

**Studies on host factors affecting the development
of charcoal rot caused by *Macrophomina
phaseolina* (Tassi) Goid in soybean (*Glycine max*
(L.) Merrill)**

THESIS



Submitted to the

Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya

In partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

In

AGRICULTURE

(PLANT PATHOLOGY)

By

RAHUL SHARMA

DEPARTMENT OF PLANT PATHOLOGY

**Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior
R.A.K. College of Agriculture, Sehore (M.P.)**

2021

CERTIFICATE-I

This is to certify that the thesis entitled “**Studies on host factors affecting the development of charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid in soybean (*Glycine max* (L.) Merrill)**” Submitted in partial fulfilment of the requirement for the degree of **MASTER OF SCIENCE in PLANT PATHOLOGY** of **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior** is a record of the bonafied research work carried out by **Mr. RAHUL SHARMA (Roll No.18131805)** under my guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee and the Director of Instruction.

No part of the thesis has been submitted for any degree or diploma or has been published. All the assistance and help received during the course of the investigation has been acknowledged by the scholar.

Signature

Place : SEHORE

Date: / /

Dr. (Smt.) Moly Saxena
Chairman of the Advisory Committee

MEMBER OF STUDENT’S ADVISORY COMMITTEE

Member – (Dr. D.R. Saxena)

Member – (Dr. Sandeep Sharma)

CERTIFICATE – II



Aadhar No.....

Id.No.....

This is to certify that the thesis entitled “**Studies on host factors affecting the development of charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid in soybean (*Glycine max* (L.) Merrill)**” submitted by **Mr. RAHUL SHARMA (Roll No. 18131805)** to the **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior** in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE in Plant Pathology in Department of Plant Pathology, College of Agriculture, Sehore** has been, after evaluation, approved by the External Examiner and by the Student’s Advisory Committee after an oral examination on the same.

Place: **SEHORE**

Date: / /

Signature

Dr. (Smt.) Moly Saxena
Chairman of the Advisory Committee

MEMBERS OF THE ADVISORY COMMITTEE

Chairman - Dr. (Smt.) Moly Saxena _____

Member - Dr. D.R. Saxena _____

Member - Dr. Sandeep Sharma _____

Head of the department- _____

Dean College of Agriculture _____

Director of Instruction - _____

ACKNOWLEDGEMENT

First and foremost, on this day I stand obliged and indebted to the chairman of my M.Sc. (Ag) advisory committee **Dr. (Smt.) Moly Saxena** (Principal Scientist), Department of Plant Pathology R.A.K. College of Agriculture Sehore (M.P.). I owe my great devotion, respect and profound gratitude towards her unfailing enthusiasm, encouragement and generous help. She gave me a patient hearing, even when she was extremely busy. Her guidance and creative criticism enriched the quality of the scientific component. Her advice, support and encouragement have no doubt enabled me to overcome all the hurdles in my M.Sc. research work. Her vision and adept discussion technically and otherwise helped me to learn and understand the challenges of this field and the potential it has. I take the pride in having been associated with such a teacher.

I am extremely obliged to the members of my advisory committee Dr. D. R. Saxena (Principal Scientist & Head of Section) Department of Plant Pathology, R.A.K. College of Agriculture Sehore, Dr. Sandeep Sharma (Principal scientist) Department of Entomology for their valuable guidance and help during the entire course of an investigation.

I am heartily thankful to Prof. S.K. Rao, Hon. Vice-chancellor; Dr. A.K.Singh, Director of Instructions; Dr. M.P. Jain, Director of Research Services, Dr. (Smt.) Mridulla Billore, Dean faculty of Agriculture Rajmata Vijayaraje Scindia Krishi VishvaVidhyalaya, Gwalior (M.P.) and Dr. H.D. Verma, Dean, R.A.K. College of Agriculture, Sehore, Madhya Pradesh for providing facilities and resources during my M.Sc. (Ag) research programme.

I feel immense pleasure to express my sincere thanks to my seniors Mr. Revendra kushwaha, Vivek viswakarma, Devendra prajapati, Abhishek Rathor, and Ms. Sumona Rai and Anamika Rathor. Juniors Sandeep, Bhagwan, Mahindra, Radha, Ayushi and Mahima, and the staff member Dr. (Smt.) Dr. Aafreen Khan and Shri A.K. Choudhary, Department of Plant Pathology R.A.K. College of Agriculture Sehore for their help in conducting the research trials and making this duration memorable.

Any appreciable word would be less to thank my parents Mr. Lakhani Lal Sharma and Mrs. Mithlesh Sharma blessings they showered on me. My career would not have progressed in this direction and to this level without their generous help, understanding and encouragement. Their love and affection brought me to the level where I am today and also kindled my enthusiasm to carry out my research. I feel a wave of sentiment toward my Younger brother Ravi Sharma and the dearest one of my life, who have a dream for me and I feel contented to have completed an important step towards their dream, whose unending effects, instinct support and moral boost provided by them, to reach up to this point, lie in each letter of this thesis. I also wish to thank all well wishers whose names are not mentioned here but are important to me.

Place :Sehore
Date :

(Rahul Sharma)

CONTENTS

S.No	Title	Page
I	Introduction	1-2
II	Review of literature	3-12
III	Materials and methods	13-21
IV	Results	22-44
V	Discussion	45-49
VI	Summary, conclusion and suggestions for further work	50-51
	Bibliography	52-59
	Appendices	60-63
	Vita	64

LIST OF TABLES

S.No.	Title	Pages
1	Reaction of charcoal rot by Soil and stem cut inoculation method	24
2	Root length and number of secondary roots of soybean varieties in infested and un-infested field	24
3	Number of trifoliolate leaves and leaves area of soybean varieties in infested and un-infested field	25
4	Fresh and dry root mass on soybean varieties in infested and un-infested field	27
5	Plant height of different soybean varieties in infested and un-infested field	28
6	Colony forming unit per gram root of different soybean varieties in infested and un-infested field	28
7	Epidermis, cortex, endodermis and pith thickness in different cultivars of soybean	29
8	Root length of soybean cultivar under different moisture regime in <i>M. phaseolina</i> infested and non-infested soil at V1 growth stage	32
9	Root length of soybean cultivar under different moisture regimes in <i>M. phaseolina</i> infested and non-infested soil at V2 growth stage	36
10	Root weight of soybean cultivars at different moisture regimes in <i>M. phaseolina</i> infested and non-infested soil at V1 growth stage	38
11	Root weight of soybean cultivars under different moisture regimes in <i>M. phaseolina</i> infested and non-infested soil at V2 growth stage	40
12	Colony forming units /gm of root tissue under different moisture regimes in <i>M. phaseolina</i> infested and non-infested soil at V1 growth stage	41
13	Colony forming units/gm of soybean root under different moisture regimes in <i>M. phaseolina</i> infested and non-infested soil at V2 growth stage	42

LIST OF PLATE

S.no	Title	Pages
1	Pathogenicity by stem cut inoculation method	23
2	Pathogenicity by soil inoculation	23
3	Transverse section of roots showing microsclerotia	31
4	Root length of different cultivars in inoculated condition at different moisture regime and V1 stage	34-35

LIST OF SYMBOL

Symbol	Abbreviation	Stand for
@		At the rate of
%		Per cent
±		Plus or minus
%		Percentage
°C		Degree centigrade
μ		Micron
	μm	Micrometer
	Avg.	Average
	WP	Wettable powder
	W / W	Weight by weight
	spp.	Species
	Conc.	Concentration
	<i>M. phaseolina</i>	<i>Macrophomina phaseolina</i>
	i.e.	That is
	viz.,	Namely
	et al.	Co- workers
	Fig.	Figure
	CD	Critical difference
	Cm	Centimeter
	Ha	Hectare
	Hrs	Hours
	Mt	Million tones
	ml	Milliliter
	Mm	Millimeter
	G	Gram
	Mg	Milligram
	NS	Non significant
	SEm±	Standard error of mean
	Psi	Pound square inch
	Ppm	Parts per million
	Temp	Temperature
	CFU	Colony forming unit
	MPa	Megapascal

CHAPTER-I INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the most significant oilseed crops of India. It is a reasonable source of vegetable oil and protein. It contains approximately 40 per cent protein, well proportional amino acids, 20 per cent oil-rich polyunsaturated fatty acids especially omega 6 and omega 3 fatty acids, 6-7 per cent aggregate mineral, 5-6 per cent crude fibre and 17-19 per cent carbohydrates (Chauhan *et al.*, 1988). Soybean production in India during 2020-21 is estimated to be 13.58 million tons from an area of 12.05 million ha and productivity of 1126 kg/ha as per 1st advance estimates of DAC&FW (Annon. 2021). Major soybean-growing states are Madhya Pradesh (5.85 m ha), Maharashtra (4.32 m ha) and Rajasthan (1.1 m ha).

Soybeans are constantly exposed to a variety of biotic and abiotic stress like high or low temperature, too much or too little water, salinity, and mineral deficiency/toxicity etc. which adversely affect their growth, metabolism, and yield (Sinclair *et al.*, 2010). Among the biotic stress charcoal rot caused by polyphagous soil fungus *Macrophomina phaseolina* (Tassi) Goid, affect the soybean at all growth stages.

During prolonged periods of drought and high temperatures (28 to 35°C) infection occurs at soybean seedling stages and the pathogen attacks the plant throughout the season. Mostly symptoms appear after midseason or when the soybean plant reaches maturity at R5, R6, and R7 stages (Hartman *et al.*, 1999). Besides soybean, this pathogen infects a wide host range of nearly 500 species in more than 100 families around the world, including other important crops like cotton (*Gossypium* spp.), chickpea (*Cicer arietinum*), corn (*Zea mays*), and common bean (*Phaseolus vulgaris*) (Srivastava *et al.*, 2001).

Soil-borne pathogens are difficult to control as they are able to produce too much microsclerotia in the soil. Excessive use of chemical pesticides not only control the disease but also lead to the appearance of resistance genes and causes environmental pollution. Therefore, cultivations of resistant cultivars are generally regarded as the most appropriate and feasible approach for controlling charcoal rot disease.

Breeders can be benefited by understanding the relationships between host and pathogen during the development of the disease will help breeders to identify varieties with promising genetic composition and disease resistance for their breeding programs. Therefore, histopathological and histochemical studies on *Macrophomina*-induced diseases, or, in other words, understanding host-pathogen interaction at cellular or tissues level is necessary before scheduling any breeding program.

Macrophomina primarily produces either microsclerotia or pycnidia in the host plant. The former one act as the primary source of inoculums when suitable environmental conditions are available to the pathogen for instance presence of a host plant, low water potential and high soil temperature favours the disease development (Dhingra and Sinclair, 1978, Purkayastha *et al.*, 2006). Looking at the importance of edaphic factors like soil moisture level on charcoal rot development and different genetic characters of soybean cultivar the present investigation was conducted with the following objectives:

1. To compare the morphological and histological characters of susceptible and resistant cultivars of soybean.
2. To compare the effect of *M. phaseolina* in artificially infested soils and non-infested soils on soybean seedling.
3. To estimate the impact of soil moisture upon *M. phaseolina* soil populations and root infection on soybean seedlings.

CHAPTER-II REVIEW AND LITERATURE

Charcoal rot, caused by the fungus *Macrophomina phaseolina* (Tassi) Goidanich, is a root and stems disease of soybean that develops in tropical and semi-tropical regions during the mid to late summer when plants are under heat and drought stress (Gupta *et al.*, 2012). *M. phaseolina* has a wide host range and in addition to soybean (*Glycine max* (L.) Merrill). It is responsible for causing economic losses on sesame (*Sesamum indicum* L.), safflower (*Carthamus tinctorius* L.), sunflower (*Helianthus annuus* L.) and more than 500 cultivated and wild plant species in 75 families (Islam *et al.*, 2012).

The fungus *M. phaseolina* is a pervasive disease of economic significance on soybeans that generally occurs when plants are under heat and drought stress (Wrather and Koenning, 2006; Mengistu *et al.*, 2015). Charcoal rot, a biotic stress (Mengistu *et al.*, 2011), is therefore impacted by drought, an abiotic stress (Sinclair *et al.*, 2007). Mengistu *et al.*, (2015) reported the charcoal rot's impact and prevalence in the US and worldwide. This pathogen causes charcoal rot, dry rot and seedling blight disease (Su *et al.*, 2001).

Soybeans are constantly exposed to a variety of biotic (i.e., infection by pathogens) and abiotic (i.e., high or low temperature, too much or too little water, salinity, and mineral deficiency/toxicity) stresses that adversely affect their growth, metabolism, and yield (Sinclair *et al.*, 2010).

Charcoal rot, also referred to as dry-weather wilt and summer wilt, is a root and stem disease that can reduce yield and seed quality as well as cause plant death (Smith and Wyllie, 1999). Yield losses due to charcoal rot vary from year to year, but usually favor years with heat stress and where rainfall is less frequent.

Taxonomy

The taxonomy of the fungus *Rhizoctonia bataticola* (*M. phaseolina*) has been studied by the number of workers all over the world to establish the exact systemic position with a valid name. Young, (1944) concluded that *R. bataticola* is a synonym of *Sclerotium bataticola* and that *Macrophomina*

phaseolina is the pycnidial form of the same fungus. Reichert and Hellinger, (1947) suggested that the *Rhizoctonia bataticola* should be discarded and *M. phaseoli* should be applied solely to stains forming pycnidia only, *S. bataticola* to those forming sclerotia only while those formatting both sclerotia and pycnidia should be reported as *M. (Sclerotium) phaseolina*

However, now it is established that the causal organism of charcoal rot of soybean is *Rhizoctonia bataticola* (Taub.) Butler and its systemic position are as follows (Alexopoulos and Mims, 1983)

Sub division	-	Deuteromycotina
Form class	-	Deuteromycetes
Form sub class	-	Hyphomycetidae
Form order	-	Agonomycetales
Form family	-	Stilbellaceae
Genus	-	<i>Rhizoctonia</i>

The perfect stage of *R. bataticola* has been reported in India (Anonymous, 1963 and Ghosh *et al.*, 1964). They obtained typical cultures of the stain of saprophytic fungus from the jute field. The fungus was described as *Orilia obscura* by Ghosh *et al.*, (1964) and was considered as the perfect stage of *R. bataticola*.

In culture, colonies of *M. phaseolina* range from white, to brown, to gray to black in color and become darker with age and some isolates may grow in concentric rings (Dhingra and Sinclair, 1978; Smith and Wyllie, 1999). This fungus produces hyphae that are hyaline with granular contents that disappear over time (Dhingra and Sinclair, 1978). Hyphae can branch at right angles or acute angles (Smith and Wyllie 1999). Sometimes aerial mycelia are produced that can reach up to the top of the Petri dish when grown in culture (Dhingra and Sinclair, 1978; Smith and Wyllie, 1999). This fast-growing fungus produces small shiny, smooth, black microsclerotia that vary from spherical, oblong to irregular in shape, are made up of thick-walled, dark anastomosing mycelial cells (Dhingra and Sinclair, 1978).

