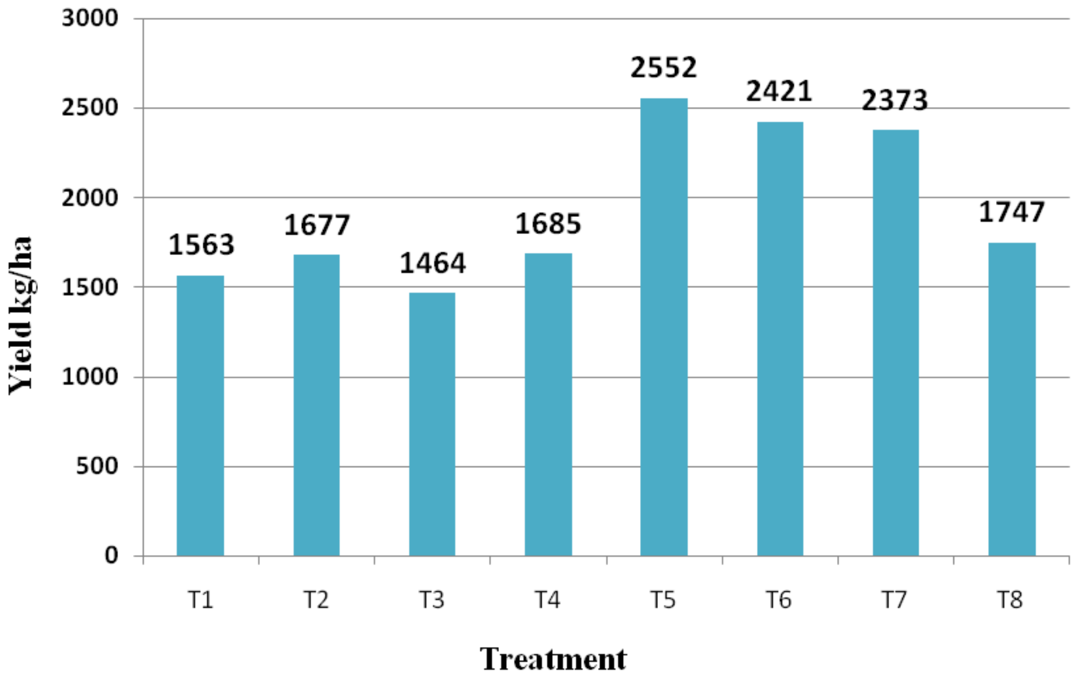

Fig. 4.44: Effect of seed treatment with systemic fungicides on the soybean yield against charcoal rot

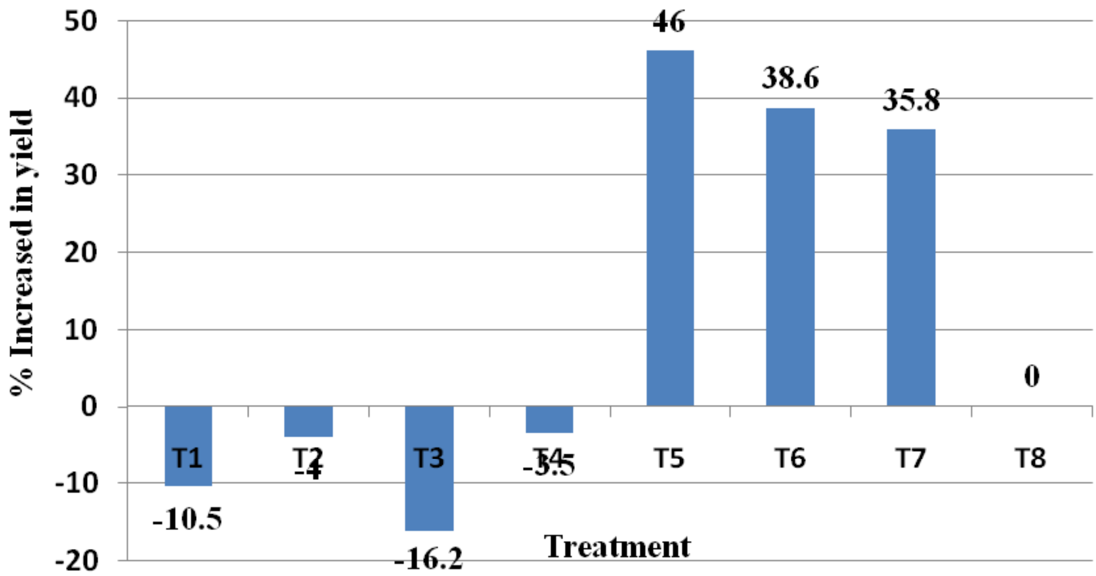

Fig. 4.45: Effect of seed treatment with systemic fungicides on the increase in soybean yield against charcoal rot over control

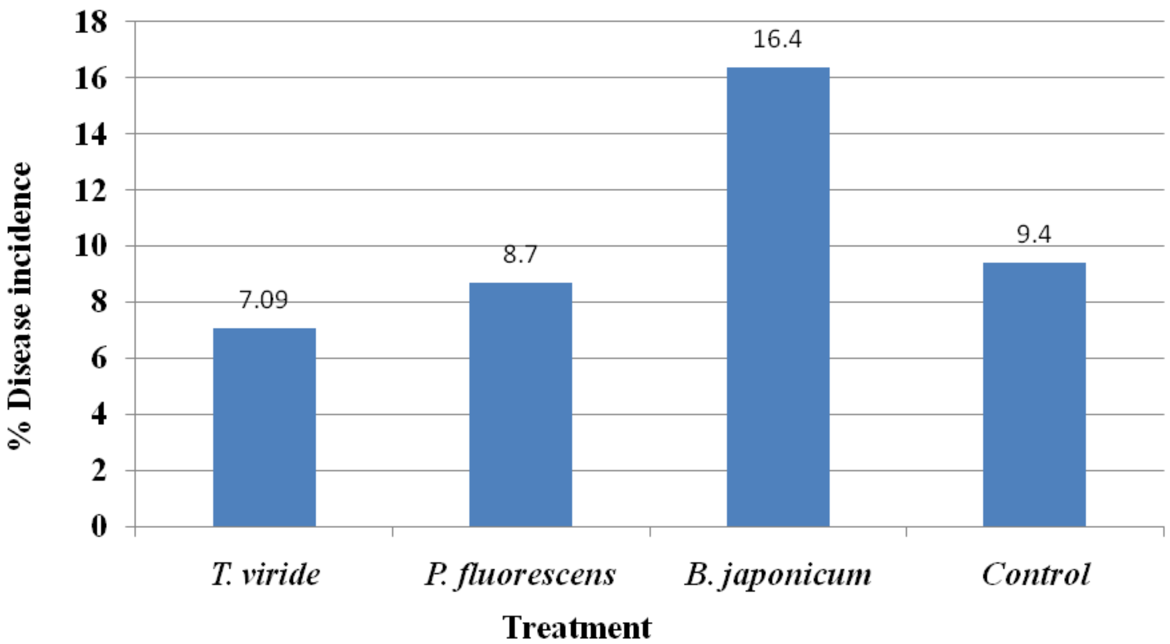

Fig. 4.46: Effect of seed treatment with biocontrol agent against the disease incidence of charcoal rot

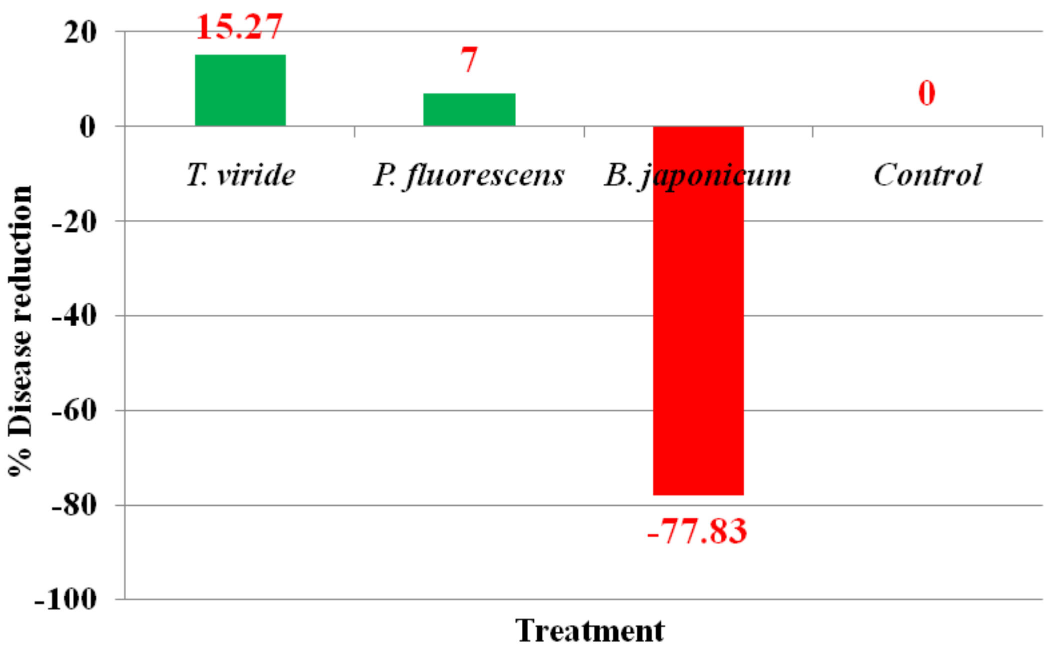

Fig. 4.47: Effect of seed treatment with biocontrol agent on % disease reduction over control

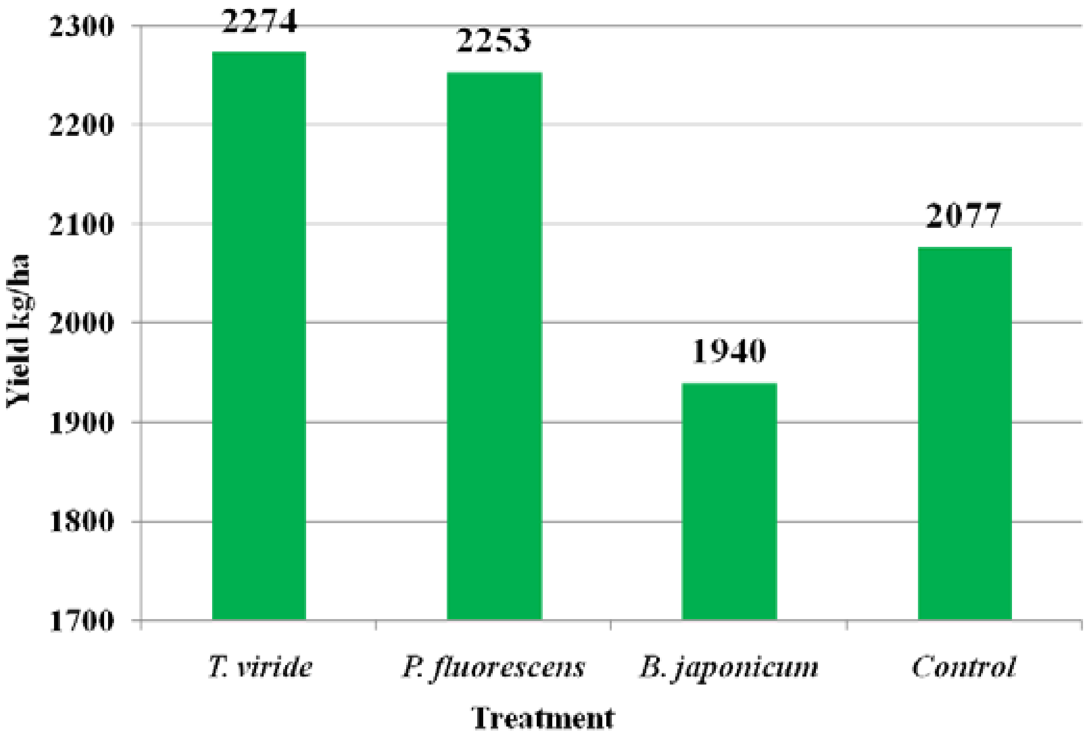

Fig.4.48: Effect of seed treatment with biocontrol agent on the yield of soybean

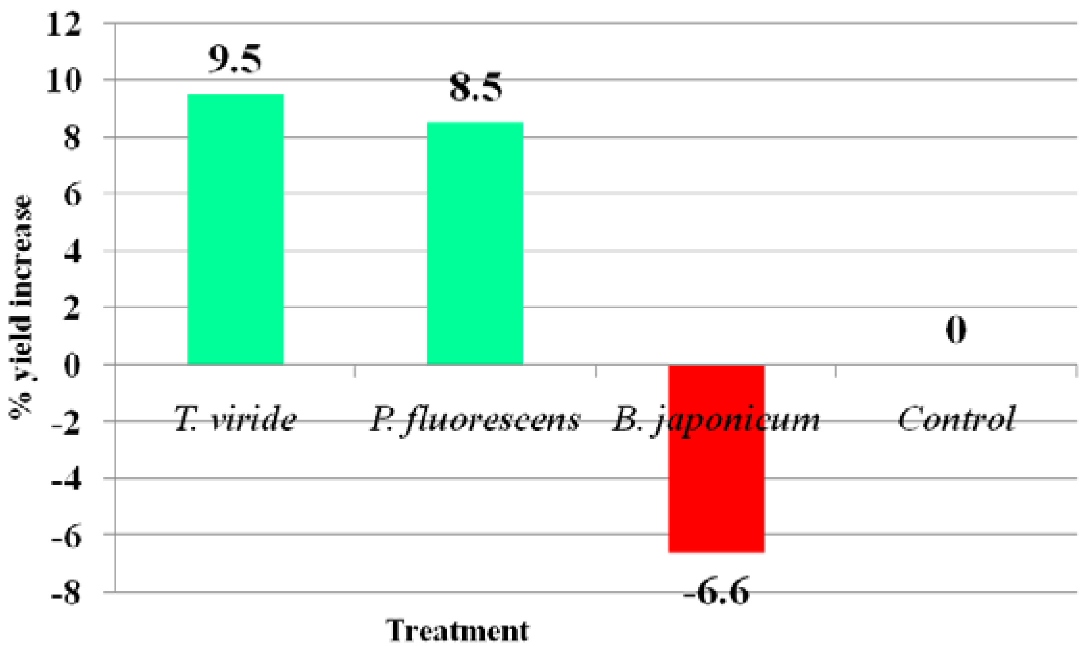

Fig. 4.49: Role of biocontrol agent on percent increased in yield of soybean

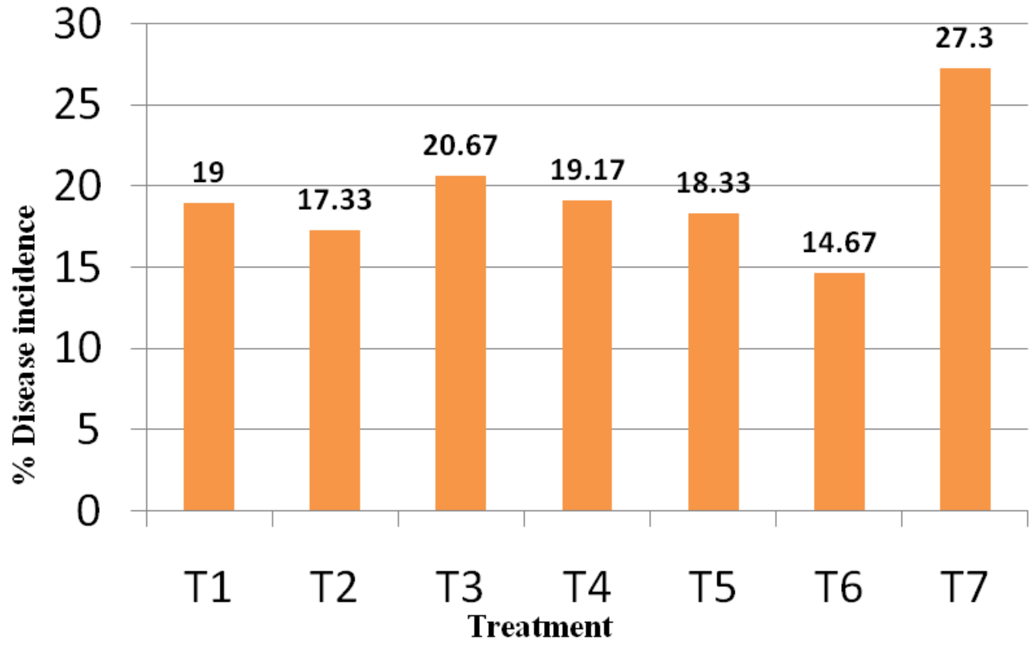

Fig. 4..50: Effect of foliar spray of systemic fungicides on disease incidence of charcoal rot

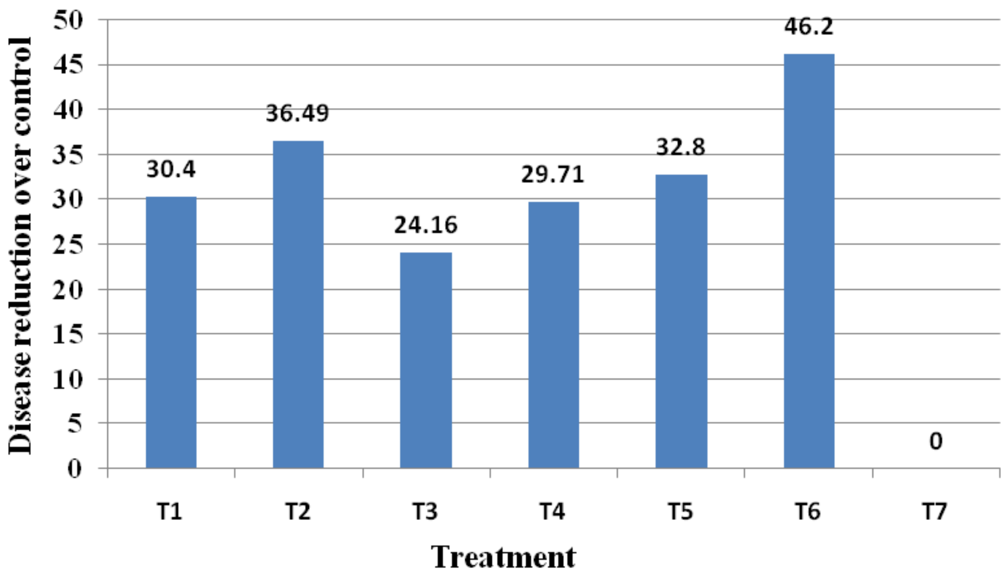

Fig. 4.51: Foliar spray of systemic fungicides reduce disease reduction of over control

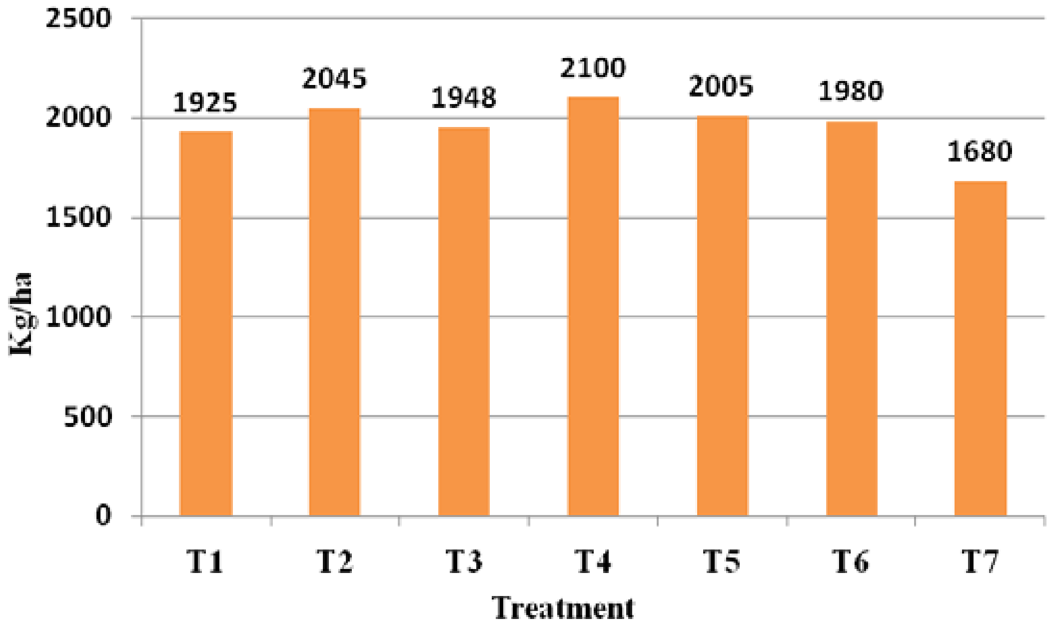

Fig. 4.52: Foliar spray of systemic fungicides on yield of soybean against charcoal rot of soybean

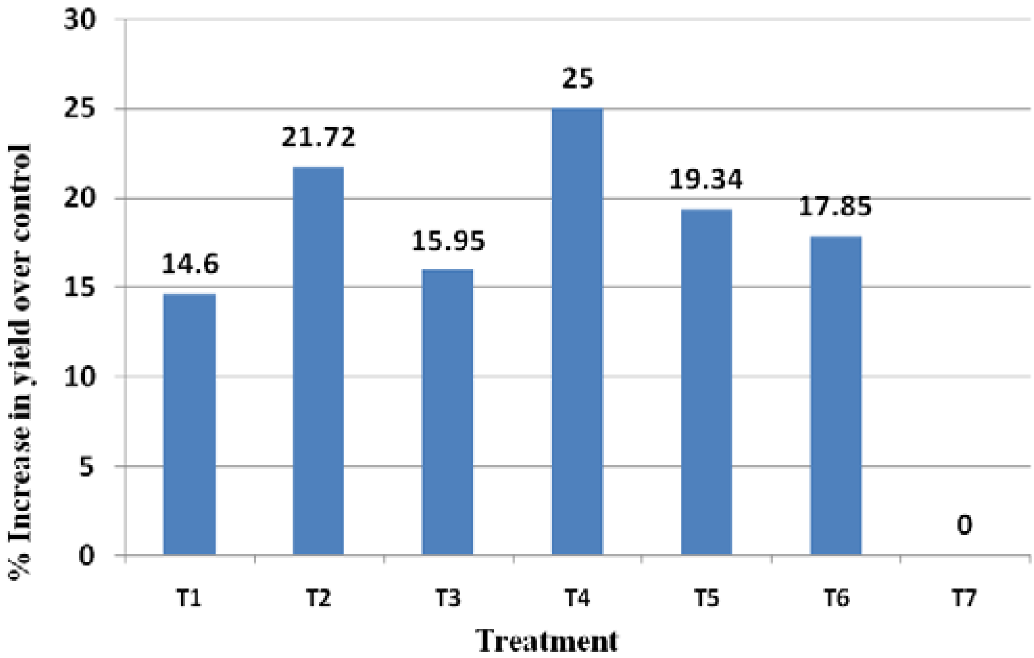

Fig. 4.53: Foliar spray of systemic fungicides on yield of soybean

4.10 Epidemiology

4.10.1 Influence of meteorological parameters on infection

Soybean cultivar JS 97-52 planted in two different season *kharif* 2015 and 2016. The progressive disease incidence was recorded at weekly interval from the reproductive stage R3 (plant can have developing pods, withering flowers and flowers buds) to R7 stage (yellow pods moving towards maturity). Simultaneously the meteorological data were also recorded from the date of sowing and association study between the weather parameters and disease incidence was carried out.