Primary inoculum of *M. phaseolina* is microsclerotia which can survive in dry soils or in decaying host debris for two years or more (Meyer et al., 1974) but for only about seven to eight weeks in wet soils (Smith and Wyllie, 1999). The germination of microsclerotia occurs on the surface of or in close proximity to soybean roots. This pathogen can survive as mycelium in the soil for up to seven days which is more than enough time to infect soybean seedlings (Meyer et al., 1974). Within the first two to three weeks after planting, eighty to one hundred per cent of seedlings can be infected (Smith and Wyllie, 1999). Since, infection with this pathogen can occur at any growth stage, this can cause seeds to fail to germinate, seedlings to develop lesions or harbor latent infections, or plants to die. Those plants that harbor a latent infection often do not show symptoms until the onset of reproductive stages or environmental stresses (Smith and Wyllie, 1999).

Baird et al., (2003) described that the *M. phaseolina* can survive in soil as microsclerotia (a resilient structure) for nearly two years or embedded in the plant's roots for longer survived. The structures are black, spherical produced in the host tissue that is released in the soil to allow the fungal survival in adverse conditions (Hartman et al., 1999). The structure consists of several glucose cells that can germinate independently when the environmental situation is convenient. The formation of sclerotia in the host as well as in culture can be seen in the pith region and below the bark surface of infected root plants (Subramanian, 1971).

Symptomology

Symptoms of charcoal rot are also referred to as dry-weather wilt or summer wilt, because it often occurs when plants are under heat and drought stresses (Smith and Wyllie, 1999). These stresses can also occur in irrigated soybeans causing losses from 6 to 33% in experimental plots (Mengistu et al., 2011) and the combination of stress and the presence of *M. phaseolina* caused higher yield loss on soybeans than drought alone.

Seedlings of soybean can be infected when temperature is continuously 35 °C for 2-3 weeks. Symptoms are not typically observed until the R5 (beginning of seed formation) to R7 (beginning of maturity) growth stages (Fehr et al., 1971, Meyer et al., 1974, Mengistu, 2015). Early symptoms include yellow leaves, reduced vigour, brown to red discoloration

on roots and stems, and a general wilting appearance (Gupta and Chauhan, 2005, Short *et al.*, 1978). Later foliar symptoms include premature senescence, wilting, and premature plant death.

The symptoms of infection are usually evident at the end of the crop cycle and can be confused with the plant senescence stage, so the disease may go unnoticed in crops (Almeida *et al.*, 2001). Symptomatic plants that die from patches or bands with yellowish leaves and, later, fallen branches with wilted leaves attached to the stems (Dhingra & Sinclair, 1978; Ndiaye, 2007; Almeida, 2001). Because infection and the early development of diseases caused by root pathogens occur below the ground, symptoms usually become apparent when they reach advanced stages.

Charcoal rot is most recognizable for its below-ground signs and symptoms which include a gray to black discoloration of the epidermal and sub-epidermal tissues of tap roots, secondary roots and the lower part of stems (Smith and Wyllie, 1999; Wyllie 1976). This discoloration is caused by the pathogen forming numerous microsclerotia in the vascular and pith tissues (Smith and Wyllie 1999). These microsclerotia resemble bits of ground up charcoal giving this disease its name. Microsclerotia may be formed up the stem and are most noticeable in the nodes of the plant (Smith and Wyllie, 1999; Wyllie 1988). Tissue discoloration and the presence of microsclerotia can be seen with a hand lens by splitting the tap root and lower stem with a knife (Smith and Wyllie, 1999). Once inside the host, the fungal hyphae grow mostly intercellular but also grow intracellular as cell integrity becomes compromised inside the xylem (Smith and Wyllie, 1999; Wyllie, 1988).

The pathogen causes disease symptoms by plugging up the xylem vessels with microsclerotia, toxin production, as well as from the enzymatic action and mechanical pressures from hyphal penetration (Bowers and Russin, 1999; Smith and Wyllie, 1999). As plant tissues start to die, the pathogen forms microsclerotia (Smith and Wyllie, 1999; Wyllie, 1988). The process can be as quick as three days in young tissue, but takes longer in more mature tissue (Wyllie, 1988). Once the cortical tissue has been invaded, fungal hyphae begin vertical colonization of the vascular elements often without symptoms (Wyllie, 1988). The pathogen then grows from the interior

of the root and stem tissue towards the outer layers producing microsclerotia in the tissue (Wyllie, 1988).

The pathogen disrupts root function and is associated with environmental stresses the above-ground symptoms of the disease are not very distinct and difficult to distinguish from other problems. These symptoms include smaller than normal leaves, a loss of vigor as well as leaves that yellow, wilt, turn brown and die while still remaining attached to the plant (Smith and Wyllie, 1999; Wyllie, 1988). Underground symptoms start with the development of lesions on taproot and the basal portion of the stems causing wilt in the susceptible plants, initial infection on above-ground parts were rapid drying of the leaves and plants gets wilted at any growth stage from flowering to maturity (Prioletta and Bazzalo, 1998). Root infestation by *M. phaseolina* was to reduce water movement in soybean and reduce yields in susceptible cultivars (Doubledee *et al.*, 2018)

Since the pathogen causes the most damage during the reproductive stages, it can interfere with pod formation and seed fill (Wyllie, 1988). This can result in pods failing to fill or seeds that are smaller than normal. Yang and Navi, (2005) noticed infected plant in late July, and by late August. Infected plants were in patches, wilted and died prematurely in many fields. The pith of infected plant had a brown discoloration in taproot and lower stems. Symptoms were observed up to the 4th or 5th nodes, typical of charcoal rot.

Some of the common methods for assessing charcoal rot disease levels are rating root, stem disease severity, percent height stem discoloration, foliar symptoms, and quantifying *M. phaseolina* microsclerotia densities in tissue using colony forming units (Mengistu *et al.*, 2007). The problem with the rating for root and stem disease severity and per cent height stem discoloration is that even if a root or stem appears visually clean of microsclerotia, the pathogen may still be present in the roots as hyphae. Also, rating for root and stem severity, per cent height stem discoloration, and quantifying microsclerotia densities all rely on destructive sampling of plants and are very labor intensive. Visual ratings of foliar symptoms are difficult to separate from the effects of drought or other diseases. Also, the appearance of foliar symptoms occurs late and so tells little about the early stages of

disease development. Non-destructive method that could be used to detect this disease during the season even when visible symptoms are not present or are too indistinct to recognize would be helpful to minimize disease losses. Plants express a variety of symptoms when under stress.

Impact of *M. phaseolina* on morphology and histology of soybean cultivars

Understanding the relationships between host and pathogen during the development of the disease will help breeders to identify varieties with promising genetic composition and disease resistance for their breeding programs. Therefore, histopathological and histochemical studies on *Macrophomina*-induced diseases, or, in other words, understanding host-pathogen interaction at cellular or tissues level, is necessary before scheduling any breeding program. Although several techniques have been used in the study of plant-microbe interactions, histopathological analyses still remains efficient in examining infection events of pathogenic fungi in plants as they contribute information of the pathogen (e.g., stages present, their location and establishment in the cells) and its influence on host plants development (Bhuiyan *et al.*, 2015).

To date, numerous studies have been conducted on *M. phaseolina* (Mengistu *et al.*, 2007; Mengistu *et al.*, 2011), and its variations in morphology and pathogenicity (Purkayastha *et al.*, 2003), however, a few studies have examined its infection process in soybean roots. Moreover, little studies have been performed with the aim of histopathological comparison between resistant and susceptible soybean cultivars. The most recent study carried out by Bressano *et al.*, (2010) have developed a new in vitro method to examine the pre-penetration and the penetration phases of *M. phaseolina*.

Paris *et al.*, (2006) and Wrather *et al.*, (2008) found variation in root tissue colonization by *M. phaseolina* in soybean genotypes. Root tissue colonization was significantly lower in PI 416937 (2640 CFU g root) and N 987288 (1525 CFU g root) than Hutcheson (4000 CFU g root) at the R6 growth stage of soybean. Later on, (Mengistu *et al.*, 2011) suggested that the growth stage R7 should be taken as the optimum stage for assessment of disease using a colony-forming unit (CFU) to evaluate soybean genotypes.

Shehbaz *et al.*, (2018) noticed that green stem colour was related to susceptible genotype while purple stem colour related to resistance in sunflower at the seedling stage

Hemmati *et al.*, (2018) studied the pre and post penetration of *M. phaseolina* in resistant (Hadgeston) and susceptible (Williams) cultivars of soybean. They found a significant difference between the two cultivars in terms of root colonization and including the appearance of adventitious roots in the resistant cultivar in response to the pathogen.

Impact of soil water on *M. phaseolina*

Environmental conditions like temperature, atmospheric humidity, and soil water potential play an important role in the viability and inoculum potential of *M. phaseolina* (Khan, 2007). *M. phaseolina* is able to produce microsclerotia under relatively low water conditions. Survival of inoculums is influenced by the soil metric water potentials. Viability of microsclerotia were drastically reduced at high water potentials (-30 J/Kg, field capacity), and was virtually not affected at low water potentials (-1.500 Kg/J, permanent wilting point) in a sandy loam soil (Olaya *et al.*, 1996).

Soil water content affects the gaseous conditions in the soil and may cause reduced microsclerotia survival by the reduction of O₂. Substances found in flooded soils such as alcohols, volatiles and increased levels of CO₂ can have a detrimental effect on the inoculums (Olaya *et al.*, 1996; Wyllie *et al.*, 1984). Microsclerotia germination is annulated in artificial atmospheres containing less than 16% of O₂ concentration in soil column systems; indicating that reduction in viability is not due to nutrient deprivation (Wyllie *et al.*, 1984). This knowledge has been used to reduce propagule densities in the soil not only for *M. phaseolina* but *Sclerotinia sclerotiorum* and *Sclerotium cepivorum* under anoxic-flooded soils (Abawi *et al.*, 1985; Banks and Edgington, 1989).

Impact of irrigation on microsclerotia survival in soil and roots has been studied by several researchers. Irrigation is one of the most effective ways to deal with charcoal rot for different plant species (Kendig *et al.*, 2000). Irrigation throughout the whole soybean growing season reduces the population and colonization of *M. phaseolina* on roots as compared with a non-irrigated crop system, even though the propagules remain during the

season in both systems and no symptoms in soybean plants were found in the irrigated field (Kendig *et al.*, 2000).

Mengistu and Boykin, (2018) reported a very weak relationship between stress tolerate index (SIT) and colony-forming unit (CFU's) at the R7 growth stage, for every 1000 CFU's there was a yield loss of 11.5 kg ha⁻¹ at that growth stage. Under low relative water conditions, *M. phaseolina* is capable to produce microsclerotia therefore the survival of microsclerotia is influenced by soil matric water potential. At the high-water potential (-30 J/kg, field capacity) the viability of inoculum was drastically reduced while at low water potential (-1.500 J/kg, permanent wilting point) it was not being affected in sandy loam soil. (Olaya *et al.*, 1996). Decreasing the metric and osmotic potentials to -1.2 and -0.6 MPa (megapascals), respectively, increased sclerotia germination and mycelial growth but any further decreased caused both sclerotial germination and mycelial growth to decline again. It was considered that the matric potential was more important as a factor than the osmotic potential (Goudarzi *et al.*, 2008).

Mengistu *et al.*, (2011) described, the infection velocity of *M. phaseolina* was considerably lowered and yields were increased by 6-30% due to irrigation and fumigation (methyl bromide) treatment. Application of irrigation is recommended as one of the management practices to reduced charcoal rot disease incidence (Grau *et al.*, 2004).

In 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 (Wokocho, 2000). Water management can limit these fungi, but not prevent the colonization of *M. phaseolina* (Kendig *et al.*, 2000).

Reduction of soil moisture and infection were synergistic to disease incidence and yield loss, but independent of colonization and disease severity. Jordaan *et al.*, (2019) reported that an increase of soil moisture cannot prevent the initial infection of the host, but can significantly reduce colonization of the stem at maturity.

A soil is considered "saturated" when all soil pores are filled with water. In a saturated state the volume of water in the soil is equal to the volume of the total soil porosity (Brady and Weil, 2004). As long as water

percolates from the macro pores due to gravitational forces the soil is at a maximum retentive capacity. As water moves downwards in the soil profile by capillarity and gravity. When the soil stops draining and water in the macro pores is replaced with air, the soil is said to be at "field capacity". The term field capacity does not apply to pots in a greenhouse because there is not underlying soil that pulls the water downwards. However, the term "pot capacity" can be used, which the amount of water is remaining after irrigation and when visible drainage has stopped (Kirkham, 2005). As the soil continues to dry, the water retained at higher tensions in the smaller pores is removed. Consequently, the amount of water leftover in the soil is held so tightly that plants are not able to take it up and will wilt. This level of available water is known as the "permanent wilting point" (Brady and Weil, 2004; Kirkham, 2005). Available water is considered to be the water retained in the soil between field capacity and the permanent wilting point.

In adequate soil conditions, soybean roots can grow to a depth of 6 feet in the soil profile. However, roots are more concentrated in the upper half of the root zone. A root depth of 3 feet is commonly used to calculate the water requirements of soybean plants (Rogers, 1997). Soybean water requirements during the season range from 18 to 24 inches per year. The higher water demand during soybean growth occurs when plants are approaching the beginning of pod development (R2-R3) and demand decreases as plants mature. For maximum yields, water availability is critical at the beginning of pod fill (Rogers, 1997; Scott *et al.*, 1986).

Studies have demonstrated that irrigation is also beneficial at the latter part of the reproductive stages because water requirements for vegetative growth can be supplied by rainfall and/or stored soil water. In the worst-case scenario, a 5% reduction in yield can result if soybean is under water stress during the vegetative growth stages (Stegman *et al.*, 1990). Soybean irrigation may be necessary at any stage of soybean in order to obtain the maximum yield a single irrigation during the early reproductive stages is enough to reduce the inoculum survival by 25 to 42% in bare soil (Lodha, 1995; Rogers, 1997). Soybean irrigation at R2-R4 full bloom and beginning of pod reduces *M. phaseolina* root colonization and favoured soybean yield (Kendig *et al.*, 2000).

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). 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).

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 (Tosi and Zazzerini, 1990).

Dry conditions favour the 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). Production of microsclerotia, as well as the severity of the charcoal rot, is also known to be influenced by cultivar (Ndiaye 2007) in cowpea.

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.