The data of *kharif* 2015 weather pattern and progress of disease summarized in table 4.24 and Fig. 4.54. Disease incidence was high during this period because weather condition favoured the disease. Correlation study of charcoal rot disease incidence and weather parameter such as maximum temperature (Tmax), minimum temperature (Tmin), rainfall (RF) and relative humidity (RH) revealed that the Tmax showed a significant correlation with the charcoal rot disease incidence (0.598 at $p \leq 0.01$) while it was reflecting significantly negative association with Tmin and RH (0.762&-0.787 respectively) at 1% significance level.

Even though rainfall and disease incidence showed no significant correlation in table 4.25. But in Fig. 4.54, it was found that, with less rainfall or no rain fall during crop period, there was sharp increase in disease occurrence (5% to 45%). This may be due to the data not sufficient to justify the fact mathematically in current study, but can be considered for future work.

The epidemiological data of *kharif* 2016 weather pattern and progress of disease summarized in table 4.26 and Fig. 4.55. Disease incidence was low during this period (5% to 15%) because weather conditions did not favour the disease development. Correlation study of charcoal rot disease incidence and weather parameter (similar as in *kharif* 2015) revealed no significant correlation association (table 4.27). The maximum temperature that showed significant correlation with the charcoal rot disease incidence during *kharif* 2015 showed no correlation during *kharif* 2016. The reason behind this may be that; Tmax solely is not responsible for occurrence of disease, other factors such as minimum temperature, relative humidity and rainfall are not favourable for disease development (5% to 15%), which can be clearly seen from graph (Fig. 4.10.2).

Graphical study of disease incidence and weather parameter indicated that disease appears when low rainfall, high maximum temperature and low relative humidity prevail during reproductive growth phase. All the abiotic factors combined and create the conditions of stress for plant. The development of *M. phaseolina* is favoured by moisture stress. This moisture stress may occur as a product of increased temperature, low rainfall and low relative humidity. Curves peak in figure 38 indicated that no rainfall was observed during period Oct 12 to Oct 25, maximum temperature (31.2°C to 32.3°C) was also high during this period but less than *kharif* 2015. Although the weather factor RH was decreased, but there was increase in disease incidence coupled to this event. This fact is in contradiction to what was observed in *kharif* 2015 as well as other similar studies. The graph (fig.34) shows that during the same period, when RH was low, there was incidence of low rainfall, which is favourable to this pathogen to flourish.

Combined study of both year data showed that the prolonged dry period prevailed during *kharif* 2015 as compared to *kharif* 2016. That is the reason why disease incidence was low in *kharif* 2016 as compared to *kharif* 2015.

The appearance of disease is also related to rainfall pattern but air temperature is the most critical factor. The timing of host reproduction is another factor that has a strong influence on charcoal rot development. Colonization of the pathogen was higher when plants were subjected to water stress and post flowering water stress resulted in greater intensity of charcoal rot (Tosi and Zazzerini 1990; Diourte *et al.* 1995). Visible symptoms of the disease in the field are most apparent under conditions e.g., poor soil fertility, high seeding rates (Sinclair and Backman 1989), low soil water content (Kendig *et al.*, 2000). Mengistu *et al.* (2011) observed that the population density of *M. phaseolina* increased slowly from the V5 to R6 growth stages and then rapidly from the R6 to R7 growth stages. Wokocha (2000) reported that the humid tropical, high soil moisture levels were unfavourable for the growth and pathogenicity of *M. phaseolina*, while low soil moisture levels favoured these fungal traits. Similarly, in cowpea, *M. phaseolina* population in the soil was negatively correlated with soil moisture and positively correlated with maximum soil temperature (Gupta and Gupta 1986). Hagedorn (1991) reported that dry conditions favour survival of microsclerotia in the soil, but mycelial growth and infection require moist conditions and are favoured by temperature above 27°C.

Table 4.24: Progress of Charcoal rot incidence during *kharif* 2015

Weeks	Max T(°C)	Min T (°C)	RF(mm)	RH (%)	%DI
Jul 22-28	31	25	23.7	80.30	0
Jun 29-5 Jul	33.2	24.4	8.0	76.45	0
Jul 6-12	32.9	25.6	8.4	77.35	0
Jul 13-19	33.4	25.8	2.5	77.65	0
Jul 20-26	30.1	25.1	6.5	85.15	0
Jul 27-2 Aug	32	25.7	0.7	72.40	0
Aug 3-9	29.2	24.3	6.7	85.25	0
Aug 10-16	32.3	25.3	15.9	82.70	0
Aug 17-23	31.9	25.6	7.1	78.60	0
Aug 24-30	31.6	25.3	7.7	83.90	0
Aug 31-6 Sep	32	25.8	1.1	79.25	5
Sep 7-13	33.5	25.5	1.4	76.45	10
Sep14-20	31.9	25.3	23.8	84.60	20
Sep 21-27	30.8	24.5	5.3	80.30	20
Sep 28-4 Oct	33.3	25.3	0	73.80	30
Oct 5-11	33.7	21.9	0	67.90	45
Oct 12-18	33.6	23.1	0	68.60	45
Oct 19-25	34.1	22.1	0	65.40	45

Table 4.25: Correlation matrix prepared from weather parameters of *kharif* 2015 and disease incidence of charcoal rot.

	Max.T(°C)	**Min.T(°C)**	**RF(mm)**	**RH(%)**	**DI (%)**
Max.T(°C)	1.00	-0.324^{NS}	-0.401^{NS}	-0.781**	0.598**
Min.T(°C)		1.000	0.292^{NS}	0.640**	-0.762**
RF(mm)			1.000	0.612**	-0.466^{NS}
RH(%)				1.000	-0.787**
RH(%)					1.000

Table 4.26: Progress of Charcoal rot incidence during *kharif* 2016

Weeks	Max T(°C)	Min T (°C)	RF(mm)	RH (%)	% DI
Jul 22-28	35.8	26.1	5.9	70.55	0
Jun 29-5 Jul	32.9	26	6.7	79.5	0
Jul 6-12	28.8	24.5	16.9	90.85	0
Jul 13-19	30.4	24.7	11.2	85.35	0
Jul 20-26	31.5	25.3	23.9	83	0
Jul 27-2 Aug	31.6	25.4	3.8	82.05	0
Aug 3-9	28.7	21.9	16.9	86.65	0
Aug 10-16	30.1	25.3	2.2	81.85	5
Aug 17-23	29.1	24.2	1.7	79.75	10
Aug 24-30	32.3	26.3	3.8	81.35	10
Aug 31-6 Sep	31.4	25.2	7.9	79.4	10
Sep 7-13	30.9	24.5	19.1	82.8	10
Sep14-20	31.8	24.7	13.9	84.2	10
Sep 21-27	31.2	25	12.3	86.4	10
Sep 28-4 Oct	29.3	24.6	13.1	88.4	10
Oct 5-11	31.2	24.4	2.2	81.05	15
Oct 12-18	32.3	20.3	0	62.1	15
Oct 19-25	31.5	18.3	0	61.6	15

Table 4.27: Correlation matrix prepared from weather parameters of *kharif* 2016 and incidence of charcoal rot.

	Max.T(°C)	**Min.T(°C)**	**RF(mm)**	**RH(%)**	**DI (%)**
Max.T(°C)	1.00	0.200^{NS}	-0.287^{NS}	-0.556*	-0.018^{NS}
Min.T(°C)		1.000	0.247^{NS}	0.607**	-0.453^{NS}
RF(mm)			1.000	0.632**	-0.432^{NS}
RH(%)				1.000	-0.422^{NS}
RH(%)					1.000

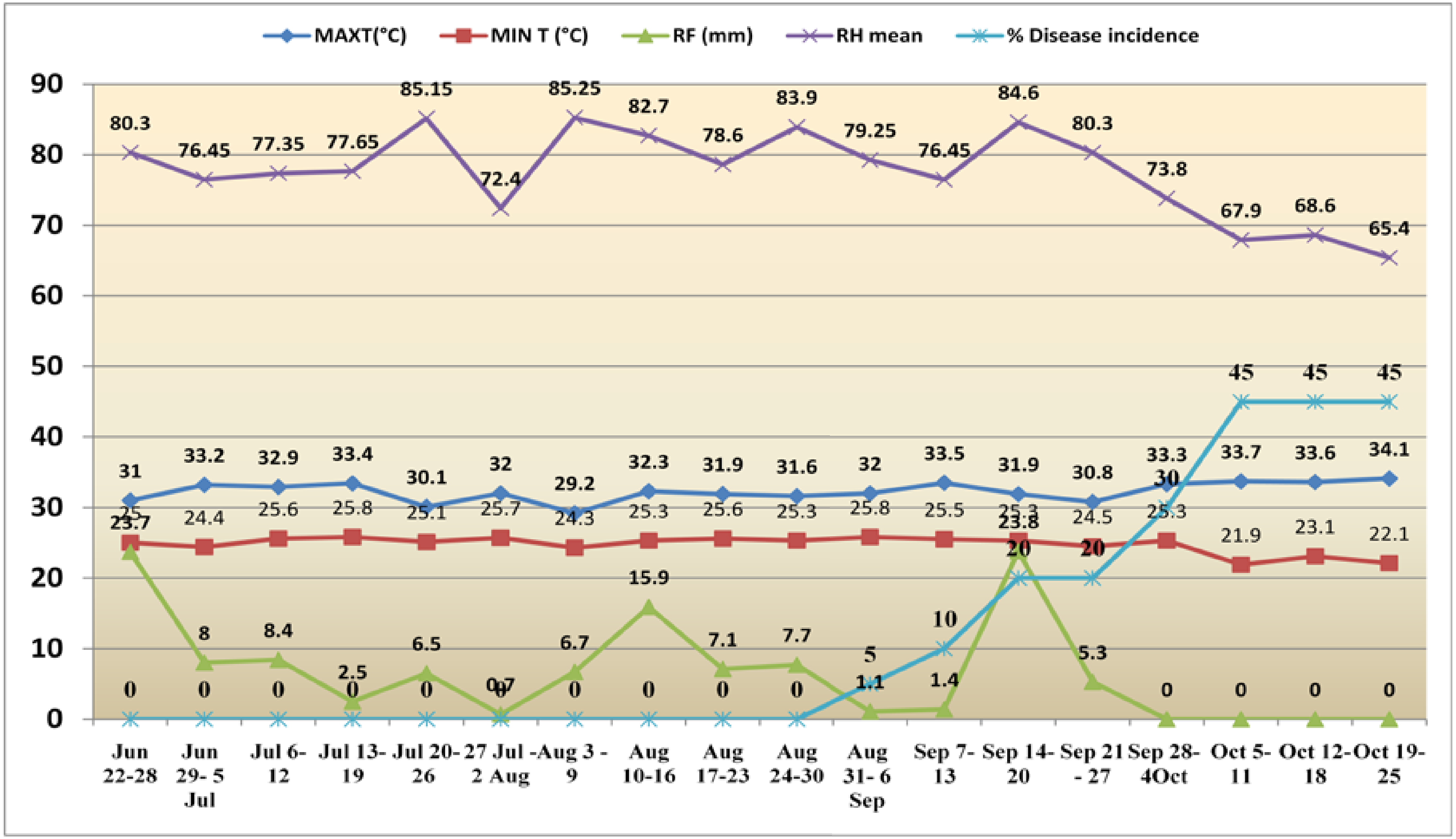

Fig 4.54: Progress of charcoal rot in relation to weather parameter during *kharif* 2015

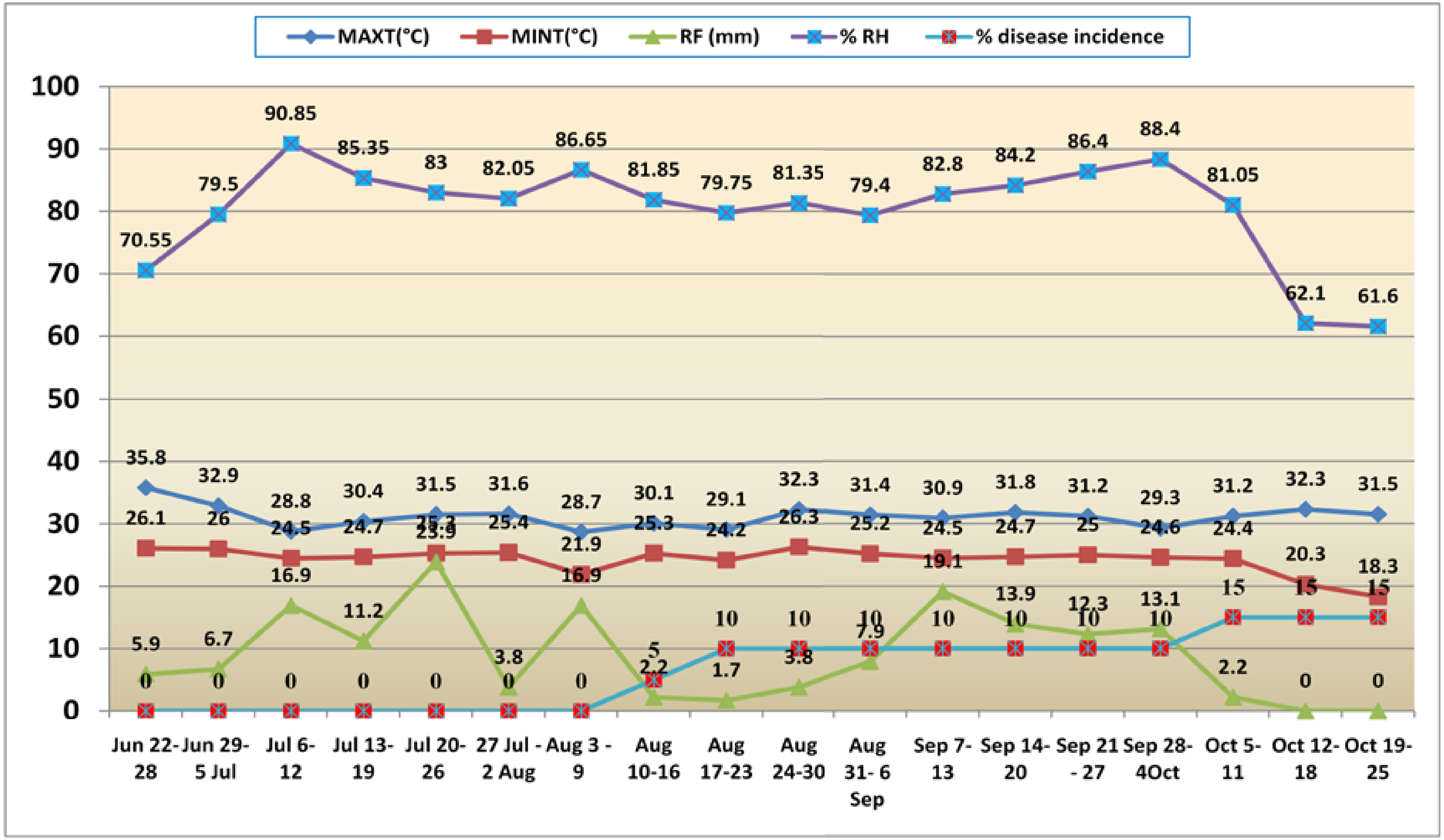

Fig 4.55: Progress of charcoal rot in relation to weather parameter during *kharif* 2016

CHAPTER – V

SUMMARY AND CONCLUSION

The finding of the investigation on "Studies on *Macrophomina phaseolina* (Tassi) Goid causing charcoal rot of soybean [*Glycine max* (L.) Merrill] and its management" were carried out at the Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The investigation mainly consist of following objectives:

1. To survey and collection of *Macrophomina phaseolina* isolates from Chhattisgarh and record the disease incidence of charcoal rot.

2. To study the morpho-cultural and pathogenic variability among the *Macrophomina phaseolina* isolates.

3. To identify the resistance source through the screening of soybean varieties/wild relatives against charcoal rot.

4. To evaluate different management practices against the charcoal rot of soybean.

The results obtained in present investigation were summarized as below:

The intensive survey was conducted on disease incidence of charcoal rot conducted in soybean growing area of Chhattisgarh. The disease incidence was recorded recorded for all surveyed villages with incidence 42.63% and 19.12% during *kharif* 2015 and *kharif* 2016 respectively.

Broad range of charcoal rot symptoms appeared on soybean plants such as non emergence of seedlings, dead seedlings, yellowing and wilting of plant, longitudinal streaks along the main stem, wilting of branches and shriveled pods Presence of microsclerotia in the vascular tissues, stems, root and pods showed greyish black appearance.

Disease samples were collected from each location to isolate *M. phaseolina* to observe the cultural, morphological and pathogenic variations among the isolates.

Fifteen isolates of *M. phaseolina* were characterized for cultural and morphological characteristics on PDA medium. The isolates showed varied pattern of mycelial growth rate. Two fast growing isolates MP1 and MP14 were completed their radial growth within 72h, Seven medium growing isolates *viz.,* MP3, MP4, MP5, MP6, MP8, MP12 and MP13 completed their radial growth within 96h and six isolates namely MP2, MP7, MP9, MP10, MP11 and MP15 were very slow growing and didn't complete the full radial growth even after (96h) of inoculation. The hyphal width of isolates was categorized in three groups. First group comprised of eight isolates namely MP1, MP3, MP6, MP9, MP12, MP13, MP14 and MP15 with hyphal width ranging between 5-6 µm. Second group has isolates having hyphal width ranging 6-7 µm, MP2, MP7, MP10 and MP11. Third group consists of only three isolates *viz.,* MP4, MP5 and MP8 with hyphal width of more than 7µm. Mycelial growth pattern observed in isolates were fluffy, partial submerged, submerged, and irregular, whereas the colony colour found to be dark black, greyish and light greyish in appearance. Among the isolates of *M. phaseolina*, sclerotial size varied from 71.5-102.4 x 62.4-85.7 µm, whereas shape of sclerotia categorized into round and oval. Highest number of sclerotia per microscopic field reported in MP4 (171.94) and least in MP5 (75.33).

The studies on the pathogenic varibility revealed that, all the isolates were found to be pathogenic under artificially inoculated condition. Aggressiveness study of *M. phaseolina* on soybean seedlings through *in vitro* blotter paper technique revealed that variation exists within the isolates. Isolates showed varying degree of disease rating after inoculation on the particular variety. The disease reaction of all the fifteen isolates on cultivar varied between the 5-9 grades. It was observed that isolate MP14 behave highly aggressive on all the five varieties CG Soya-1, JS 97-52, JS 93-05, JS 335 and RSC 10-46 of soybean. Similarly cut stem inoculation technique was also used for aggressiveness study. The disease reaction of all the fifteen isolates on cultivar CG Soya-1, JS 335 and JS 97-52 was observed in the form of linear stem necrosis. It was observed that like *in vitro* blotter paper technique isolate MP14 showed highly aggressive behavior on all the three varieties of soybean.

Early diagnosis of healthy looking soybean plant showed microsclerotia on the stem, root and nodule portion of soybean plant. Which indicated that infection of *Macrophomina phaseolina* took place at early stage of plant growth but did not show any symptom of charcoal rot like wilting and yellowing of leaves till the climatic conditions favour the disease development such as temperature in the range of 30-35 °C and low rainfall or no rainfall.

Field screening of soybean conducted during *kharif* 2015 and *kharif* 2016 showed variation in the performance against charcoal rot of soybean. During the *kharif* 2015 out of 237 germplasm 24 germplasm lines found to be moderately resistant, 86 were moderately susceptible, 104 susceptible and 23 germplasm as highly susceptible. Similarly field screening of 237 germplasm during *kharif* 2016 revealed that, 46 soybean entries behaved as moderately resistant and 191 as moderately susceptible. Field screening of soybean entries was also conducted during *kharif* 2015. Six entries were found to be moderately resistant, thirty five entries were found to be moderately susceptible and sixteen as susceptible. Similarly during screening of soybean entries during *kharif* 2016-17 identified forty two entries moderately resistant and twenty two entries exhibited moderately susceptible.