Variation in root tissue colonization by *M. phaseolina* among soybean genotypes was observed by many researchers (Bristow and Wyllie, 1984; Pearson *et al.*, 1984; Smith and Carvil, 1997; Kendig *et al.*, 2000; Paris *et al.*, 2006). Weimer (1947) found that the cultivars Roanoke, Volstate, FG 30261-1 and Woods Yellow of soybean showed tolerance to charcoal rot. 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. Few soybean genotypes, including Delta, Pineland 3478, Hamilton, Jackson II, Davis and Asgrow 3715

have been identified as either moderately resistant or tolerant under field conditions (Smith and Carvil, 1997; Smith and Wyllie, 1999). They also recommended the cultivation of cultivars that do not have a late reproductive stage that coincides with periods of drought stress.

CHAPTER -III MATERILAS AND METHODS

(A) Materials

3.1 General

Corning and Borosil made glassware were used throughout the experimental study. Glasswares were cleaned with a chronic acid solution followed by washing them with a detergent-based solution and finally rinsed with tap water. After drying of glass ware were sterilized in a hot air oven at 180 °C for 2 hours. The other metallic equipment such as forceps, needle and cork borer was sterilized by dipping in alcohol and thereafter heating over a flame till red hot on a spirit lamp. Surface sterilization of plant parts and diseased materials were done by dipping them in 0.1% mercuric chloride solutions for 1-2 minutes and thereafter washed in sterilized water for 3 times.

The culture media was sterilized in an autoclave at 15 lbs pressure per square inch (1.05 kg/cm²) for 20 minutes. The soil and sand were sterilized at 30 lbs per square inch (3.1 kg/cm²)

3.2 Media

The following media was used in the experiment with the following ingredients

3.2.1 Potato Dextrose Agar (Riker and Riker, 1939)

Peeled and Sliced Potato	-	200g
Dextrose	-	20g
Agar	-	20g
Distilled water	-	1000ml

3.3 Source of seed and other materials

The soybean seed varieties were acquired from AICRP of soybean R.A.K. College of Agriculture Sehore (M.P.).

3.4 Experimental site

All the experiments were executed at the experimental field of plant pathology, greenhouse and plant pathology lab, R.A.K. College of Agriculture Sehore, during *Kharif* season 2019-20.

(B) Methods

3.5 Symptomatology

The crops of different soybean varieties were regularly observed at the research farm, greenhouse and in lab conditions and the appearance of different symptoms on plants were regularly noted and illustrated.

3.5.1 Isolation and purification of the pathogen

The plants and plant parts were obtained from the soybean field where the diseased plants showing charcoal rot symptom were kept in a transparent polythene bag thereafter its washed with tap water and sclerotia carriage plant parts were chosen. The tissue was cut into 5 mm long and 2-3 mm slices. These slices were surface-sterilized with 0.1% HgCl₂ solution for nearly one minute followed by 3 changes in sterilized water. The slices were dried on sterilized blotting papers and thereafter transferred to potato dextrose agar (PDA) medium in 9 cm diameter Petri dishes and incubated at 25±2 °C and examined at frequent intervals. Purification of the isolated was done by hyphal tip technique as described by Dhingra and Sinclair, (1985). Isolated fungus was identified according to their morphological character according to Barnett and Hunter, (1986).

3.6. Pathogenicity tests

3.6.1 Soil inoculation method

The test fungus was multiplied on sorghum seeds for 7-14 days. The soil was collected from the local field and dried thoroughly in sun and sterilized. Pot of 25 cm face diameter was used during the experiments.

The pots were filled with 1kg sterilized soil and then 20g of inoculums were added in a pot. One inch of soil was placed over the inoculums. The contents were then mixed and surface-sterilized seeds were sown and covered by a half-inch layer of soil. In control, no inoculums were added and the pot was filled with sterilized soil.

3.6.2 Stem cut inoculation method

The six weeks old age V2 stage soybean plants were selected for the stem cut inoculation method. The apex of each plant was cut 25 mm above the unifoliate node with a sharp sterilized razor blade. Plastic straw were poked on apex trimmed stem of the plant with an actively growing *M. phaseolina* culture in a circular dish from potato dextrose agar to established

mycelium over the trimmed stem. In control, without inoculated plastic straw were poked in each control plant. Stem cut technique was used by Twizeyimana, *et al.* (2012) to inoculate *Phasseeolia spp.* plant.

Based on Plant Disease Index (PDI), varieties were classified as follows.

Percent Disease	Resistant Category
0	Absolutely resistant (AR)
0.01-11.11	Highly resistant (HR)
12.22-33.33	Moderately resistant (MR)
34.44-55.55	Moderately susceptible (MS)
56.66-77.77	Susceptible (S)
78.88-100.00	Highly susceptible (HS)

3.7 Morphological character of resistant and susceptible varieties

The present experiment observations were carried out under Plant Pathology College's sick field by *M. phaseolina* and were taken on following growth parameters such as Number of trifoliolate leaves, plant height, roots length, number of secondary roots, leaves area, roots biomass, and roots colony forming units in charcoal rot resistant (RVS-18) and susceptible (JS-335, JS-9560 and RVS-24) cultivars.

Design : Two way factorial RBD
 Varieties : JS-335, JS-9560, RVS-18, RVS 24
 Replication : 4
 Treatment : 2

Following observations were taken after 80 to 85 days of sowing at the R7 growth stage.

3.7.1. Root length

The root length of the selected plant was measured in cm from the root tip to collar region of plant thereafter average root length was calculated.

3.7.2 Number of secondary roots

The number of secondary roots of each plant was counted in the form of a number and then the average number of secondary roots was calculated.

3.7.3 Number of trifoliolate leaves

The number of trifoliolate leaves was counted in number from throughout the plant and then the average numbers of trifoliolate leaves were calculated.

3.7.4 Leaf area

The leaf area of each plant was measured in cm². The average leaves area was calculated.

3.7.5 Root biomass

The fresh weight and dry weight of each plant were measured in gram. The average weight was recorded.

3.7.6 Plant height

The height of the selected plants was measured in cm from the ground surface up to the tip of the plant and then average plant height was calculated.

3.7.7 CFUs from root

The colony-forming units were recorded from each sample units and then average CFU's was recorded.

3.8 A pot trial was conducted to study histological characters and histopathology

The histological character and histopathological observation of soybean roots were recorded in *M. phaseolina* inoculated pot condition in resistant and susceptible cultivars of soybean.

3.8.1 Histological characters

The determination of histological character of different cultivars was recorded at V6 growth stage in both resistance and susceptible cultivars. The following histological characters such as epidermis, cortex, endodermis and pith thickness were studied.

Design	:	CRD
Varieties	:	JS-335, JS-9560, RVS-18, RVS 24
Replication	:	4
Treatment	:	4

The following characters were taken after 65 to 70 days of sowing at the V6 growth stage.

3.8.1.1 Epidermis thickness

The root epidermis thickness was measured from each samples unit and then average measurement of epidermis thickness was calculated.

3.8.1.2 Cortex thickness

The root cortex thickness was measured from each samples units and their average measurement was calculated.

3.8.1.3 Endodermis thickness

The root endodermis thickness was calculated from each units and their average calculation was recorded.

3.8.1.4 Pith thickness

The root pith thickness was measured from each plant samples and then its average was calculated.

3.8.2 Histopathology

3.8.2.1 Plant material and growing conditions

In this study, one tolerant RVS-18 and three susceptible cultivar JS-9560, JS-335 and RVS-24 soybean were selected. Soybean seeds were surface-sterilized using 5% sodium hypochlorite for 5 min and rinsed several times with sterile double distilled water for the next 5 min. The seeds were sown in plastic pots containing sterilized soil and sand mixture. Plants were grown in a greenhouse under natural light conditions during *Kharif* season. The plants were hand irrigated daily until field capacity was reached. The field capacity was determined by slowly saturating soil-filled pots until water started to drip from the bottom (Gholamhoseini *et al.*, 2018).

3.8.2.2 Inoculation method

The root immersion method was used to inoculate seedling of soybean plants (Gaige *et al.*, 2010). Sixty-five days after seed sowing at the V6 growth stage, soybean seedlings were extracted from the pots, and then sand and soil mixture were gently removed from the roots by washing with de-ionized water, avoiding any damage to the roots. Then the roots were immersed for 1h in microsclerotia suspension before the seedlings were transplanted in new pots. To ensure the microsclerotia growth, while placing the roots in the suspension, a sample of microsclerotia was grown on the PDA media and microsclerotia was monitored daily by placing a drop of suspension on a slide and examined under an optical microscope.

3.8.2.3 Observations

On the second day after inoculation, the microsclerotia started to germinate, so sampling was started 3 days after inoculation to ensure that the pathogen uniformly penetrated the roots. Five plants of each cultivar were harvested every day for two weeks for root microscopic studies. For this purpose, the plants were extracted from the pots and potting mix was removed from the roots by washing with de-ionized water. Then five roots were cut into 2-3 mm piece and soaked in 0.5 M sodium hydroxide to soften the tissue for 3 min. Safranin was used to stain the cut root pieces for easier microscopic identification. Root segments were placed on a microscope slide and then a cover slip was placed over the root sections and pressed firmly to facilitate analysis at high magnification. The specimens were examined using an optical microscope (Leica instrument inc., magnification 150 to 600) equipped with a digital camera connected to a computer.

3.9 Evaluation of different moisture regime on root colonization by *M. phaseolina* in seedling stage

Experiment on moisture level for root colonization by *M. phaseolina* was performed under pots condition. The soil mixtures were constructed by mixing sand and black cotton soil at equal proportions. The soil was sterilized as described above and dried on the bench for two days.

The observation of different soil moisture regimes at pot saturation, pot capacity and permanent wilting point in inoculated with *M. phaseolina* were taken at V1(first trifoliolate leaves unfolded) and V2 (second trifoliolate leaves unfolded) growth stages

Design	:	Three way factorial CRD
Replications	:	4
Treatments	:	3 moisture levels
		(1).Pot saturation
		(2). Pot capacity
		(3). Permanent wilting point

3.9.1 Inoculum production

M. phaseolina microsclerotia were obtained from infested soybean plants as described above. Ten kg of sorghum (*Sorghum bicolor*) seeds were soaked for twenty hours; afterwards, seeds were dried under the sunlight until the appropriate moisture in the seeds. Each kg of seeds was divided into three transparent plastic autoclave bags with a 5 cm diameter a 10 cm long PVC plastic tube inserted into each autoclave bag approximately half the length of the tube. The autoclave bags were plugged with non-absorbent cotton and cover with aluminium foil. Inoculation bags were autoclaved at 121 °C for 30 min. The subculture of ~ 1 cm² of *M. phaseolina* (prepared from an *M. phaseolina* isolates taken from infested soybean roots) grown on full strength PDA, were added to the sterilized sorghum autoclaved bags. Inoculated bags were stored at 21 °C temperature. One week after inoculation, bags content were mixed by shaking and left to incubate for an additional 10 days. After incubation, the colonized millet was dried on a plastic sheet and passed through a sieve. Dried microsclerotia were collected after sieving and stored in the refrigerator until used.

3.9.2 Setting variables

The soil mixture was infested with *M. phaseolina* microsclerotia @ 20g/kg soil and seedling were exposed to different soil moisture contents - pot saturation (PS), pot capacity (PC), and permanent wilting point (PWP). The ability of the inoculum to infest seedling root tissue was qualitatively confirmed by visualization under the microscope. *M. phaseolina* root populations were quantified at two soybean development stage V1 (first trifoliate leaves unfolded) and V2 (second trifoliate leaves unfolded).

3.9.3 Calibration of soil moisture levels

Since the soil water retention properties for each soil differ, three levels of soil moisture *i.e.* pot saturation, pot capacity, and permanent wilting points were set for each soil. Pots containing 1200 ml of dried soil were watered to saturation. One day after the pots was saturated and water drainage has stopped. The pot was weighted once a day for five days.

After obtaining, weighing a calculation of volumetric water content was performed for the pot using the following equation.

Equation

$$\theta v = \frac{V_w}{V_s}$$

Where θv is the volumetric water content of soil, V_w is the volume of water, and V_s is the volume of soil. However, to calculate θv , V_w was calculated as follows:

Equation

$$V_w = \text{weight of watered pot} - \text{soil weight}$$

With this calibration, it was possible to set three levels of soil moisture or irrigation regimes based on volumetric water content for the soil: pot saturation (PS), pot capacity (PC), and permanent wilting point (PWP). Every 24 hours pots were weights and water added to maintain the desired level of soil moisture.

3.9.4 Experimental design

The experimental design was three way factorial completely randomised design (CRD), containing four-factor: soil volumetric water content (θV) (pot saturation, pot capacity, and permanent wilting point), soil (inoculated soil, non-inoculated soil), soybean developmental stage (V1, V2) and four independent experiments (four replication).

The soybean variety used for these experiments were RVS-18, RVS-24, JS-9569 and JS-335 obtained from the college seeds department.

A constant volume of soil was kept in each pot to maintain a regular concentration of *M. phaseolina* inoculums. 1200 mL of soil for each pot were measured by weighing machine and inoculated with 0.02 g of *M. phaseolina* microsclerotia obtained from sorghum. 1200 mL of soil and inoculums were mixed by shaking in a plastic bag.

After inoculating soils with microsclerotia, soybean seeds were surface-sterilized with a 5% sodium hypochlorite for 5 min and rinsed with distilled water for 5 min. nine seeds per pot were planted immediately after sterilization at a depth of 2cm. Pots were watered every day until the seedling reached the VC (cotyledon) stage. Pot saturation, pot capacity and, permanent wilting point irrigation regimes were introduced at the VC stage until the experiments were completed.

3.9.5 Evaluation of *M. phaseolina* root infection

Soybean seedlings were collected and cut below the cotyledonary node, transported to the lab rinsed with sterile distilled water to remove soil particles. Roots were dried and scanned under the stereoscopic microscope and root length were measured by a scale 'root weights were taken from each sample immediately after rinsed and dried' and left for one day under the paper towel at room temperature (21 °C). Roots were maintained in plastic bags at 4°C until processing. The root samples were ground separately. For each root, a 0.05 g sub-sample of ground tissue was obtained to calculate roots CFUs using a modification of the procedure described by Mengistu *et al.*, (2007), Mihail (1992) and Pearson *et al.*, (1984).

The crushed tissue was blended in 250ml of a 0.5% NaOCl for 3 min with 30-sec idle intervals, collected in a sieve and rinsed with distilled water for 1 min. The root tissue was concentrated in one side of the sieve using a squeeze bottle containing sterile distilled water and poured into a 50 mL sterile tube. Afterwards, PDA media was added to each tube, mixed, and poured in three 100 mm-diameter Petri dishes. Poured dishes were placed in the incubator at 30 °C in the dark for 5 days. After the incubation period, CFU's of *M. phaseolina* were counted and transformed to CFUs/g of root tissue.

Statistical analysis:

The data were subjected to statistical analysis after transformation. The data converted into percentage were transformed to angular values. The different between the two means was subjected to further testing by computing critical difference at 5% probability level.