Efficacy of antagonists, botanicals and fungicides were evaluated *in vitro* against *M. phaseolina*. Antagonist *T. viride* was found to be effective against *Macrophomina phaseolina* and maximum radial growth inhibition (59.72%) was observed in the isolates of MP5 and the minimum percent of inhibition was observed in the isolate MP8 (32.55%). Among the botanicals, maximum inhibition of mycelial growth was recorded in the neem oil followed by garlic (58.50%), neem oil + castor oil + karanj oil (29.93%) and karanj oil (17.03%). Ginger extract and castor oil showed no inhibition on the radial growth of mycelium and not found suitable. Efficacy of seven different systemic fungicides was tested under *in vitro* condition against virulent isolate of *Macrophomina phaseolina* MP14. At 100ppm complete inhibition of *M. phaseolina* was observed with tebuconazole, hexaconazole, propiconazole and carbendazim+mancozeb and carbendazim, whereas complete inhibition of mycelium was observed by all the fungicides at 200ppm.

The study on the highly aggressive isolates for testing the performance of five soybean varieties, using sick pot method. The survival per cent of soybean plant after 30 days of sowing varied from 50% to 53.33% among all the varieties under sick pot condition compared to control (70.00% to 80.00%). *In vivo* sick pot method was used to test the efficacy of different biocontrol agents under glass house condition. Minimum disease incidence 18.33 percent was when seed was treated with *T. viride* followed by *P. fluorescens* 23.33 percent and *B. japonicum* 36.67 percent. The soybean seed treated with *T. viride* showed maximum shoot length (27.43cm) followed by *P. fluorescens* (26.97cm) and *B. japonicum* (26.87), whereas seed treatment with *B. japonicum* showed maximum root length (11.90cm) followed by *T. viride* (11.63cm) and *P. fluorescens* (8.5cm).

Efficacy of organic soil amendments, fungicides and biocontrol agents was evaluated under natural field condition for the management of charcoal rot of soybean. Soil application of organic amendments was found effective for reducing the disease incidence of charcoal rot. The percent disease incidence was low (10.60%), when the neem cake was used as organic amendment followed by karanj cake (15.60%), castor cake 16.00 percent, linseed cake 18.67 percent, FYM (19.82 %) and mustard cake (21.60%). Among all the organic amendments, Neem cake was highly effective for reducing the disease incidence by 66.34 percent, lowered the the number of chaffy pods (3.63). Soil application of neem cake recorded highest yield (2166kg/ha) followed by castor cake (1956 kg/ha),karanj cake (1937 kg/ha), mustard cake (1893kg/ha) linseed cake (1883 kg/ha) and 1797kg/ha in FYM.

Performance of botanicals as seed treatment was found effective for disease management. The percent disease incidence was low 10.67 percent, when the neem oil was used as seed treatment for disease management followed by neem oil + castor oil + karanj oil (13.13%), garlic extract (14.00%), karanj oil (16.00%), castor oil (18.33%) and ginger extract (21.13%) over control (23.13%) . Seed treatment with neem oil was found highly effective for managing the disease. It reduced the disease incidence by 53.83 percent, maximum number of pods/plant (86.33), reduced chaffy pods (3.67) and seed index 9.51g. With regards to yield, the highest yield (2165kg/ha) was recorded in case of neem oil followed by garlic extract (2152 kg/ha), neem + castor + karanj (2145 kg/ha), karanj oil (2044 kg/ha), castor oil (1846 kg/ha) and ginger extract (1789kg/ha).

The performance of systemic fungicides used as seed treatment for the management of charcoal rot was studied. Seed treatment with carbendazim + mancozeb, carboxin + Thiram and carbendazim resulted significant reduction in the disease incidence 7.33 percent, 8.66 percent and 9.00 percent respectively, whereas seed treatment with tebuconazole, hexaconazole, propiconazole and trifloxystrobin + tebuconazole did not show significant percent disease reduction over control and instead of decrease the percent disease increased over control *i.e.,* 69.40, 65.69, 91.71 and 76.25 percent respectively. With regards to yield, the highest yield (2552 kg/ha) was recorded in seed treatment with carbendazim + mancozeb followed by treatment with carboxin + thiram (2421 kg/ha) and carbendazim (2373 kg/ha). Treatment T1, T2, T3 and T4 did not show increase in yield over control (1563, 1677, 1464 and 1685 kg/ha respectively).

With the use of biological agent as seed treatment against charcoal rot of soybean. Among the all treatment minimum percent disease incidence was observed when seeds were treated with *T. viride* (7.09%) followed by *P. fluorescens* (8.70%) while maximum incidence was recorded in *B. japonicum* (16.40%) which was greater than the control (9.40%). *T. viride* treated seed significantly reduced the number of chaffy pod over control (8.46%) followed by *P. fluorescens* (5.82%). Highest seed index (9.95g) was recorded in seed treatment with *T. viride* followed by *P. fluorescens* (9.62g). With regard to yield, highest yield (2274 kg/ha) was recorded in *T. viride* followed by *P. fluorescens* (2253kg/ha). Least yield was recorded in *B. japonicum* (1940kg/ha) which was lower than the control (2077kg/ha).

Foliar spray of systemic fungicides indicated that all the treatments showed significant reduction in disease incidence. Minimum incidence of charcoal rot (14.67%) was observed, when foliar spray of carboxin + thiram was done. This was followed by hexaconazole (17.33%), carbendazim + mancozeb (18.33%), tebuconazole (19.00%) and trifloxystrobin + tebuconazole (19.17%). Disease reduction over control due to fungicides showed that maximum reduction was in carboxin + thiram (46.20%) followed by hexaconazole (36.49%), carbendazim+ mancozeb (32.80%), tebuconazole (30.40%) and trifloxystrobin + tebuconazole (29.71%) while lowest was in propiconazole (24.16%). Maximum number of pods/plant (82.2%) were obtained with foliar spray of trifloxystrobin + tebuconazole while least number of pods per plant were observed in plants sprayed with propiconazole. Minimum number of chaffy pods per plant were observed when plants were sprayed with trifloxystrobin+ tebuconazole (3.53) and maximum number of chaffy pods were in carboxin +thiram (4.30). Highest seed index was (9.83) recorded in the plants sprayed with trifloxystrobin+ tebuconazole and lowest seed index was observed in foliar spray of hexaconazole (9.45). With regards to yield the highest yield (2100kg/ha) was recorded in case of trifloxystrobin+ tebuconazole and lowest yield was recorded in tebuconazole (1925kg/ha).

The meteorological parameters played significant role on progress of charcoal rot. The weather data of *kharif* 2015 and disease progress indicated that disease was high during this period because weather condition favoured the disease. Correlation study of charcoal rot disease incidence and weather parameter such as maximum temperature (Tmax), minimum temperature (Tmin), rainfall (RF) and relative humidity (RH) revealed that the Tmax showed a significant correlation with the charcoal rot disease incidence (0.598 at $p \leq 0.01$). It was found that, with less rainfall or no rain fall during crop period, there was sharp increase in disease occurrence (5% to 45%). The similar study conducted during *kharif* 2016 on progress of disease, the disease incidence was low during this period (5% to 15%) because weather conditions did not favour the disease development. Correlation study of charcoal rot disease incidence and weather parameters revealed no significant correlation association. Study also revealed that disease incidence appeared when low rainfall, high maximum temperature and low relative humidity prevailed during reproductive growth phase. RH was low, there was incidence of low rainfall, which is favourable to this pathogen to flourish. Combined study of both year data showed that the prolonged dry period prevailed during *kharif* 2015 as compared to *kharif* 2016.

CONCLUSION

In the light of the findings of present investigation, some conclusion could be drawn like

- The intensive survey of major soybean growing area of Chhattisgarh recorded high disease incidence during *kharif* 2015 as compared to *kharif* 2016.
- Isolates of *M. phaseolina* exhibited a high degree of variability for its cultural, morphological characteristics.
- Isolates of *M. phaseolina* exhibited high degree of pathogenic variability.
- Early diagnosis of healthy looking soybean plant, can be used as effective tool for identification of charcoal rot infected soybean plant.
- Screening of soybean germplasm against the charcoal rot of soybean could identify the moderately resistant lines. These lines can be used for future breeding programme.
- Efficacy of antagonists, botanicals and fungicides was found effective for inhibition of mycelial growth under *in vitro* condition.
- The highly aggressive isolates, MP14 will be highly useful for further screening programme of soybean varieties against charcoal rot.
- Biocontrol agents *T. viride* and *P. fluorescens* showed strong antagonistic effect against the *Macrophomina phaseolina* under glass house and field condition.
- The charcoal rot management study indicated that in organic soil amendment, neem cake was found to be most effective treatment for disease management.
- Among the botanicals, neem oil was found to be most effective seed treatment for disease management.

- Seed treatment with systemic fungicides carbendazim + mancozeb was found to be very effective for charcoal rot disease management.
- Foliar spray of systemic fungicides trifloxystrobin+ tebuconazole was found to be effective for disease management.
- The meteorological parameters played significant role on progress of disease charcoal rot. Weather condition such as less or no rainfall, high temperature and low relative humidity create the condition of dryness and development of disease.

SUGGESTIONS FOR FUTURE RESEARCH WORK

- A regular survey for the assessment of charcoal rot in soybean growing areas and evaluation of performance of variety under natural condition should be done.
- Work should be done in the area of *in vitro* pycnidial stage production, that can be used for diversity analysis and also for resistant breeding programme.
- Molecular level diversity analysis with the help of molecular markers such as ITS, SSR, RFLP and rDNA sequence analysis will be better for understanding the genetic diversity and pathogenic variability among the isolates of *M. phaseolina.*
- Effect of toxin on disease development can also be used for further study.
- Early diagnosis of soybean plant infection with charcoal rot should be used for screening the varieties and germplasm and also the extent of pathogen development in the plant tissue.
- Regular screening of soybean germplasm and entries should be performed under natural field condition to identify the resistant source with less error.
- The genotypes found resistant during screening, should be utilized in breeding programmes to build disease resistant or tolerant varieties.
- More intensive study need to be conducted in the area of *B. japonicum* to identify the potential biocontrol agent as well as nitrogen fixation for soybean crop.

- Further study need attention in the field of seed treatment with fungicides especially triazoles group fungicides.
- Detailed study in the field of integrated disease management of disease charcoal rot with the use of all management practices for effective manage the disease should be done.

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Survey to Record the Disease Incidence of Charcoal Rot of Soybean Caused by *Macrophomina phaseolina* from Chhattisgarh

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ABSTRACT

The intensive survey of charcoal rot of soybean conducted in soybean growing area of Chhattisgarh *i.e*. Raipur, Bemetara, Kawardha, Rajnandgaon and Durg. The disease incidence recorded during *kharif* 2015, ranged from 30 to 60 per cent. The maximum percent of disease incidence (60%) was observed in Jaibra and lowest percent of incidence was found in Bemetara and Basni (30.00%). The average percent disease incidence in different soybean growing area of Chhattisgarh was about 42.63%. The survey report of charcoal during *kharif* 2016 was ranged from 15 to 25.10 percent with average disease incidence of 19.12 percent compare to the last year average (42.63%). The maximum percent of disease incidence (25.10%) was observed in Ataria and lowest disease incidence (15.00%) recorded in Bemetara and Parpondi.

Key words *Record, Disease Incidence, Charcoal Rot, Soybean, Macrophomina phaseolina, Chhattisgarh*

Soybean [*Glycine max*.(L.) Merrill] designated as miracle bean established its potential as an industrially vital and viable oilseed crop in many areas of India. Soybean is a world's first rank crop as a source of vegetable oil. The major soybean producing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh, Karnataka and Chhattisgarh. In Chhattisgarh, soybean occupies 134.00 thousand ha area with a yield of 9.75 qt. ha^{-1}(SOPA, 2016). The major soybean growing districts are Rajnandgaon, Durg, Mungeli, Bemetara, and Kawardha (Anon., 2015). Soybean crop can be attacked by more than 100 pathogens (Sinclair and Shurtleff, 1975). Among the different soil borne pathogen which infect soybean, *Macrophomina phaseolina* is an important fungus that causes charcoal rot having broad host range like soybean, common bean, mung bean, sorghum, maize, cotton, peanut, sesame, cowpea, chickpea and cluster bean producing the symptoms of dry root rot, dry weather wilt, ashy stem blight and seedling blight (Su *et al*., 2001). *Macrophomina phaseolina* is capable of infecting soybean at any crop growth stage, but usually, it infects at post flowering stage. The disease cycle for *M. phaseolinia* begins with germination of microsclerotia when temperatures are between 28 °C and 32 °C. In general symptoms in adult plants, red to brown lesions on roots and stems, and produces dark mycelia and black microsclerotia. The stem shows longitudinal dark lesions and the plant becomes defoliated and wilted (Abawi and Pastor-Corrales, 1990). In India, the charcoal rot, which is used to be a minor disease of soybean until 2004, became serious due to altered weather conditions particularly on the account of longer drought spells during crop growth period. There are report of few epiphytotics occur in areas where temperature ranges from 35–40°C during the crop season, disease can cause up to 80% yield losses. During the year 1997, charcoal rot caused substantial loss to plant stand and yield in soybean in Guna District of Madhya Pradesh State (Gupta and Chauhan, 2005). In Chhattisgarh, detailed survey of charcoal rot was conducted during *kharif* 2015 and the disease incidence recorded 5.0 to 30.0% (Anon., 2015).

MATERIALS AND METHODS

An intensive survey was conducted during *kharif* 2015 and 2016 on the incidence of charcoal rot in soybean growing districts of Chhattisgarh. Soybean plants showing typical symptoms of stems bearing microsclerotia of *Macrophomina phaseolina* and characteristic symptoms of charcoal rot were collected from the infected plants from farmer's field different locations of Chhattisgarh (Rajnandgaon, Durg, Mungeli, Bemetara, and Kabirdham and Raipur). Samples of 3–5 plants were sampled per field. Total 10-12 spots were selected randomly for taking root samples representing the whole field. Wherever required, the complete infected plants were also collected for isolation of the pathogen and other studies. The per cent disease incidence was calculated by using the following formula.

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

RESULTS AND DISCUSSION

The intensive survey was conducted at the R7 (Beginning Maturity i.e. Physiological maturity) to record the incidence of charcoal rot in major soybean growing district of Chhattisgarh, Raipur, Bemetara, Kawardha, Rajnandgaon and Durg. Among the five district, twenty two locations (Dharsiwa, Ranka, Jaibra, Bemetara, Saigona, Kanhera, Kodia, Patharra, Chorbhatti, Chimagondi, Maharajpur, Lohara, Udiya Khurd, Ataria, Narmada, Kanhar, Salebharri, Bhulatola, Parpondi , Basni, Pendri and Rajpur were surveyed during *Kharif* 2015-16 and 2016-17. Observations were recorded from farmer's fields under natural condition.

Results of survey; conducted during *kharif* 2015-16, presented in table1 and figure 1 indicated that the appearance of disease in all soybean growing area of Chhattisgarh. Disease incidence ranged from 30 to 60 per cent during the year 2015-16 with average of (42.63%). The maximum percent of disease incidence (60%) was observed in Jaibra, followed by Chorbhatti (55.30%), Parpondi (50.50%), Udiya Khurd (50.40%), Patharra (50.00), Kodia

Table 1. Incidence of soybean charcoal rot in different location of Chhattisgarh during *Kharif* 2015-16

S. No.	District	Block	Location	Variety sown	% Disease incidence
1.	Raipur	Dharsiwa	Dharsiwa	JS 97-52	30.70
2.	Bemetara	Bemetara	Ranka	JS 335	40.50
3.	Bemetara	Bemetara	Jaibra	JS 335	60.00
4.	Bemetara	Bemetara	Bemetara	JS 335	30.00
5.	Bemetara	Bemetara	Saigona	JS 335	40.40
6.	Bemetara	Bemetara	Kanhera	JS 335	40.60
7.	Bemetara	Bemetara	Kodia	JS 335	45.80
8.	Bemetara	Bemetara	Patharra	JS 335	50.00
9.	Bemetara	Bemetara	Chorbhatti	JS 335	55.30
10.	Kawardha	Kawardha	Chimagondi	JS 335	45.50
11.	Kawardha	Kawardha	Maharajpur	JS 335	45.55
12.	Kawardha	Lohara	Lohara	JS 335	35.50
13.	Kawardha	Lohara	Udiya Khurd	JS 335	50.40
14.	Rajnandgaon	Ataria	Ataria	JS 97-52	45.60
15.	Rajnandgaon	Narmada	Narmada	JS 335	40.70
16.	Rajnandgaon	Kanhar	Kanhar	JS 335	40.00
17.	Rajnandgaon	Salebharii	Salebharri	JS 97-52	40.10
18.	Rajnandgaon	Bhulatola	Bhulatola	JS 335	40.60
19.	Durg	Dhamdha	Parpondi	JS 335	50.50
20.	Durg	Dhamdha	Basni	JS 335	30.00
21.	Durg	Dhamdha	Pendri	JS 97-52	40.30
22.	Durg	Dhamdha	Rajpur	JS 335	40.00
	Mean				**42.63**

(45.80%), Ataria (45.60%), Maharajpur (45.55%), Chimagondi (45.50%), Narmada (40.70%), Kanhera and Bhulatola (40.60%), Ranka (40.50%), Saigona (40.40%), Pendri (40.30%), Salebharri (40.10%), Kanhar and Rajpur (40.00%), Lohara (35.50%), Dharsiwa (30.70%). The lowest percent of incidence was found in Bemetara and Basni (30.00%). The soybean crops damage substantially in the Chhattissgarh state due to the disease charcoal rot (plate 4.1). The average percent disease incidence in different soybean growing area of Chhattisgarh was about 42.63%.

Survey of soybean charcoal rot was also carried out during the *kharif* 2016-17. The results presented in table 2 and figure 1 indicate that less disease incidence recorded compare to the previous year crop. It was ranged from 15 to 25.10 percent with average disease incidence of 19.12 percent compare to the last year average (42.63%). The maximum percent of disease incidence (25.10%) was observed in Ataria followed by Jaibra (25.00%), Lohara and Kodia (20.70%), Saigona (20.60%), Chorbhatti (20.50%), Ranka, Bhulatola, Basni and Pendri (20.40%), Dharsiwa (20.30%), Chimagondi, Udiya Khurd, Narmada and Salebharri (20.00%), Kanhera (15.70%), Rajpur (15.60), Kanhar (15.30%), Maharajpur (15.20%) and Patharra (15.10%). Bemetara and Parpondi record to be minimum charcoal rot disease incidence (15.00%).

Data of disease incidence presented in table 1 & 2 indicates the seasonal variation of disease. It was reported that during *kharif* 2015-16 Chhattisgarh recorded less rainfall and high temperature that favours the high disease incidence without consideration of variety. During *kharif* (2016-17) less disease incidence was reported, because during this period state reported the high rain fall and low temperature which is not suitable for disease charcoal rot.

Anonymous (2015) conducted a survey of soybean growing area of Chhattisgarh i.e. Durg, Rajnandgaon, Mungeli, Bemetara, and Kawardha and observe the disease incidence of charcoal rot vary from 3-30%. Similar report was also found by Gupta and Chauhan (2005) about the charcoal rot epiphytotics occur in India, where temperature ranges from 35–40°C during the crop season and the disease can cause up to 80% yield losses. During the 1997 season, charcoal rot caused substantial loss to plant stand and yield in soybean in Guna District of Madhya Pradesh State.

Wrather *et al*. (1997) estimated yield loss due to charcoal rot in soybean in the top 10 soybean-producing countries during 1994 was 1.234 million metric tones. Yang and Navi (2005) also reported the charcoal rot epidemics caused by *Macrophomina phaseolina* in Soybean in Iowa. It was observed that during the 2003 growing season, a severe epidemic of charcoal rot was observed throughout

Table 2. Incidence of soybean charcoal rot in different location of Chhattisgarh during *Kharif* 2016-17

S. No.	District	Block	Location	Variety sown	% Disease incidence
1.	Raipur	Dharsiwa	Dharsiwa	JS 335	20.30
2.	Bemetara	Bemetara	Ranka	JS 335	20.40
3.	Bemetara	Bemetara	Jaibra	JS 335	25.00
4.	Bemetara	Bemetara	Bemetara	JS 97-52	15.70
5.	Bemetara	Bemetara	Saigona	JS 335	20.60
6.	Bemetara	Bemetara	Kanhera	JS 335	15.70
7.	Bemetara	Bemetara	Kodia	JS 335	20.70
8.	Bemetara	Bemetara	Patharra	JS 97-52	15.10
9.	Bemetara	Bemetara	Chorbhatti	JS 335	20.50
10.	Kawardha	Kawardha	Chimagondi	JS 335	20.00
11.	Kawardha	Kawardha	Maharajpur	JS 97-52	15.20
12.	Kawardha	Lohara	Lohara	JS 335	20.70
13.	Kawardha	Lohara	Udiya Khurd	JS 335	20.00
14.	Rajnandgaon	Gandai	Ataria	JS 97-52	25.10
15.	Rajnandgaon	Gandai	Narmada	JS 335	20.00
16.	Rajnandgaon	Gandai	Kanhar	JS 335	15.30
17.	Rajnandgaon	Khairagarh	Salebharri	JS 335	20.00
18.	Rajnandgaon	Khairagarh	Bhulatola	JS 335	20.40
19.	Durg	Dhamdha	Parpondi	JS 97-52	15.00
20.	Durg	Dhamdha	Basni	JS 335	20.40
21.	Durg	Dhamdha	Pendri	JS 97-52	20.40
22.	Durg	Dhamdha	Rajpur	JS 335	15.60
	Mean				**19.12**