1) **Standard error of different between two treatment means:**

$$S.E.m.\pm = \sqrt{\frac{Em.s}{r}}$$

2) **Critical difference:**

$$C.D.5\% = S. Em. \pm \times \sqrt{2} \times t \text{ value at } 5\%$$

Where,

Em.s	=	Error means sum of square
r	=	Number of replication
t	=	't' value at 5%probability levels

CHAPTER-IV RESULTS

4.1. Isolation and identification of pathogen

The charcoal rot affected soybean plants were recognised in the field according to their symptoms at the reproductive stage such as yellowing and wilting of plants. The roots of diseased plants were black in colour and showed light silvery discolouration of the epidermal and sub-epidermal tissues on taproot. On the lower parts of stem, microsclerotia were formed in the vascular tissue of the soybean plants.

On PDA colony growth of isolated fungus was gray or white in colour. Mycelium inclined towards the growth direction with absolute margins, changing to black colour with the age of colonies. Hyphae branched at right angles from the main hyphae, septate, 3-5µm wide and hyaline to dark brown in colour. The microsclerotium was black, spherical to oblong or irregular (80-110 × 70-90 µm) after 6-7 days of incubation. On the basis of fungal growth and sclerotia it was identified as *M. phaseolina*.

4.2 Pathogenicity tests

Pathogenicity of the isolated fungus was tested by soil inoculation and stem cut inoculation method.

In the pot inoculation method, symptoms were observed after 20-25 days of sowing. Necrotic lesions were formed on taproots and collar region of plant. Initially yellowing of leaves with stunted or wilted growth was observed when the necrotic patches were collapse or shrink together and completely. The *M. phaseolina* affected vascular tissues were clogged by sclerotia, consequently leaf yellowing, wilting and ultimately plants were die (plate-2A) and visual microsclerotia were appeared on stem surface (plate-2B).

In stem cut inoculation method symptoms were produced after 72 hours of inoculation. Plants showed yellowing and drooping of leaves. Later on leaves fall off and plants died within seven days. Necrotic dark brown lesions were produced on stem, which contained numerous microsclerotia (plate-1A) while in control plant non-symptoms were observed (plate-1B).

The plant infested fungus that were causing plant wilting, yellowing, decaying and dying was re-isolated from the infested plant and confirm that

the same fungus was causing diseases with similar symptoms, it was proving the Koch's postulates.



Plate-1A



Plate-1B

Plate no 1: Pathogenicity by stem cut inoculation method



Plate-2A



Plate-2B

Plate no 2: Pathogenicity by soil inoculation

4.2.1. Reaction of different varieties

Four soybean varieties viz. JS-335, JS-9560, RVS-18 and RVS-24 were tested against charcoal rot by soil inoculation and stem cut inoculation method and results are presented in table 1.

Table 1: Reaction of charcoal rot by Soil and stem cut inoculation method

S. No	Varieties	Soil inoculation	Stem cut inoculation
1	JS-335	S	S
2	JS-9560	S	S
3	RVS-18	R	R
4	RVS-24	S	MR

S= Susceptible, R= Resistance, MR=Moderate resistance

Data in the table 1 indicates that out of four varieties RVS-18 exhibited resistant reaction in both the inoculation methods. RVS-24 indicated moderately resistant reaction while JS-335 and JS-9560 showed susceptible reaction.

Field study

4.3. Morphological characters of soybean varieties in *Macrophomina phaseolina* infested and un-infested field

4.3.1. Root length and number of secondary roots

The Root heights and number of secondary roots of soybean plant were recorded at R7 growth stage in *M. phaseolina* infested and un-infested soil conditions as shown in the table 2.

Table 2: Root length and number of secondary roots of soybean varieties in infested and un-infested field

Treatments	Root length (cm)*			Number of secondary root*		
	Infested	Un-infested	Mean B	Infested	Un-infested	Mean B
JS-335	18.10	18.50	18.30	20.50	21.00	20.75
JS-9560	19.00	20.00	19.50	14.25	15.50	14.88
RVS-18	21.13	20.75	20.94	15.75	17.00	16.38
RVS-24	19.38	20.88	20.13	14.25	14.75	14.50
Mean A	19.40	20.03		16.19	17.06	
Factors	S.Em±	C.D. at 5%		S.Em±	C.D. at 5%	
A) Infested and un-infested	0.41	NS		0.42	NS	
(B) Varieties	0.58	1.71		0.59	1.74	
(A X B)	0.82	NS		0.83	NS	

* Mean of four replications

NS- Non Significant

Data in the table 2 shows that root length and number of secondary root of soybean were not affected by presence of *M. phaseolina* in soil. Only the effects of varietal characters were observed.

The root length ranged between 18.10 cm to 21.13 cm in infested soil. Significantly higher root length was recorded in RVS-18 (21.38 cm) followed by RVS-24 (19.38 cm), JS-9560 (19 cm) and JS-335 (18.10 cm). Mean root length in infested soil was 19.40 cm. In un-infested soil, the root length ranged between 18.50 to 20.88 cm. significantly higher root length was recorded in RVS-24 (20.88 cm) followed by RVS-18 (20.75 cm), JS-9560 (20 cm) and JS-335 (18.5 cm). Mean root length of un-infested soil was 20.03 cm.

The number of secondary roots ranged between from 14.25 to 20.5 in infested soil. Significantly higher number of secondary roots was recorded in JS-335 (20.5) followed by RVS-18 (15.75), JS-9560 (14.25) and RVS-24 (14.25). Mean of number of secondary root in infested soil was 16.19. In un-infested soil, the number of secondary root ranged between from 14.75 to 21. Significantly higher number of secondary roots was recorded in JS-335 (21) followed by RVS-18 (17), JS-9560 (15.5) and RVS-24 (14.75). Mean of number of secondary roots of un-infested soil was 17.06.

4.3.2 Number of trifoliolate leaves and leaves area

The Number of trifoliolate leaves and leaves area of soybean plant were recorded at R7 growth stage in infested and un-infested soil conditions as presented in the table 3.

Table 3: Number of trifoliolate leaves and leaves area of soybean varieties in infested and un-infested field

Treatments	Number of trifoliolate leaves*			Leaves area (cm ²)*		
	Infested	Un-infested	Mean B	Infested	Un-infested	Mean B
JS-335	19.25	19.50	19.38	31.39	32.82	32.10
JS-9560	9.00	9.25	9.13	25.39	26.13	25.76
RVS-18	18.50	19.25	18.88	40.01	41.82	40.92
RVS-24	15.00	15.25	15.13	27.43	28.31	27.87
Mean A	15.44	15.81		31.05	32.27	
Factors	S.Em±	C.D. at 5%		S.Em±	C.D. at 5%	
(A) Infested and un-infested	0.36	NS		0.54	NS	
(B) Varieties	0.50	1.49		0.76	2.25	
(A X B)	0.71	NS		1.08	NS	

* Mean of four replications
NS- Non Significant

Data in the table 3 shows that number of trifoliolate leaves and leaves area of soybean were not affected by presence of *M. phaseolina* in soil. Only the effects of varietal characters were observed.

The number of trifoliolate leaves ranged between from 9 to 19.25 in infested soil. Significantly higher number of trifoliolate leaves were recorded in JS-335 (19.25) followed by RVS-18 (18.5), RVS-24 (15.43) and JS-9560 (9). Mean number of trifoliolate leaves in infested soil was 15.44. In un-infested soil, the number of trifoliolate leaves ranged between from 9.25 to 19.25. Significantly higher number of trifoliolate leaves was recorded in RVS-18 (19.25) followed by JS-335 (19.5), RVS-24 (15.25) and JS-9560 (9.25). Mean number of trifoliolate leaves of un-infested soil was 15.81. There was no significantly difference between infested and un-infested soil condition of soybean cultivars.

The leaves area ranged between 25.39 to 40.01 cm² in infested soil. Significantly higher leaves area was recorded in RVS-18 (40.01 cm²) followed by JS-335 (31.39 cm²), RVS-24 (27.43 cm²) and JS-9560 (25.39 cm²). Mean leaves area in infested soil was 31.05 (cm²). In un-infested soil, the leaves area ranged between 26.13 to 41.82 cm². Significantly higher leaves area was recorded in RVS-18 (41.82 cm²) followed by JS-335 (32.82 cm²), RVS-24 (28.31 cm²) and JS-9560 (26.13). Mean leaves area of un-infested soil was (32.27 cm²). There was no significantly difference between infested and un-infested soil condition.

4.3.3 Fresh and dry root mass

The fresh and dry root mass of soybean plant were recorded at R7 growth stage in infested and un-infested soil conditions as shown in the table 4.

Data in the table 4 shows that fresh and dry root mass of soybean were not affected by presence of *M. phaseolina* in soil. Only the effects of varietal characters were observed.

The fresh root mass ranged between from 3.82 to 4.49 gm in infested soil. Significantly higher fresh root mass were recorded in RVS-18 (4.49 gm) followed by JS-335 (4.24 gm), RVS-24 (4.17 gm) and JS-9560 (3.82 gm). Mean fresh root mass in infested soil was (4.18 gm). In un-infested soil, the fresh root mass ranged between from 3.96 to 4.61 gm. Significantly higher

fresh root mass was recorded in JS-335 (4.61 gm) followed by RVS-18 (4.50 gm), RVS-24 (4.36 gm) and JS-9560 (3.96 gm). Mean fresh root mass of un-infested soil was 4.35 gm. There was no significantly difference between infested and un-infested soil condition of soybean cultivars.

Table 4: Fresh and dry root mass on soybean varieties in infested and un-infested field

Treatments	Fresh root mass (gm)*			Dry root mass (gm)*		
	Infested	Un-infested	Mean B	Infested	Un-infested	Mean B
JS-335	4.24	4.61	4.42	1.69	2.15	1.92
JS-9560	3.82	3.96	3.89	1.36	1.43	1.39
RVS-18	4.49	4.50	4.49	2.04	2.18	2.11
RVS-24	4.17	4.36	4.26	1.68	1.86	1.77
Mean A	4.18	4.35		1.69	1.90	
Factors	S.Em±	C.D. at 5%		S.Em±	C.D. at 5%	
(A) Infested and un-infested	0.06	0.17		0.07	0.20	
(B) Varieties	0.08	0.25		0.10	0.28	
(A X B)	0.12	NS		0.14	NS	

* Mean of four replications

NS- Non Significant

The dry root mass ranged between 1.36 to 2.04 gm in infested soil. Significantly higher dry root mass was recorded in RVS-18 (2.04 gm) followed by JS-335 (1.69 gm), RVS-24 (1.68 gm) and JS-9560 (1.36 gm). Mean dry root mass in infested soil was 1.69 gm. In un-infested soil, the dry root mass ranged between 1.43 to 2.18 gm. Significantly higher dry root mass was recorded in RVS-18 (2.18 gm) followed by JS-335 (2.15 gm), RVS-24 (1.86 gm) and JS-9560 (1.43 gm). Mean dry root mass of un-infested soil was (1.90 gm). There was no significantly difference between infested and un-infested soil condition.

4.3.4 Plant height

The plant height and of soybean plant were recorded at R7 growth stage in infested and un-infested soil conditions as shown in the table 5.

The plant height ranged between 32 cm to 46.5 cm in infested soil. Significantly higher plant height was recorded in RVS-18 (46.5 cm) followed by JS-335 (44.75 cm), RVS-24 (40 cm) and JS-9560 (32 cm) (plate no 3). Mean plant height in infested soil was 40.81 cm.

Table 5: Plant height of different soybean varieties in infested and un-infested field

Treatments	Plant height (cm)*		
	Infested	Un-infested	Mean B
JS-335	44.75	45.38	46.06
JS-9560	32.00	32.38	32.19
RVS-18	46.50	47.38	46.94
RVS-24	40.00	40.75	40.38
Mean A	40.81	41.47	
Factors	S.Em±	C.D. at 5%	
(A) Infested and un-infested	0.58	NS	
(B) Varieties	0.82	2.43	
(A X B)	1.16	NS	

* Mean of four replications

NS- Non Significant

In un-infested soil, the plant height ranged between 32.38 cm to 47.38 cm. significantly higher plant height was recorded in RVS-18 (47.38 cm) followed by JS-335 (45.38 cm), RVS-24 (40.75 cm) and JS-9560 (32.38). Mean plant height of un-infested soil was 41.47 cm. There was no significantly difference between infested and un-infested soil condition on plant height of soybean cultivars. Only varietal differences were found.

4.3.5 Colony forming unit

Colony forming unit of soybean plant were recorded at R7 growth stage in infested and un-infested field as given in the table 6.

Table 6: Colony forming unit per gram root of different soybean varieties in infested and un-infested field

Treatments	Colony forming units/gram root*		
	Infested	Un-infested	Mean B
JS-335	2820.00	1195.00	2007.50
JS-9560	2780.00	1160.00	1970.00
RVS-18	1690.00	1140.00	1415.00
RVS-24	2140.00	1160.00	1650.00
Mean A	2357.50	1163.75	
Factors	S.Em±	C.D. at 5%	
(A) Infested and un-infested	22.75	67.36	
(B) Varieties	32.17	95.26	
(A X B)	45.50	134.71	

* Mean of four replications

The colony forming units/gram of root tissue ranged between from 1690 to 2820 CFU's in infested soil. Significantly higher colony forming units was recorded in JS-335 (2820.00) followed by JS-9560 (2780.0 CFU's) and

RVS-24 (2140.0 CFU's) while the minimum CFU's were recorded in RVS-18 (1690.0 CFU's). Mean of colony forming units in infested soil was 2357.50 CFU's.

In un-infested soil the colony forming units/gram tissue ranged between from 1160 to 1140 CFU's. Higher colony forming units was recorded in JS-335 (1195 CFU's) followed by JS-9560 (1970 CFU's) and RVS-24 (1160 CFU's) while the minimum CFU's was recorded in RVS-18 (1140 CFU's). Mean of colony forming units of un-infested soil was 1163.75 CFU's

Pot studies

4.4 Histological characters

Histological observations *viz.* epidermis, cortex, endodermis and pith of four soybean cultivars are presented in table 7.

Table 7: Epidermis, cortex, endodermis and pith thickness in different cultivars of soybean

Varieties	Thickness (μm)			
	Epidermis*	Cortex*	Endodermis*	Pith*
RVS-24	15.00	293.75	22.50	1518.75
RVS-18	30.63	228.13	46.25	1650.00
JS-335	46.25	165.63	22.50	1562.50
JS-9560	26.88	208.75	34.38	1412.50
S.Em \pm	2.30	11.11	2.39	20.01
C.D. at 5%	7.15	34.60	7.44	62.34

* Mean of four replications

Date in table 7 indicates significantly more epidermis thickness in JS-335 (46.25 μm). RVS-18 (30.63 μm) was at par with JS-9560. The minimum epidermis thickness was observed in RVS-24 (15.0 μm)

Significantly maximum root cortex thickness (228.12 μm) was in RVS-24 followed by RVS-18 (228.13). The cortex thickness of RVS-18 was at par with JS-9560 (208.75 μm). The minimum root cortex thickness was observed in JS-335 (165.62 μm).

Root endodermis thickness was significantly more (46.25 μm) in RVS-18 followed by JS-9560. The endodermis thickness was similar in RVS-24 and JS-335 (22.5 μm).

Pith thickness was significantly (1650.0 μm) in RVS-18 followed by JS-335 and RVS-24. The pith thickness of JS-335 and RVS-24 were at par to each other.

4.4.1 Histopathological observation

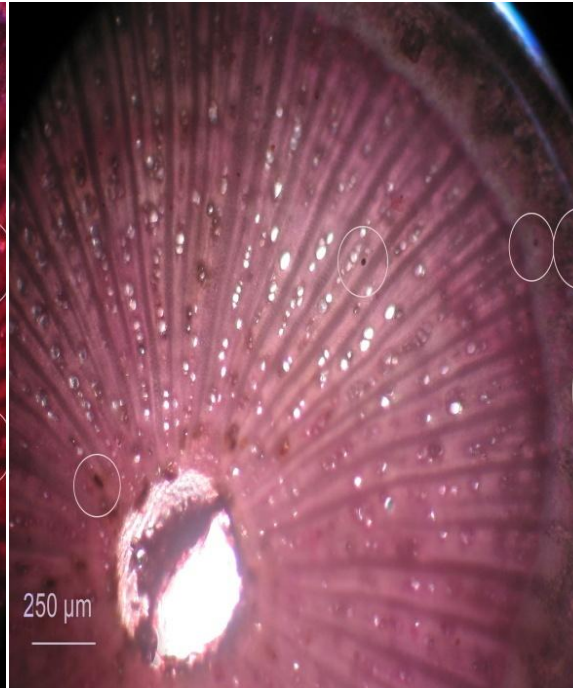
On PDA microsclerotia started germinating on second day, therefore three days after inoculation soybean plant were uprooted to ensure whether that the pathogen uniformly penetrated the roots or not. Attachment of microsclerotia on root surface was found on all the four cultivars and by 5th day pathogen colonized the epidermis of all the soybean cultivars. Thus, difference in epidermal thickness of resistant and susceptible cultivar showed no effect on penetration of pathogen.

On 10 days of inoculation difference between the resistant and susceptible cultivars were noticed. Necrotic lesions found on roots of JS-335, JS-9560 and RVS-24 were larger than RVS 18 (plate-4). Transverse section of roots showed growth of mycelium towards cortex and endodermis and more number of developing microsclerotia. While in RVS-18 the development of microsclerotia was limited to epidermis only (Plate-4).

On 14th days of inoculation, the density of microsclerotia significantly increased in all JS-335, JS-9560 and RVS-24 cultivars especially near the pith regions while no significant change were observed in RVS-18.



JS-335



JS-9560



RVS-18



RVS-24

Plate no 3: Transverse section of roots showing microsclerotia

4.5 Effect of different moisture regime on roots of soybean cultivars at V1 and V2 stage.

4.5.1 Root length at V1 stage

The data in table 8 indicates root length of soybean cultivars at three different moisture regimes viz. pot saturation (PS), pot capacity (PC) and permanent wilting point (PWP) in *M. phaseolina* infested and non-infested soil.

Table 8: Root length of soybean cultivar under different moisture regime in *M. phaseolina* infested and non-infested soil at V1 growth stage

Treatments	Root length (cm)								
	Infested*				Non-infested*				Mean C
	PWP	PC	PS	Mean	PWP	PC	PS	Mean	
JS-335	6.13	6.13	8.85	7.03	6.45	6.78	11.85	8.36	7.70
RVS-18	15.13	19.75	21.00	18.63	16.25	19.88	21.13	19.08	18.85
JS-9560	7.38	8.75	9.88	8.67	9.88	12.00	14.75	12.21	10.44
RVS-24	9.25	13.00	16.50	12.92	12.00	14.00	22.25	16.08	14.50
Mean A	9.47	11.91	14.06	11.81	11.14	13.16	17.49	13.93	
Factors	S.Em±			C.D. at 5%					
(A) Infested and un-infested	0.16			0.46					
(B) Moisture	0.20			0.56					
A X B	0.28			0.80					
(C) Varieties	0.23			0.65					
A X C	0.32			0.92					
B X C	0.40			1.13					
A X B X C	0.57			1.60					

* Mean of four replications

Varieties	Root length (cm)			
	PWP	PC	PS	Mean C
JS-335	6.29	6.45	10.35	7.70
RVS-18	15.69	19.81	21.06	18.85
JS-9560	8.63	10.38	12.31	10.44
RVS-24	10.63	13.50	19.38	14.50
Mean B	10.31	12.53	15.78	

PWP= Permanent wilting point PC= Pot capacity PS= Pot saturation

Table 8 indicates that among the cultivars root length was significantly more in RVS-18 (18.85 cm) as compare to RVS-24 (14.5 cm), JS-9560 (10.44 cm) and JS-335 (7.70 cm). However, maximum root lengths were observed in PS (15.78 cm) as compare to PC (12.53 cm) and PWP (10.31 cm).







Significantly higher root length was found in (13.93 cm) non-infested soil than infested soil (11.81 cm).

The root length ranged between 6.13 cm to 15.13 cm in infested soil at PWP. At permanent wilting point significantly higher root length was recorded in RVS-18 (15.13 cm) followed by RVS-24 (9.25 cm), JS-335 (6.13 cm) and JS-9560 (7.38 cm). Mean root length in infested soil at PWP was 9.47 cm. while in non-infested soil at PWP it ranged between 6.45 cm to 16.25 cm. Significantly higher root length was recorded in RVS-18 (16.25 cm) followed by RVS-24 (12 cm) , JS-335 (6.45) and JS-9560 (9.88 cm). Mean root length in non-infested soil at PWP was 11.14 cm.

In the infested soil at PC, the root length reached between 6.13 cm to 19.75 cm. Significantly higher root length was recorded in RVS-18 (19.75 cm) followed by RVS-24 (13.00 cm) as compared to JS-335 (6.13 cm) and JS-9560 (8.75 cm). Mean root length in infested soil at PC was 11.91 cm. while in non-infested soil at PC the root length reached between 6.78 cm to 19.88 cm. Significantly higher root length was recorded in RVS-18 (19.88 cm) followed by RVS-24 (14.0 cm) as compared to JS-335 (6.78 cm) to JS-9560 (12.00 cm). Mean root length in non-infested soil at PC was 13.16 cm.

In the infested soil at PS the root length reached between 8.85 cm to 21 cm. Significantly higher root length was recorded in RVS-18 (21 cm) followed by RVS-24 (16.50 cm) as compared to JS-335 (8.85 cm) and JS-9560 (9.88 cm). Mean root length in infested soil at PS was 14.06 cm. While in non-infested soil at PS the root length reached between 11.85cm to 22.25 cm. significantly higher root length was recorded in RVS-24 (22.25 cm) which was at par with RVS-18 (21.13 cm) followed by JS-9560 (14.75 cm) as compared to JS-335 (11.85 cm)

It showed strong evidence that the root length were affected by moisture per cent as well fungal infestation. Significantly more root length was measured in non-infested soil [PS (17.49 cm), PC (13.16 cm) and PWP (11.14 cm)] as compared to infested soil [PS (14.04 cm), PC (11.91 cm) and PWP (9.47 cm)].

JS-335			
	PWP	PC	PS
RVS-18			
	PWP	PC	PS

JS-9560			
	PWP	PC	PS
RVS-24			
	PWP	PC	PS
<p>Plate no 4: Root length of different cultivars in inoculated condition at different moisture regime and V1 stage</p>			

4.5.2 Root length at V2 stage

Root length of soybean varieties in *M. phaseolina* infested and non-infested soil at three different moisture regimes are presented in table 9.

Table 9: Root length of soybean cultivar under different moisture regimes in *M. phaseolina* infested and non-infested soil at V2 growth stage

Treatments	Root length (cm)								
	Infested*				Non-infested*				Mean C
	PWP	PC	PS	Mean	PWP	PC	PS	Mean	
JS-335	10.88	10.25	12.13	11.08	12.50	17.75	20.00	16.75	13.92
RVS-18	15.75	21.25	23.75	20.25	16.88	21.75	23.13	20.58	20.42
JS-9560	9.25	16.25	19.25	14.92	11.75	20.50	25.75	19.33	17.13
RVS-24	10.50	14.25	18.38	14.38	14.38	20.25	25.38	20.00	17.19
Mean A	11.59	15.50	18.38	15.16	13.88	20.06	23.56	19.17	
Factors	S.Em±				C.D. at 5%				
(A) Infested and un-infested	0.19				0.56				
(B) Moisture	0.24				0.68				
A X B	0.34				0.97				
(C) Varieties	0.28				0.79				
A X C	0.39				1.12				
B X C	0.48				1.37				
A X B X C	0.69				1.94				

* Mean of four replications

Varieties	Root length (cm)			
	PWP	PC	PS	Mean C
JS-335	11.69	14.00	16.06	13.92
RVS-18	16.31	21.50	23.44	20.42
JS-9560	10.50	18.38	22.50	17.13
RVS-24	12.44	17.25	21.88	17.19
Mean B	12.73	17.78	20.97	

PWP= Permanent wilting point PC= Pot capacity PS= Pot saturation

Table 9 indicates that the overall root length was significantly more in RVS-18 (20.42 cm) as compare to RVS-24 (17.19 cm), JS-9560 (17.13 cm) and JS-335 (13.92 cm) cultivars while the significantly maximum root length was observed in PS (20.97 cm) as compare to PC (17.78 cm) and PWP (12.73 cm). The maximum root lengths were observed in (19.17 cm) non-infested soil as compared to infested soil (15.16 cm)

The root length ranged between 9.25 cm to 15.75 cm in infested soil at PWP. Significantly higher root length was recorded in RVS-18 (15.75 cm)

followed by JS-335 (10.88 cm) as compared to JS-9560 (9.25 cm) and RVS-24 (10.50 cm). Mean root length in infested soil at PWP was 11.59 cm. while in non-infested at PWP it ranged between 11.75 cm to 16.88 cm. significantly higher root length was recorded in RVS-18 (16.88 cm) followed by RVS-24 (14.38 cm) as compared to JS-9560 (11.75) and JS-335 12.50 cm). Mean root length in non-infested at PWP was 13.88 cm.

In the infested soil at PC the root length reached between 10.25 cm to 21.25 cm. significantly higher root length was recorded in RVS-18 (21.25 cm) followed by JS-9560 (16.25 cm) as compared to JS-335 (10.25 cm) and RVS-24 (14.25 cm). Mean root length in infested soil at PC was 15.50 cm. while non-infested soil at PC the root length reached between 17.75 cm to 21.75 cm. Significantly higher root length was recorded in RVS-18 (21.75 cm) which was at par with JS-9560 (20.50 cm) followed by JS-335 (17.75 cm) as compared to RVS-24 (20.25 cm).

In the infested soil at PS the root length reached between 12.13 cm to 23.75 cm. significantly higher root length was recorded in RVS-18 (23.75 cm) followed by JS-9560 (19.25 cm) as compared to JS-335 (12.13 cm) and RVS-24 (18.38 cm). Mean root length in infested soil at PS was 18.38 cm. while non-infested soil at PS the root length reached between 20. cm to 25.75 cm. significantly higher root length was recorded in JS-9560 (25.75 cm) which was at par with RVS-24 (25.38 cm) followed by RVS-18 (23.13 cm) as compared to JS-335 (20. cm).

It showed strong evidence that the root weight were affected by different moisture regimes as well fungal infestation. Significantly more root length was measured in non-infested soil [PS (18.38 cm), PC (15.50 cm) and PWP (11.59 cm)] as compared to infested soil [PS (23.56 cm), PC (20.06 cm) and PWP (13.88 cm)].

4.5.3 Root weight at V1 stage

Table 10 shows the root weight of different cultivars at V1 growth stage in *M. phaseolina* infested and non-infested soil at three moisture regimes.

Table 10: Root weight of soybean cultivars at different moisture regime in *M. phaseolina* infested and non-infested soil at V1 growth stage

Treatments	Root weight (gm)								
	Infested*				Non-infested*				Mean C
	PWP	PC	PS	Mean	PWP	PC	PS	Mean	
JS-335	0.05	0.06	0.07	0.06	0.07	0.10	0.12	0.10	0.08
RVS-18	0.78	0.85	1.10	0.91	0.80	0.89	1.01	0.90	0.91
JS-9560	0.07	0.09	0.20	0.12	0.13	0.33	0.95	0.47	0.29
RVS-24	0.12	0.16	0.63	0.30	0.18	0.21	0.92	0.44	0.37
Mean A	0.25	0.29	0.50	0.35	0.29	0.38	0.75	0.48	
Factors	S.Em±			C.D. at 5%					
(A) Infested and un-infested	0.011			0.031					
(B) Moisture	0.013			0.037					
A X B	0.019			0.053					
(C) Varieties	0.015			0.043					
A X C	0.022			0.061					
B X C	0.027			0.075					
A X B X C	0.038			0.106					

* Mean of four replications

Varieties	Root weight (gm)			
	PWP	PC	PS	Mean C
JS-335	0.06	0.08	0.10	0.08
RVS-18	0.79	0.87	1.06	0.91
JS-9560	0.10	0.21	0.58	0.29
RVS-24	0.15	0.19	0.77	0.37
Mean B	0.27	0.34	0.63	

PWP= Permanent wilting point saturation

PC= Pot capacity

PS= Pot

The data (Table 10) revealed that the overall root weight was significantly more in RVS-18 (0.91 gm) as compare to RVS-24 (0.37 gm), JS-9560 (0.29 gm) and JS-335 (0.08 gm) cultivars. Maximum root weights were observed in PS (0.63 gm), as compared to PC (0.34 gm) and PWP (0.27 gm). Mean root weight was in non-infested soil (0.48 gm) than infested soil (0.35 gm).

At permanent wilting point root weight measured between 0.05 gm to 0.78 gm. Significantly higher root weight was measured in RVS-18 (0.78 gm) followed by RVS-24 (0.12 gm), JS-335 (0.05 gm) and JS-9560 (0.07 gm). Mean root weight in infested soil at PWP was 0.25 gm. While in non-infested

soil at PWP the root weight measured between 0.07 gm to 0.80 gm. Significantly higher root weight was recorded in RVS-18 (0.80 gm) followed by RVS-24 (0.18 gm) JS-9560 (0.13 gm) and JS-335 (0.07 gm). Mean root length in non-infested soil at PWP was 0.29 gm.