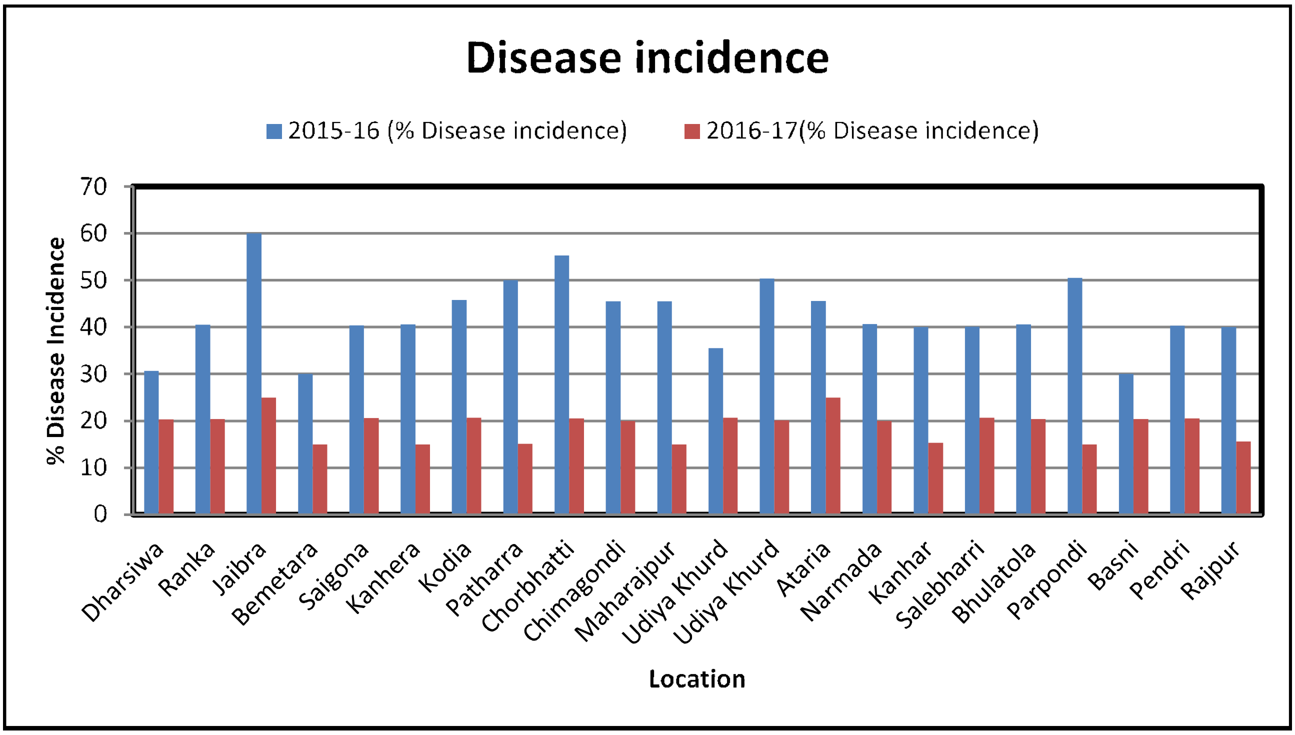

Fig. 1. Yearly variation of charcoal rot disease incidence from soybean growing areas of Chhattisgarh

the state. A systematic survey was conducted between late August and early September, 2003 to determine the prevalence and severity of charcoal rot in Iowa.

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Evaluation of Different Biological Agents Used as Seed Treatment to Manage Charcoal Rot of Soybean

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ABSTRACT

Seed treatment with biocontrol agents found suitable to manage the disease charcoal rot. Disease incidence was lowest in *T. viride* (7.09%) followed by *P. fluorescens* (8.70%) while maximum incidence was recorded in *B. japonicum* (16.40%) which was greater than the control (9.40%). Highest percent disease reduction over control was observed in *T. viride* (15.27%) and lowest was in *P. fluorescens* (7.0%). There was significant differences observed in plant height. Highest seed index (9.95g) was recorded in seed treatment with *T. viride* followed by *P. fluorescens* (9.62g). With regard to yield, highest yield (2274 kg/ha) was recorded in *T. viride* followed by *P. fluorescens* (2253kg/ha).

Key words *Evaluation, Biological Agents, Seed Treatment, Charcoal Rot, Soybean*

Soybean [*Glycine max*.(L.) Merrill] is the cheapest source of vegetable oil and protein. It contains about 40% protein, well balanced in essential amino acids, 20% oil rich with poly unsaturated fatty acid specially omega 6 and Omega 3 fatty acids, 6-7% total mineral, 5-6% crude fiber and 17-19% carbohydrates (Chauhan *et al*.,1988). Soybean is a world's first rank crop as a source of vegetable oil. The major soybean producing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh, Karnataka and Chhattisgarh. Soybean crop can be attacked by more than 100 pathogens (Sinclair and Shurtleff, 1975). The major economically important diseases of the soybean are rust, wilts, leaf spots, rots, web blight, powdery mildew, bacterial and viral diseases. Among the different soil borne pathogen which infect soybean, *Macrophomina phaseolina* is an important fungus that causes charcoal rot having broad host range like soybean, common bean, mung bean, sorghum, maize, cotton, peanut, sesame, cowpea, chickpea and cluster bean producing the symptoms of dry root rot, dry weather wilt, ashy stem blight and seedling blight (Su *et al*., 2001). *Macrophomina phaseolina* is capable of infecting soybean at any crop growth stage, but usually, it infects at post flowering stage. The disease cycle for *M. phaseolinia* begins with germination of microsclerotia when temperatures are between 28 °C and 32 °C. Germinated microsclerotia produce germ tubes that develop hyphopodia which, in turn, either penetrate plant epidermal cell walls or through natural openings (Bressano *et al*., 2010; Dhingra and Sinclair 1978). During the early stages of infection, hyphae are restricted to intercellular spaces of the root cortex, but subsequently, the fungus uses both mechanical pressure and chemical softening (Ammon *et al*., 1974) to facilitate its intracellular colonization of xylem in vascular tissues (Wyllie, 1988). This fungus provokes disease development in plants by secreting one or more phytotoxins that facilitate host penetration, invasion, and colonization. Several phytotoxic metabolites produced by *M. phaseolina* have been identified in individual isolates (Bhattacharya *et al.* 1992). These phytotoxins include asperlin, isoasperlin, phomalactone, phaseolinic acid, phomenon, and phaseolinone (Dhar *et al*., 1982; Mahato *et al*., 1987). Phaseolinone is a non-host-specific toxin that causes wilting of seedlings and formation of necrotic lesions on leaves, similar to those incited by the pathogen (Bhattacharya *et al.*, 1987). In adult plants, the pathogen causes red to brown lesions on roots and stems, and produces dark mycelia and black microsclerotia. The stem shows longitudinal dark lesions and the plant becomes defoliated and wilted (Abawi and Pastor-Corrales, 1990). The asexual structures formed by the fungus are pycnidia and microsclerotia. The black, 0.1–1.0 mm sized microsclerotia are formed in soil, infected seeds or host tissues constitute the primary inoculum source of the pathogen (Dhingra and Sinclair, 1978). Microsclerotia is the heat tolerant structure could withstand a temperature range of 60–65ºC and could survive up to 15 years under different weather condition (Bega and Smith, 1962; Mihail and Alcorn, 1984). A number of management approaches *viz.*, use of tolerant varieties, application of fungicides, cultural practices and combination of approaches leading to integrated management of the disease have been evaluated. In spite of all these measures, charcoal rot continues to be one of the major constraints in soybean production. In addition, there are large variations among different genotypes and cultural practices in different regions. It is necessary to know the severity of the disease and factors associated with disease development, which will help in devising suitable and effective management practices feasible to each location, looking into the prevailing conditions.

MATERIALS AND METHODS

Seed treatment with different biocontrol agents viz., *Trichoderma viride, Psedomonas fluorescens* and *Bradyrhizobium japonicum*, commercial product of IGKV,

Table 1. The doses of biocontrol agents according to treatment are as follows

Treatments	Fungicides	Dosage (g) / kg seed
T1	*T. viride*	10
T2	*P. fluorescens*	10
T3	*B. japonicum*	4
T4	Carbendazim	2

Table 2. Effect of seed treatment with biocontrol agents against disease incidence of charcoal rot of soybean

Treatment	Plant height (cm)	Branches/plant (No)	Pods/ plant (No)	Chaffy pods/ plant (No)	%Disease incidence PDI	%Disease reduction over control	% reduction in chaffy pods over control	100 seed weight (g)	Yield Kg/ha	% Increase in yield over control
T. viride	71.20	3.98	75.00	3.46	7.09 *(2.84)	15.27	8.46	9.95	2274	9.50
P. fluorescens	69.60	3.70	72.40	3.56	8.70 (3.11)	7.00	5.82	9.62	2253	8.50
B. japonicum	74.00	3.70	77.20	6.00	16.40 (4.16)	-77.83	-58.73	9.10	1940	-6.60
Control	69.40	3.80	72.00	3.78	9.40 (3.21)	0.00	0.00	9.26	2077	0.00
SEm(±)	**0.859**	**0.140**	**1.682**	**0.296**	**0.075**	**9.007**	**8.644**	**0.153**	**53.40**	
CD(5%)	**2.667**	**NS**	**NS**	**0.921**	**0.233**	**28.061**	**26.929**	**0.477**	**166.38**	

**Arc sine transformed value*

Raipur. Before the sowing of crop seed treated with biological agent *Bradyrhizobium japonicum* culture @4gm/kg, *Pseudomonas fluorescens* @10gm/kg, *Trichoderma viride* 10gm/kg of soybean seed. Seed treatment with Carbendazim @2gm/kg is used as control (Table 1). The field experiment was laid out in RBD with four treatment and five replications during *kharif* 2016 at Plant Pathology Research Farm, IGKV Raipur (C.G.). Seed of JS 97-52 varieties were sown with 30cm × 10cm spacing in plots measuring 4m x 3m. All other cultural and pest control practices were followed as recommended in package of practices. Following observation were recorded during experiment during experiment such as total plant population, pre and post emergence mortality caused by disease, plant height (cm), branches/plant, pods/plant, chaffy pods/plant, percent disease incidence (PDI), percent disease reduction over control, percent reduction in chaffy pods over control, 100 seed weight and yield per plot kg/ha.

RESULTS AND DISCUSSION

Among the all treatments minimum percent disease incidence was observed when seeds were treated with *T. viride* (7.09%) followed by *P. fluorescens* (8.70%) while maximum incidence was recorded in *B. japonicum* (16.40%) which was greater than the control (9.40%) fig. 1 and table 2. Highest percent disease reduction over control was observed in *T. viride* (15.27%) and lowest was in *P. fluorescens* (7.0%) fig. 2. There was significant differences observed in plant height. Seed treatment with *B. japonicum* showed increased plant height (74.00 cm) over control and minimum was in *P. fluorescens* (69.60 cm).