In the infested soil at PC the root weight measured between 0.06 gm to 0.85 gm. significantly higher root weight was measured in RVS-18 (0.85 gm) followed by RVS-24 (0.16 gm) as compared to JS-335 (0.06 gm) and JS-9560 (0.09 gm). Mean root weight in infested soil at PC was 0.29 gm. While non-infested soil at PC the root weight was measured between 0.10 gm to 0.89 gm. Significantly higher root weight was recorded in RVS-18 (0.89 gm) followed by JS-9560 (0.33 gm) as compared to JS-335 (0.10 gm) to RVS-24 (0.21 gm). Mean root weight in non-infested soil at PC was 0.38 gm.

In the infested soil at PS the root weight measured between 0.07 gm to 1.10 gm. significantly higher root weight was measured in RVS-18 (1.10 gm) followed by RVS-24 (0.63 gm) as compared to JS-335 (0.07 gm) and JS-9560 (0.20 gm). Mean root weight in infested soil at PS was 0.50 gm. While non-infested soil at PS the root length was measured between 0.12 gm to 1.01 gm. significantly higher root weight was recorded in RVS-18 (1.01 gm) which was at with JS-9560 (0.95 gm) followed by RVS-24 (0.92 gm) as compared to JS-335 (0.12 gm).

Table 9 clearly exhibits that the root weight were affected by moisture per cent as well fungal infestation. The maximum root weight was measured in non-infested conditions [PS (0.75 gm), PC (0.38 gm) and PWP (0.29 gm)] as compare to infested soil conditions [PS (0.50 gm), PC (0.29 gm) and PWP (0.25 gm)].

4.5.4 Root weight at V2 stage

The data table 11 indicates the effects of three moisture regimes on root weight of different cultivars under *M. phaseolina* infested and non-infested soil.

Data in the table 11 indicates that the overall root weight was significantly more in RVS-18 (0.77 gm) followed by RVS-24 (0.61 gm) as compared to JS-9560 (0.46 gm) and JS-335 (0.21) cultivars while the maximum root weight were observed in PS (0.73 gm) as compare to PC (0.48

gm) and PWP (0.32 gm). Significantly more root weights were found in (0.68 gm) non-infested soil cultivars than (0.35 gm) infested soil cultivars

Table 11: Root weight of soybean cultivars under different moisture regimes in *M. phaseolina* infested and non-infested soil at V2 growth stage.

Treatments	Root weight (gm)								
	Infested*				Non-infested*				Mean C
	PWP	PC	PS	Mean	PWP	PC	PS	Mean	
JS-335	0.08	0.16	0.17	0.13	0.19	0.30	0.39	0.29	0.21
RVS-18	0.63	0.54	0.98	0.72	0.63	0.74	1.09	0.82	0.77
JS-9560	0.10	0.19	0.23	0.17	0.27	0.83	1.15	0.75	0.46
RVS-24	0.12	0.23	0.76	0.37	0.56	0.86	1.12	0.85	0.61
Mean A	0.23	0.28	0.53	0.35	0.41	0.68	0.94	0.68	
Factors	S.E.m±			C.D. at 5%					
(A) Infested and un-infested	0.009			0.025					
(B) Moisture	0.011			0.031					
A X B	0.015			0.043					
(C) Varieties	0.013			0.035					
A X C	0.018			0.05					
B X C	0.022			0.061					
A X B X C	0.031			0.086					

* Mean of four replications

Varieties	Root weight (gm)			
	PWP	PC	PS	Mean C
JS-335	0.13	0.23	0.28	0.21
RVS-18	0.63	0.64	1.03	0.77
JS-9560	0.18	0.51	0.69	0.46
RVS-24	0.34	0.54	0.94	0.61
Mean B	0.32	0.48	0.73	

PWP= Permanent wilting point PC= Pot capacity PS= Pot saturation

The root weight measured between 0.08 gm to 0.63 gm in infested soil at PWP. Significantly higher root weight was measured in RVS-18 (0.63 gm) followed by RVS-24 (0.12 gm) as compared to JS-335 (0.08 gm) and JS-9560 (0.10 gm). Mean root weight in infested soil at PWP was 0.23 gm. While in non-infested soil at PWP the root weight was measured between 0.19 gm to 0.63 gm. significantly higher root weight was recorded in RVS-18 (0.63 gm) which was at par with RVS-24 (0.56 gm) as compared to JS-335 (0.19 gm) and JS-9560 (0.27 gm).

In the infested soil at PC the root weight measured between 0.16 gm to 0.54 gm. significantly higher root weight was measured in RVS-18 (0.54 gm) followed by RVS-24 (0.23 gm) as compared to JS-335 (0.16 gm) and JS-9560

(0.19 gm). Mean root weight in infested soil at PC was 0.28 gm. While in non-infested soil at PC the root weight was measured between 0.30 gm to 0.86 gm. significantly higher root weight was recorded in RVS-24 (0.86 gm) which was at par with JS-9560 (0.83 gm) as compared to JS-335 (0.30 gm) to RVS-18 (0.74 gm).

In the infested soil at PS the root weight measured between 0.17 gm to 0.98 gm. significantly higher root weight was measured in RVS-18 (0.98 gm) followed by RVS-24 (0.76 gm) as compared to JS-335 (0.17 gm) and JS-9560 (0.23 gm). Mean root weight in infested soil at PS was 0.53 gm. While in non-infested soil at PS the root weight was measured between 0.39 gm to 1.15 gm. significantly higher root weight was recorded JS-9560 (1.15 gm) which was at par with RVS-24 (1.12 gm) followed by RVS-18 (1.09 gm) as compared to JS-335 (0.39 gm).

Table 12 clearly showed that the root weight was influenced by different water level as well as fungal infestation. The maximum root weight was measured in non-infested conditions [PS (0.53 gm), PC (0.0.28 gm) and PWP (0.23 gm)] as compare to infested soil conditions [PS (0.94 gm), PC (0.68 gm) and PWP (0.41 gm)].

4.5.5 Colony forming units per gram of root tissue at V1 stage

The data on colony forming units of different cultivars under *M. phaseolina* infested and non-infested soil at three moisture regimes are presented in table 12.

Data in the table 12 indicates the significant effect of different moisture levels on CFU. The maximum CFU were observed in JS-9560 (594.17) followed by JS-335 (577.5), RVS-24 (545) and RVS-18 (389.17) while in non-infested soil none of the cultivar exhibited the presence of *M. phaseolina* Maximum colony forming units were observed under moisture regimes at PWP (559.38), followed by PC (543.13) and PS (476.88).

Under PWP condition colony forming unit/gm of root tissue measured between 800 CFU's to 1315 CFU's in infested soil. Significantly more colony forming unit was recorded in JS-9560 (1315 CFU), JS-335 (1210 CFU), RVS-24 (1150 CFU) while the minimum colony forming units were observed in RVS-18 (800 CFU). Mean colony forming unit in per gram of root tissue was

1118.75 CFU in infested soil, whereas non colony forming units were observed in non-infested cultivars.

Table 12: Colony forming units /gm of root tissue under different moisture regime in *M. phaseolina* infested and non-infested soil at V1 growth stage

Treatments	CFU/gm of root								
	Infested*				Non-infested*				Mean C
	PWP	PC	PS	Mean	PWP	PC	PS	Mean	
JS-335	1210.00	1200.00	1055.00	1155.00	0	0	0	0	577.50
RVS-18	800.00	780.00	755.00	778.33	0	0	0	0	389.17
JS-9560	1315.00	1235.00	1015.00	1188.33	0	0	0	0	594.17
RVS-24	1150.00	1130.00	990.00	1090.00	0	0	0	0	545.00
Mean A	1118.75	1086.25	953.75	1052.92	0	0	0	0	
Factors	S.Em±			C.D. at 5%					
(A) Infested and un-infested	3.22			9.09					
(B) Moisture	3.95			11.13					
A X B	5.58			15.74					
(C) Varieties	4.56			12.85					
A X C	6.45			18.18					
B X C	7.90			22.26					
A X B X C	11.17			31.48					

* Mean of four replications

Varieties	CFU's/gm of root			
	PWP	PC	PS	Mean C
JS-335	605.00	600.00	527.50	577.50
RVS-18	400.00	390.00	377.50	389.17
JS-9560	657.50	617.50	507.50	594.17
RVS-24	575.00	565.00	495.00	545.00
Mean B	559.38	543.13	476.88	

PWP= Permanent wilting point PC= Pot capacity PS= Pot saturation

In the infested soil at PC the colony forming unit measured between 780 CFU's to 1235 CFU's. Significantly more colony forming units were observed in JS-9560 (1235 CFU) JS-335 (1200 CFU) and RVS-24 (1130 CFU) while the minimum colony forming units were observed in RVS-18 (780 CFU). Mean colony forming unit per gram of root tissue was 1086.25 CFU in infested soil condition, whereas non colony forming units were observed in non-infested cultivars.

In the infested soil at PS the colony forming units were measured between 755 CFU's to 1055 CFU's. Significantly more colony forming units were observed in JS-335 (1055 CFU), JS-9560(1015 CFU) and RVS-24 (990 CFU) while the minimum colony forming units were observed in RVS-18 (755 CFU). Mean colony forming unit per root gram of tissue in infested soil at PS

was 953.75 CFU whereas non colony forming units were observed in non-infested cultivars.

The table 10 clearly indicates that the moisture regimes along with soybean cultivars affected the CFU's/gm of root tissue. The maximum colony forming units were measured in [PWP (1118.75) followed by PC (1086.25) and PS (953.75)] in infested soil conditions while non colony observed in non-infested cultivars.

4.5.6 Colony forming units per gram of root tissue at V2 stage

The data table 13 revealed that the effects of three moisture regimes on colony forming units of different cultivars under *M. phaseolina* infested and non-infested conditions are presented.

Table 13: Colony forming units/gm of soybean root under different moisture regime in *M. phaseolina* infested and non-infested soil at V2 growth stage

Treatments	CFU's/gm of root								
	Infested*				Non-infested*				Mean C
	PWP	PC	PS	Mean	PWP	PC	PS	Mean	
JS-335	1310.00	1225.00	1130.00	1221.67	0	0	0	0	610.83
RVS-18	830.00	800.00	780.00	803.33	0	0	0	0	401.67
JS-9560	1360.00	1300.00	1170.00	1276.67	0	0	0	0	638.33
RVS-24	1345.00	1245.00	1010.00	1200.00	0	0	0	0	600.00
Mean A	1211.25	1142.50	1022.50	1125.42	0	0	0	0	
Factors	S.Em±			C.D. at 5%					
(A) Infested and un-infested	3.78			10.65					
(B) Moisture	4.63			13.04					
A X B	6.54			18.45					
(C) Varieties	5.34			15.06					
A X C	7.55			21.30					
B X C	9.25			26.09					
A X B X C	13.08			36.89					

* Mean of four replications

Varieties	CFU's/gm of root			
	PWP	PC	PS	Mean C
JS-335	655.00	612.50	565.00	610.83
RVS-18	415.00	400.00	390.00	401.67
JS-9560	680.00	650.00	585.00	638.33
RVS-24	672.50	622.50	505.00	600.00
Mean B	605.63	571.25	511.25	

PWP= Permanent wilting point PC= Pot capacity PS= Pot saturation

Data in table 13 indicates that the significant effect of different moisture levels on CFU. The maximum CFU's were observed in JS-9560 (638.33) followed by JS-335 (610.83), RVS-24 (600) and RVS-18 (401.67) while in non-infected soil none of the cultivar exhibited the presence of *M. phaseolina* colony whereas the maximum colony forming units were observed under moisture regimes at PWP (605.63), followed by PC (571.25) and PS (511.25).

Under PWP condition colony forming unit/gm of root tissue measured between 830 CFU's to 1360 CFU's in infested soil. Significantly more colony forming unit was recorded in JS-9560 (1360 CFU), RVS-24 (1345 CFU), JS-335 (1310 CFU), while the minimum colony forming units were observed in RVS-18 (830 CFU). Mean colony forming unit in per gram of root tissue was 1211.25 CFU in infested soil, whereas non colony forming units were observed in non-infested cultivars.

In the infested soil at PC the colony forming unit measured between 800 CFU's to 1300 CFU's. Significantly more colony forming units were observed in JS-9560 (1300 CFU) RVS-24 (1245 CFU) and JS-335 (1225 CFU) while the minimum colony forming units were observed in RVS-18 (800 CFU). Mean colony forming unit per gram of root tissue was 1142.50 CFU in infested soil condition, whereas non colony forming units were observed in non-infested cultivars.

In the infested soil at PS the colony forming units were measured between 780 CFU's to 1170 CFU's. Significantly more colony forming units were observed in JS-9560 (1170 CFU), JS-335 (1130 CFU) and RVS-24 (1010 CFU) while the minimum colony forming units were observed in RVS-18 (780 CFU). Mean colony forming unit per root gram of tissue in infested soil at PS was 1022 CFU whereas non colony forming units were observed in non-infested cultivars.

The table 13 surely revealed that the both water per cent and soybean cultivars affected the colony forming units/gm of root tissue. The maximum colony forming units were measured in [PWP (1211.25) followed by PC (1142.50) and PS (1022.50)] in infested conditions while non colony observed in non-infested cultivars.

CHAPTER -V

DISCUSSION

The present investigation was carried out to find out the morphological and histological characters of soybean genotypes responsible for charcoal rot resistance and susceptibility in soybean plants. The emphasis has been given to find out the impact of soil moisture on soybean cultivars and *M. phaseolina* root infection.

The pathogenicity of isolated fungus (*M. phaseolina*) test was confirmed by the pot inoculation method and stem cut inoculation method. In the pot inoculation method, the symptoms were produced by the plant after 20-25 days of inoculation while, stem cut inoculation method the inoculated plants showed the symptoms after 3 days of inoculation. Initially water soaked lesion was formed and they turn into a soft black near the collar region of the plant. The infection of charcoal rot reached the roots and produced microsclerotia. Leaves became yellow and dried. In stem cut method necrotic lesion gradually reached the downside of plant roots and plants died within 7 days. Similar symptoms of charcoal rot were described by Twizeyimana *et al*, (2012); Prioletta and Bazzalo (1998) and Bradley and Rio (2003).

In the field no significant impact on morphological characters of such as root length of soybean plants were noticed in *M. phaseolina* infested and un-infested field because the environmental condition like rainfall and temperature were not favourable for the charcoal rot development. However, effect of varietal differences was noticed. Resistant variety RVS-24 showed significantly higher root length as compared to susceptible ones. While number of secondary roots were highest in susceptible cultivar JS-335. Saima and Wu (2019) found reduction in growth, development and metabolism in plants such as shoot length, root length, photosynthetic pigment, relative water content and increase in sugar contents due to *M. phaseolina* infection.

Susceptible variety JS-335 exhibited the highest number of trifoliolate leaves and, resistant variety, RVS-18 exhibited highest leaves area among the cultivars. Resistant cultivars (RVS-18) not only showed the highest fresh root mass but also showed the highest dry root mass in all four cultivars. Similarly the plant heights of all the four cultivars at R7 growth stages were

not affected by *M. phaseolina*. RVS-18 showed the highest length of plant height among four cultivars. Disruption of root function by pathogen is associated with environment stresses, the above-ground symptoms of the disease are difficult to distinguish from other problems. These symptoms include loss of vigor as well as leaves that yellow, wilt, turn brown and die while still remaining attached to the plant (Smith and Wyllie, 1999; Wyllie,1988)

Quantity of microsclerotia in root tissue (CFU/g of tissue) is the most common method of measurement of charcoal rot development in soybeans (Mengistu *et al.* 2007; Smith and Carvil 1997). Number of colony forming unit /gram of root showed significantly higher 2820 CFU, 2780 CFU in susceptible cultivar JS-335, JS-9560 respectively as compared to resistant cultivar RVS-18 (1690 CFU). Sick plots plants did not expressed the symptoms of charcoal rot due to unfavourable condition for disease development. This shows that although pathogen was present in roots, but symptoms were not expressed. This indicates, that environmental factors plays an important role in charcoal rot development. Mengistu *et al.*, (2007); Mengistu *et al.*, (2011) and Paris *et al.*, (2006) noticed higher CFU/g tissue in susceptible cultivar than resistant cultivars during the genotype assessment experiment.

In the present investigation resistant and susceptible soybean cultivars were compared at the cellular level. Histopathology of soybean roots provides a better understanding of the cause and mechanism of resistance or susceptibility in soybean cultivars. Histological observations of soybean roots by Mengistu *et al.*, (2007); Mengistu *et al.*, (2011) and Bhuiyan *et al.*, (2015) showed black and dusty spotted appearance of microsclerotia on tissue like vascular, cortical and pith tissue and its pathogenic event gathered information of the pathogen (e.g. their present stage, location and establishment in the cells) and its influence on host plant growth. Epidermis thickness was significantly more in susceptible cultivar as compare to resistant one. It shows that epidermis thickness is not playing role in resistance. Thomas *et al.* (2007) observed thicker endodermis in roots of phytophthora blight resistant/ tolerant soybean cultivars. They demonstrated higher amount of suberin content in endodermis of roots than epidermis and cortex. In the present investigation also root endodermis thickness was

greater in RVS 18 as compared to other cultivars, which may be containing higher suberin content.

The histopathological observations were carried out to find the difference in host-plant relation at cellular level in resistant and susceptible cultivars of soybean. Three days after inoculation mycelium growth of *M. phaseolina* was observed on epidermis of all the four cultivars of soybean viz. JS-335, JS-9560, RVS-24 and RVS-24. On the 5th day, the pathogen colonized the epidermis tissue in all cultivars. Thus, there was no difference between susceptible and resistant genotype of soybean in pathogen colonization. On 10th days, larger necrotic lesions were observed on JS 335, JS 9560 and RVS 24. Transverse section clearly exhibited the formation of microsclerotia in cortex and vascular region of susceptible cultivars. While in RVS-18 microsclerotia was observed in epidermal region only. On 14th days of inoculation, the density of microsclerotia increased in all three susceptible cultivars of soybean (JS-335, JS-9560 and RVS-24). This shows that in susceptible cultivars pathogen continue to grow and reach the vascular and pith region of root and block the passage by the formation of microsclerotia. In chickpea plant, Singh *et al.*, (1990) demonstrated the colonization of pathogen in deeper root tissues in susceptible cultivars. After epidermal and cortex invasion, *M. phaseolina* colonized the vascular system and developed microsclerotia in xylem vessels which may lead to their blockage. Xylem vessel blocking causes wilt symptoms (Ilyas and Sinclair, 1974).

Soil moisture level is an important factor affecting the survival and activity of microsclerotia in soil and root infection. Therefore, in the present study effect of three moisture levels permanent wilting point, pot capacity and pot saturation on soybean infection by *M. phaseolina* was studied. *M. phaseolina* may infect the soybean plants at seedling stages i.e. one or two weeks after planting. Infection stays dormant if stressful environmental conditions are not present during this period of time. When the plants reach reproductive stages, physiological stress occurs and infection will progress (Hartman *et al.*, 1999). Till date, information on charcoal root of soybean disease progress at seedling stages is poorly documented.

Reyes-Franco *et al.*, (2006) demonstrated that at low water potential and high soil temperature and in the presence of the host plants,

microsclerotia germinate and produce a mass of hyphal threads. The hyphae grow towards the host root and colonize the seedlings roots during the first weeks of seed germination. In the present study at three moisture levels, higher root length and root weight was observed in pot saturation (PS) as compare to pot capacity (PC) and permanent wilting point (PWP). Root length was significantly less in *M. phaseolina* infested soil. This proves the earlier finding (Saima and Wu, 2019; Bowers and Russin 1999 and Kendig *et al.*, 2000) that *M. phaseolina* cause reduction in growth, development and metabolism in plants such as shoot length, root length, root volume and root weight.

Significantly higher colony forming units were found in infested soil at PWP (1118.75 CFU/g of root), PC (1086.25 CFU/g of root) while lower at PS (953.75 CFU/g of root). Substances such as alcohols, volatiles and increased levels of CO₂ found in flooded soils have a detrimental effect on the inoculums (Olaya *et al.*, 1996; Wyllie *et al.*, 1984). According to Wyllie *et al.*, (1984), microsclerotia germination is annulated in artificial atmospheres containing less than 16% of O₂ concentration in soil column systems. Several workers (Abawi *et al.*, 1985; Banks and Edgington, 1989) used this knowledge to reduce propagule densities of *M. phaseolina*, *Sclerotinia sclerotiorum* and *Sclerotium cepivorum* in soil under anoxic-flooded soils. *M. phaseolina* soil populations are reduced at high levels of soil moisture, mainly by low concentrations of soil oxygen and the proliferation of actinomycetes and bacteria that proliferates in high moisture conditions and degrade microsclerotia and germ tubes (Srivastava and Dhawan, 1980; Dhingra and Sinclair, 1975).

Brady and Weil, (2004) obtained high numbers of root CFUs, when soybean seedlings were exposed to a water deficit (PWP) regime. In contrast, PS was detrimental to root infection and possessed the lowest numbers of *M. phaseolina* root CFUs, except for a few exceptions in loam soil, in which the lowest populations were present under the PC water content regime results in higher levels of stress for the plant and consequently greater root infection. Root infections at low soil water content have been reported by Kendig *et al.*, (2000) and Olaya *et al.* (1996). They described strong effects of water stress at R2 soybean stages in *M. phaseolina* root populations and high rates of

colonization in soybean root segments buried in the soil at low water potentials.

Results of present investigation indicate increase in numbers of root CFUs from V1 to V2 soybean stages, which indicates that *M. phaseolina* root colonization progressed throughout vegetative growth. Root infection increased when plants were under water stress (PWP), but this effect was more evident at V2. In contrast, soil saturation (PS) had a significant detrimental effect on root populations significantly different from the PWP treatment at V2 stage. If moisture remains relatively constant, plants may produce new root tissues without further infection. These results also show that rains during the early vegetative stages prevent root colonization by *M. phaseolina*. Previous studies by Kendig, (2000) have also indicated the strong effect of water stress early in the season under field conditions. Optimum water conditions are an integrated management strategy for the charcoal rot disease. Dry soil conditions predispose the plant to stress and subsequent *M. phaseolina* colonization.

CHAPTER-VI

SUMMARY, CONCLUSION AND SUGGESTION FOR FUTHER WORK

Studies on host factors affecting development of charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid in soybean (*Glycine max* (L.) Merrill) executed during *kharif* season 2019-20, at Department of plant pathology laboratory and field of R.A.K. College of Agriculture, Sehore (M.P.), RVSKVV, Gwalior (M.P.)

❖ Summary

- The morphological characters of soybean cultivars were not affected in *M. phaseolina* sick plot during incessant minimum temperature, high relative humidity and frequently precipitation of rain.
- Colony forming unit/gram of dry root tissue was significantly higher in susceptible cultivars viz. JS-335, JS-9560 and RVS-24 as compared to cultivar RVS-18.
- The histological observations showed thicker endodermis and pith in resistant cultivar RVS-18 than susceptible cultivars JS-335, JS-9560 and RVS-24.
- Colonization of *M. phaseolina* on epidermis was found in all the four cultivars. At 10th day of inoculation the transverse section of roots showed mycelium growth towards cortex and endodermis in JS-335, JS-9560 and RVS-24 while in resistance cultivar RVS-18 the growth was limited to epidermis only. On 14th day, number of microsclerotia increased in all these susceptible cultivars.
- At pot saturation, pot capacity and permanent wilting point of moisture levels, RVS 18 plants exhibited significantly more root length and root weight than JS-335, JS-9560 and RVS-24 in *M. Phaseolina* infested and uninfected soil at V1 and V2 growth stage.
- The colony forming units of *M. phaseolina* in per gram of root tissue was significantly higher in all three cultivars viz. JS-335, JS-9560 and RVS-24 while in resistance cultivar RVS-18 the CFU was considerably lower at V1 and V2 growth stages.

- Among permanent wilting point, pot capacity and pot saturation condition root length and root weight was considerably more at pot saturation in inoculated state.

❖ **Conclusion**

- *M. phaseolina* assessment by colony forming units shows difference between resistant and susceptible cultivars even if persistence unfavorable condition for disease development.
- Thicker endodermis and pith of roots lead to resistance against *M. phaseolina* of soybean cultivars.
- Preliminary mycelium penetration of *M. phaseolina* occurs both in resistant and susceptible cultivars.
- Penetration of mycelium and micro-sclerotia formation is restricted to epidermis in resistance cultivar.
- The root length and root weight of all four cultivars was significantly higher at pot saturation compare to permanent wilting point and pot capacity in both V1 and V2 growth stages.
- At V1 and V2 stages, root length and root weight of resistant cultivar was found significantly more at all the three moisture regimes.
- Number of colony forming units were significantly less in resistant cultivar than susceptible cultivar at all three moisture in both growth stages from V1 to V2.

❖ **Suggestion for further work**

- Study on the effect of infestation and colonization on plant throughout the season is needed.
- Research is needed to understand the resistance to charcoal rot disease and how resistance can be measured in soybean.
- Studies should be conducted on interaction of environment and different cultivars on charcoal rot development.
- There is need to study the methods to decrease the colony forming units in the field.
- A broad study should be carried out to find the disease reactions in different types of soil types along with different moisture regimes.

BIBLIOGRAPHY

- Abawi, G.S.; Grogan, R.G. and Duniway, J.M. (1985). Effect of water potencial of survival of sclerotia of *Sclereotina minor* in two California soils. *Phytopathol.* **75**:221-221.
- Alexopoulos, C.J. and Mims, C.W. (1983). Introductory mycology (3rded.) Published by M.S. Sejwal for Willey Eastern Limited, 483/24, New Delhi. 632pp.
- Almeida A.M.R.; Torres, E.; Farias, J.R.B.; Benato, L.C.; Pinto, M.C. and Marin, S.R.R. (2001). *Macrophomina phaseolina* em restos de cultura e diversidade genética. Londrina PR. Embrapa Soja. Circular Técnica No. 34.
- Anonymous (1963). Annual report of the *Jute Agric. Res. Inst.*, 1961-1962, 108pp.
- Anonymous, (2021). Online, <https://iisrindore.icar.gov.in/pdfdoc/AicrpsAR2020-21.pdf>
- Baird, R.E.; Watson, C.E. and Scruggs, M. (2003). Relative longevity of *Macrophomina phaseolina* and associated mycobiota on residual soybean roots in soil. *Plant Dis.* **87**:563-566.
- Banks, E. and Edgington, L.V. (1989). Effect of integrated control practices on the onion white rot pathogen in organic soil. *Can J. Plant Pathol-Rev Can Phytopathol.* **11**:268-272.
- Barnett, H.L. and Hunter, B.B. (1986). Illustrated genera of imperfect fungi. 3rd Ed., Burgess Publishing Co. New York. 273 pp.
- Bhuiyan, M.A.H.B.; Groom, T.; Nicolas, M.E. and Taylor, P.W.J. (2015). Histopathology of *S.tanacetii* infection in pyrethrum leaf lamina. *Australas. Plant Pathol.* **44**:629–636.
- Bowers, G.R. and Russin, J.S. (1999). Soybean disease management. In soybean production in the midsouth, Eds. L.G. Heatherly and H.F. Hodges. Boca Raton, Florida: *CRC press*. pp 235-237.
- Bradley, C. A. and Rio, L. E. (2003). First report of charcoal rot on soybean caused by *Macrophomina phaseolina* in North Dakota. *Plant Dis.* **87**:601.

- Brady, N.C. and Weil, R.R. (2004). Soil water: characteristics and behavior. In Elements of the nature and properties of soils, N.C. Brady, and R.R. Weil, eds. (New Jersey, Prentice Hall), pp. 134-161.
- Bressano, M.; Lorena, G.M.; Luna, C.M. and Ducasse, D.A. (2010). An in vitro method for examining infection of soybean roots by *Macrophomina phaseolina*. *Physiol. Mol. Plant Pathol.* **74**:201-204.
- Bristow, P.R. and Wyllie, T.D. (1984) Reaction of soybean cultivars to *Macrophomina phaseolina* as seedlings in the growth chamber and field. *Trans. Mo. Acad. Sci.* **18**:5-10.
- Chauhan, G.S.; Verma, N.S. and Bains, G.S. (1988). Effect of extrusion processing on the nutritional quality of protein in rice legume blends. *Nahrung.* **32**(1):43.
- Dhingra, O.D. and Sinclair, J.B. (1985). Basic Plant Pathology Methods. *CRC Press, Inc. Boca Raton, Florida.* pp 132-163.
- Dhingra, O.D. and Sinclair, J.B. (1978). Biology and pathology of *Macrophomina phaseolina*. O.D. Dhingra and J.B. Sinclair eds. Universidade Federal De Vicosa, Minas Gerais. 166p.
- Diourte, M.; Starr, J.L.; Jeger, M.J.; Stack, J.P. and Rosenow, D.T. (1995) Charcoal rot (*Macrophomina phaseolina*) resistance and the effects of water stress on disease development in sorghum. *Plant Pathol.* **44**:196-202.
- Doubledee, M.D.; Rupe, J.C.; Rothrock, C.S. and Bajwa, S.G. (2018). Effect of root infection by *Macrophomina phaseolina* on stomatal conductance, canopy temperature and yield of soybean. *Can. J. Plant Pathol.* **40**:272-283.
- Fehr, W.R.; Caviness, C.E.; Burmood, D.T. and Penningt, J.S. (1971). Stage of development descriptions for soybean, *Glycine max* (L) Merr. *Crop Sci.* **11**:929-931.
- Gaige, A.R.; Ayella, A and Shuai, B. (2010). Methyl jasmonate and ethylene induce partial resistance in *Medicago truncatula* against the charcoal rot pathogen *Macrophomina phaseolina*. *Physiol. Mol. Plant Pathol.* **74**(5): 412-418.
- Gangopadhyay, S.; Agarwal, D.K.; Sarbhoy, A. and Wadhi, S.R. (1973). Charcoal rot disease of soybean in India. *Indian Phytopath.* **26**:730-732.