There was no significant results were observed in

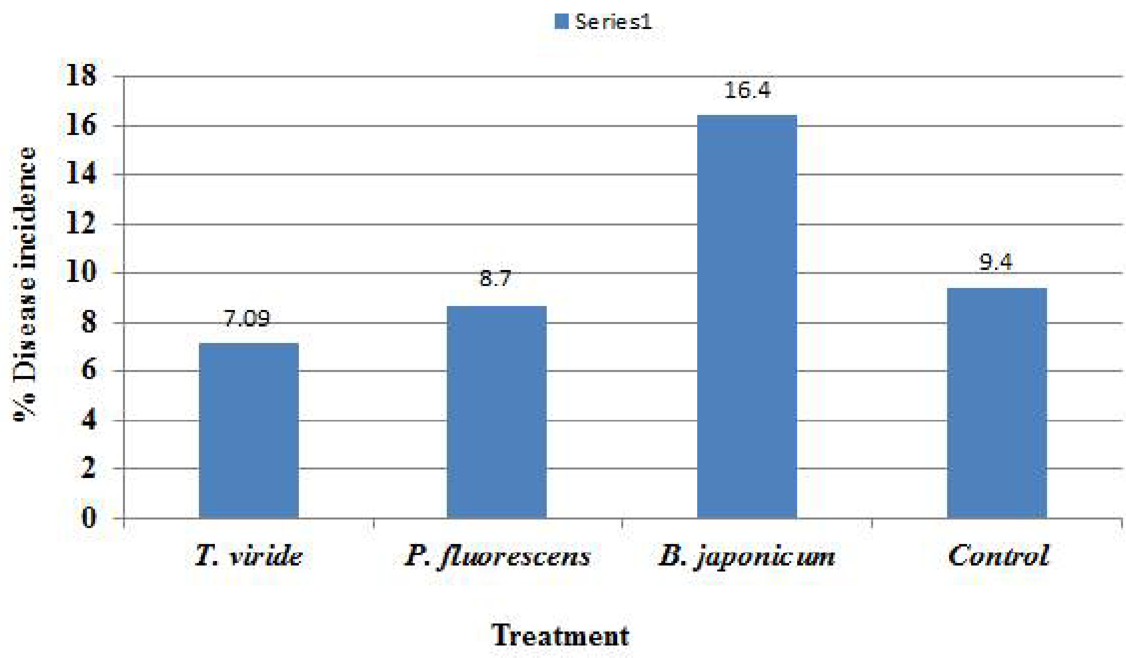

Fig. 1. Effect of seed treatment with biocontrol agent against the disease incidence of charcoal rot

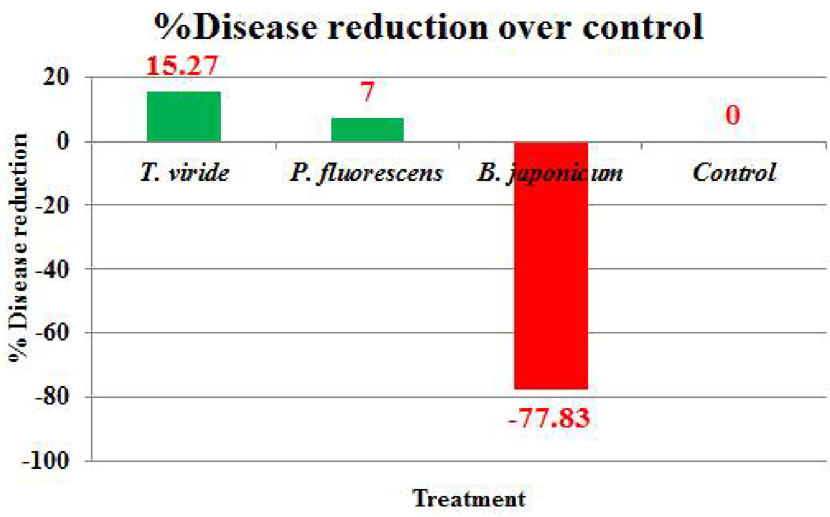

Fig. 2. Effect of seed treatment with biocontrol agent on % disease reduction over control

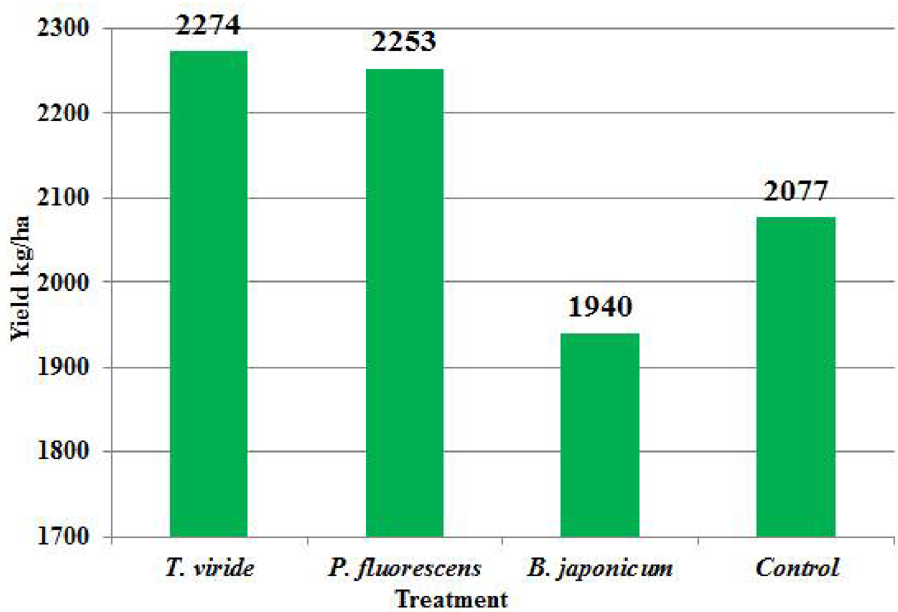

Fig.3. Effect of seed treatment with biocontrol agent on the yield of soybean against the disease charcoal rot

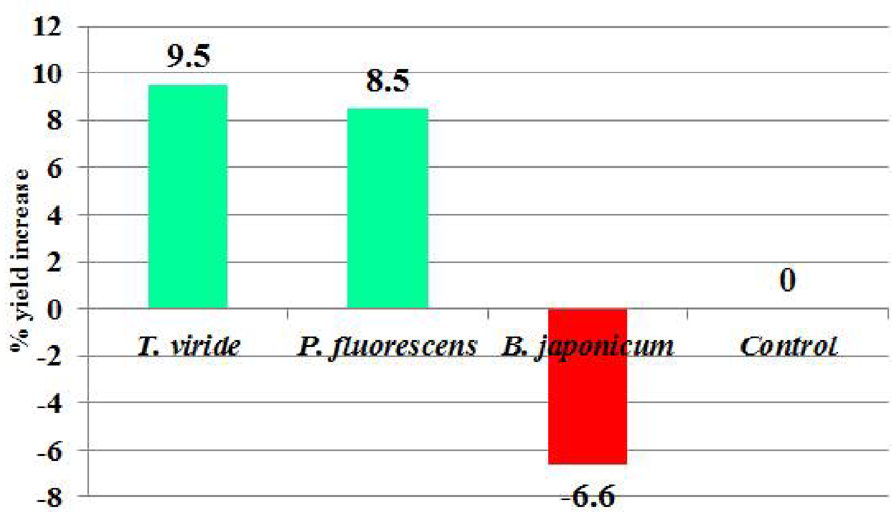

Fig. 4. Effect of seed treatment with biocontrol agent on the % increased in yield of soybean against the disease charcoal rot over control

effect of seed treatment on number of branches per plant and pods per plant. Seed treatment with *T. viride* significantly reduced the number of haffy pod over control (8.46%) followed by *P. fluorescens* (5.82%). Seed treatment with *B. japonicum* increased number of chaffy pods than the control. Highest seed index (9.95g) was recorded in seed treatment with *T. viride* followed by *P. fluorescens* (9.62g). Seed treatment with *B. japonicum* recorded comparatively lower seed index (9.10g) than the control (9.26g). With regard to yield highest yield (2274 kg/ha) was recorded in *T. viride* followed by *P. fluorescens* (2253kg/ha). Least yield was recorded in *B. japonicum* (1940kg/ha) which was lower than the control (2077kg/ha) fig 3, table 2.

The fact is in agreement with the findings of Srivastava *et al.* (1996) regarding management strategies to control charcoal rot using biocontrol agents *T. harzianum* and *Pseudomonas fluorescens* prevents the host infection or to suppress the growth of the pathogen *M. phaseolina* and significantly reduced the germination of sclerotia by 60 % in natural field soil. Adekunle *et al*. (2001) also experienced that cowpea seed treated with three *Trichoderma* spp. and planted in soils amended with *M. phaseolina* significantly greater plant stands percentage than the control. Seed treated with *T. harzianum* at sowing was effective to provide a considerable reduction of the disease caused by *M. phaseolina* in sesame (Pineda, 2001). Black gram seeds treated with *Trichoderma viride* significantly reduced the sclerotial germination of *M. phaseolina* (Rettinassababady *et al.*, 2000). The incidence of root rot in blackgram was significantly reduced by 50 % when treated with *Trichoderma* spp. alone or in combination with biofertilizer both under glass house and field conditions (Indra and Gayathri, 2003). Gupta and Chauhan (2005) also recommended the soybean (4–5 gD kg seed) treatment with *T. viride* or *T. harzianum* found to be very effective for managing soil borne pathogen *M.phaseolina.* Triveni *et al.* (2015) studied the influence of bio filmed formulations composed of *Trichoderma viride* and *Anabaena torulosa* against *Macrophomina phaseolina* (Tassi) Goid. infected cotton crop, in terms of plant growth and biocontrol parameters. Scanning electron microscopy revealed significant colonisation of biofilms on the root surface, which could be correlated with lowest mortality of 5.67%, recorded using *T. viride–B. subtilis* biofilm. Manjunatha and Naik (2010) evaluated several isolates of two bio-control agents, *T. viride* and *P. fluorescens*. Bio-control agents were assessed for their ability to reduce the growth of *M. phaseolina* under laboratory conditions and subsequently used for field studies. It was found that trial combining with soil application through bioagent enriched farm-yard manure, along with seed treated with the bio-control agent showed maximum germination, least root rot incidence and highest yield, as compared to the other biological or chemical seed treatments included. Govindappa *et al.* (2010) reported that seed treatment with bioagents, *P. fluorescens* and *T. harzianum* (10 g/kg) proved to be effective in controlling disease under laboratory, greenhouse and field conditions. The efficacy of these biocontrol agents are equivalent to the standard fungicide Bavistin (carbendazim). Seed treatment with these biocontrol agents enhanced the seed germination and growth parameters against root-rot disease. Nagamani *et al.* (2014) reported that seed treatment with carbendazim and *T. viride* along with soil application of FYM (FarmYard Manure) fortified with *T. viride* as the most effective in decreasing the mortality per cent of chick pea agaist dry root rot caused by *R. bataticola*. Chakraborty and Purkayastha (1984) reported the antagonistic property of *Rhizobium japonicum*. It reduced the severity of charcoal rot disease in soybean on account of the fungitoxic action of rhizobitoxine. Arora *et al*., 2001 and Deshwall *et al*., 2003 also reported that the strains of *Bradyrhizobium* sp. and *Rhizobium meliloti* were antagonistic against *M. phaseolina* and to have plant growth promoting properties in groundnut. Choudhary (2011) characterized plant growth-promotion activities of Rhizobacteria A5F and FPT721 and *Pseudomonas* sp. strain GRP3 for their antagonistic activities against *M. phaseolina*. Contrary to this, in our experiment with *B. japonicm* was not found effective to manage the pathogen *M. phaseolina* at field level, as compare to systemic fungicide carbendazim as control and also not effective as biocontrol agent *T. viride* and *P. fluorescens.*

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