- Gholamhoseini, M.; Habibzadeh, F.; Ataei, R.; Hemmati, P. and Ebrahimian, E. (2018). Interaction of irrigation strategy and substrate amendment on some physiological traits and fruit yield of cucumber grown under soilless culture. *Rhizosphere* **6**:7-10.
- Ghosh, T.; Mukherji, N. and Basak, M. (1964). On the occurrence of new species of *Orbilia*. *Fr. Jute Bull.*, **27**:134-141.
- Goudarzi, A.; Banihashemi, Z. and Maftoun, M. (2008). Effect of water potential on sclerotial germination and mycelial growth of *Macrophomina phaseolina*. *Phytopathol Mediterr.* **47**:107-114.
- Grau, C.R.; Dorrance, A.E.; Bond, J. and Russin, J.S. (2004). Fungal diseases. 684–687 H.R. Boerma, J.E. Specht (Eds.), Soybeans: Improvement, Production, and Uses (third ed.), ASA, CSSA, and SSSA, Madison, WI (2004) Agron Monogr. 16.
- Gupta, G.K. and Chauhan, G.S. (2015). Symptoms, Identification and management of soybean diseases. *Tech. bull.*, 10. NRCS, Indore, India, 92p.
- Gupta, G.K.; Sharma, S.K. and Ramteke, R. (2012). Biology, epidemiology and management of the pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (*Glycine max* (L.) Merrill). *J. Phyto.* **160**: 167-180.
- Hagedorn, D.J. (1991) Ashy stem blight. In: Hall R. (ed.) *Compendium of Bean Diseases*. St. Paul, MN, USA, APS Press, pp 70.
- Hartman, G.L.; Sinclair, J.B. and Rupe, J.C. (1999). Compendium of soybean diseases, 4th ed. St. Paul Minnesota.
- Hemmati, P.; Zafari, D.; Mahmoodi, S.B.; Hashemi, M.; Gholamhoseini, M.; Dolatabadian, A. and Ataei R. (2018). Histopathology of charcoal rot disease (*Macrophomina phaseolina*) in resistant and susceptible cultivars of soybean. *Rhizosphere* **7**:27-34
- Ilyas, M.B. and Sinclair, J. (1974) Effects of plant age upon development of necrosis and occurrence of intraxylem sclerotium in soybean infected with *Macrophomina phaseolina*. *Phytopathology* **64**:156–157.
- Islam, M.S.; Haque, M.S.; Islam, M.M.; Emdad, E.M.; Halim, A.; Hossain, M.Z.; Ahmed, B.; Rahim, S.; Rahman, M.S.; Alam, M.M.; Hao, S.; Wan, X.; Saito, J.A. and Alam, M. (2012). Tool to kit: genome of one of the

- most destructive plant pathogen fungi *Macrophomina phaseolina*. *BMC Genom.* **19** (13): 493-503.
- Jordaan, E.; Waals, J.E.V.D. and McLaren, N.W. (2019). Effect of irrigation on charcoal rot severity, yield loss and colonization of soybean and sunflower. *Crop prot.* **122**:63-69.
- Kendig, S.R.; Rupe, J.C. and Scott, H.D. (2000). Effect of irrigation and soil water stress on densities of *Macrophomina phaseolina* in soil and roots of two soybean cultivars. *Plant Dis.* **84**(8):895-900.
- Khan, S.N. (2007). *Macrophomina phaseolina* as causal agent for charcoal rot of sunflower. *Mycopathology* **5**:111-118.
- Kirkham, M.B. (2005). Field capacity, wilting point, available water, and the non-limiting water range. In Principles of soil and water relations, M.B. Kirkham, ed. (San Diego, California, Elsevier Academic Press), pp. 101-115.
- Kirkpatrick, M.T.; Rupe, J.C. and Rothrock, C.S. (2006) Soybean response to flooded soil conditions and the association with soilborne plant pathogenic genera. *Plant Dis.* **90**:592-596.
- Lodha, S. (1995). Soil solarization, summer irrigation and amendments for the control of *Fusarium oxysporum* f.sp. *cumini* and *Macrophomina phaseolina* in arid soils. *Crop Prot.* **14**:215-219.
- Mengistu, A. (2015). Charcoal rot, pp. 67-69. In G.L. Hartman; J.B. Sinclair and J.C. Rupe (eds.), Compendium of soybean diseases and pests. *American Phytopathological Society, St. Paul, MN.*
- Mengistu, A.; Arelli, P.A.; Bond, J.P.; Shannon, G.J.; Wrather, A.J.; Rupe, J.B.; Chen, P.; Little, C.R.; Canaday, C.H.; Newman, M.A. and Pantalone, V.R. (2011). Evaluation of soybean genotypes for resistance to charcoal rot. Online: *Plant Health Prog.* [Http/www. Plantmanagement network.org /php](http://www.plantmanagementnetwork.org/php)
- Mengistu, A.; Ray, J.D.; Smith, J. R.; and Paris, R. L. (2007). Charcoal rot disease assessment of soybean genotypes using a colony-forming unit index. *Crop Sci.* **47** (6) :2453-2461.
- Mengistu, A.; Ray, J.D.; Smith, J.R.; Arelli, P.R.; Bellaloui, N.; Chen, P.; Shannon, G. and Boykin, D. (2018). Effect of charcoal rot on selected

- putative drought tolerant soybean genotypes and yield. *Crop Prot.* **105**:90-101
- Mengistu, A.; Smith, R.J.; Ray, D.J. and Bellaloui, N. (2011). Seasonal progress of charcoal rot and its impact on soybean productivity. *Plant Dis.* **95**: 1159-166.
- Mengistu, A.; Wrather, A. and Rupa, C.J. (2015). Compendium of soybean diseases. *APS, St. Paul, MN.* **5**: 67-69
- Meyer, W.A.; Sinclair, J.B. and Khare, M.M. (1974). Factors affecting charcoal rot of soybean seedlings. *Phytopathology* **64**: 845-849.
- Mihail, J.D. (1992). *Macrophomina*. In Methods for research on soilborne phytopathogenic fungi. (*St. Paul, MN, APS*), pp. 134-136.
- Ndiaye, M. (2007). Ecology and management of charcoal rot (*Macrophomina phaseolina*) on cowpea in the Sahel. the Netherlands, Wageningen, Wageningen University, PhD Thesis.
- Olaya, G. and Abawi, G.S. (1996). Effect of water potential on mycelial growth and production and germination of sclerotia of *Macrophomina phaseolina*. *Plant Dis.* **80**:1347-1350.
- Olaya, G.; Abawi, G.S. and Barnard, J. (1996). Influence of water potential on survival of sclerotia in soil and on colonization of bean stem segments by *Macrophomina phaseolina*. *Plant Dis.* **80**:1351-1354.
- Paris, R.L.; Mengistu, A.; Tyler, J. and Smith, J. (2006). Registration of soybean germplasm line DT97-4290 with moderate resistance to charcoal rot. *Crop sci.* **46**:2324-2325.
- Pearson, C. A.; Kelley, S., K.; Crow, F. J. and Schwenk, F. W. (1984). Colonization of soybean roots by *Macrophomina phaseolina*. *Plant dis.* **68**(12):1086-1088.
- Pearson, C.A.S.; Schwenk, F.W. Crowe, F.J. and Kelley, K. (1984). Colonization of soybean roots by *Macrophomina phaseolina*. *Plant Dis.* **68**:1086-1088.
- Prioletta, S. And Bazzalo, M.E. (1998). Sunflower basal stalk rot (*Sclerotinia sclerotirum*): its relationship with some yield components reduction. *Helia.* **21** (29) : 33-44.
- Purkayastha, S.; Kaur, B.; Dilbaghi, N. and Chaudhury, A. (2006). Characterization of *Macrophomina phaseolina*, the charcoal rot

- pathogen of cluster bean, using conventional techniques and PCRRAPD based molecular markers. *Plant Pathol.* **55**:106-116.
- Purkayastha, S.; Kaur, B.; Dilbaghi, N.; Power, J.B.; Davey, M.R. and Chaudhury, A. (2003). Studies on cultural characteristics and nitrogen assimilation in *Macrophomina phaseolina* infecting cluster bean (*Cyamopsis tetragonoloba*). Plant pathogen genomics – from sequence to application. Proceedings of the *British Society for Plant Pathology* Presidential Meeting, Nottingham, UK. 2003, **33**.
- Reichert, I. and Hellinger, E. (1947). On the occurrence, morphology, and parasitism of *Sclerotium bataticola*. *Palest. J. Bot. R. Ser. VI*, **1,2**: 107-147.
- Reyes-Franco, M.C., Hernández-Delgado, S.; Beas-Fernández, R.; MedinaFernández, M.; Simpson, J.; Mayek-Pérez, N. (2006). Pathogenic and Genetic variability within *Macrophomina phaseolina* from Mexico and other countries. *J. Phytopathology* **154**:447-453.
- Riker, A.T. and Riker, A.S. (1939). An Introduction to Research on Plant Disease. Johns. Swill. New York. 117 p.
- Rogers, D.H. (1997). Soybeans irrigation. In Soybean production handbook, D.H. Rogers, ed. (Kansas State University Agricultural Experiment Station and Cooperative Extension Service).
- Saima, S. and Wu, G. (2019). Effect of *Macrophomina phaseolina* on growth and expression of defense related genes in *Arabidopsis thaliana*. *J. Natl. Sci. Found. Sri Lanka* **47**(1)113–120.
- Scott, H.D.; Ferguson, J.A.; Sojka, R.E. and Batchelor, J.T. (1986). Response of Lee-74 soybean to irrigation in Arkansas. Arkansas Agricultural Experiment Station Bulletin, 1-44.
- Shehbaz, M.; Rauf, S.; Al-Sadi, A.M.; Nazir, S.; Bano, S.; Shahzad, M. and Hussain, M.M. (2018). Introgression and inheritance of charcoal rot (*Macrophomina phaseolina*) resistance from silver sunflower (*Helianthus argophyllus* Torr. & A. Gray) into cultivated sunflower (*Helianthus annuus* L.). *Australas. Plant Pathol.* **47**(4):413-420.
- Short, G.E. and Wyllie, T.D. (1976). A quantitative technique for measuring vegetative growth and sclerotial production by *Macrophomina phaseolina* in soybean tissues. *Proc Am Phytopathological Soc.* **3**:222.

- Short, G.E.; Wyllie, T.D. and Ammon, V.D. (1978). Quantitative enumeration of *Macrophomina phaseolina* in soybean tissues. *Phytopathology* **68**:736-741.
- Sinclair, R.T.; Purcell, L.C.; King, C.A.; Sneller, C.H.; Chen, P. and Vadez, V. (2007). Drought tolerate and yield increase of soybean resulting from improved symbiotic N₂ fixation. *Field Crops Res.* **101**:68-71.
- Sinclair, T.R.; Messina, C.D.; Beatty, A.P. and Samples, M. (2010). Assessment across the United States of the benefits of altered soybean drought traits. *Agron. J.* **102**: 475-482.
- Singh, S.K.; Nene, Y.L. and Reddy, M.V. (1990). Some histopathological observation of chickpea roots infected by *Rhizoctonia bataticola*. *Int, Chickpea Newsletter* **23**:24-25.
- Smith, G.S. and Carvil, O.N. (1997). Field screening of commercial and experimental soybean cultivars for their reaction to *M. phaseolina*. *Plant Dis.* **81**:363-368.
- Smith, G.S. and Wyllie, T.D. (1999). Charcoal rot. *In*: G.L. Hartman; J.B. Sinclair, and J.C. Rupe (Eds.), *Compendium of Soybean Disease*, 4th. (pp. 29-31). *St Paul, MN, USA: American Phytopathological Society.*
- Srivastava, A.K.; Singh, T.; Jana, T.K. and Arora, D.K. (2001). **Microbial colonization of *Macrophomina phaseolina* and suppression of charcoal rot of chickpea** Sinha A. (Ed.), *Microbes and plants*, Vedams eBooks (P) Ltd, New Delhi, pp. 269-319.
- Srivastava, S.K. and Dhawan, S. (1980). Pathogenicity of *Macrophomina phaseolina* isolates causing stem and root rot of *Brassica juncea* effect of varying soil texture, soil reaction and soil moisture. *Proc. Natl. Acad. Sci. India Sect. Bio. Sci.* **46**, 723-727.
- Stegman, E.C.; Schatz, B.G. and Gardner, J.C. (1990). Yield sensitivities of short season soybeans to irrigation management. *Irrig. Sci.* **11**:111-119.
- Su, G.; Suh, O.S.; Schneider, R.W. and Russin, J.S. (2001). Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. *Phyto.* **91**(2): 120-6.
- Subramanian, C.V. (1971). "The Hyphomycetes". *ICAR*, New Delhi.
- Thomas, R.; Fang, X.; Ranathunge, K.; Anderson, T.R.; Peterson, C.A, and Bernards, M.A. (2007). Soybean root suberin: Anatomical distribution,

- chemical composition, and relationship to partial resistance to *Phytophthora sojae*. *Plant Physiol.* **144**(1):299-311.
- Tosi, L. and Zizzerini, A. (1990) Influence of environmental factors and cultural techniques on *Sclerotium bataticola* Taub. on sunflower. *Inf Fitopatol.* **40**:73-76.
- Twizeyimana, M.; Hill, C.B.; Pawlowski, M.; Paul, C. and Hartman, G.L. (2012). A cut-stem inoculation technique to evaluate soybean for resistance to *Macrophomina phaseolina*. *Plant Dis.* **96**(8):1210-1215.
- Weimer, J.L. (1947). Disease survey of soybean nurseries in the South. *Plant Dis. Rep. Suppl.* **168**:27-53.
- Wokocho, R.C. (2000). Effect of different soil moisture regimes on the development of the charcoal rot diseases of soybean by *Macrophomina phaseolina*. *Global J. Pure Appl. Sci.* **6**:599-602.
- Wrather, J.A. and Koenning, S.R. (2006). Estimation of disease effects on soybean yields in the United States 2003-2005. *J. Nematol.* **38**: 173-180.
- Wrather, J.A.; Shannon, J.G; Carter, T.E.; Bond, J.P. Rupe, J.C. and Almeida, A.M.R. (2008). Reaction of drought-tolerant soybean genotypes to *Macrophomina phaseolina*. Online. *Plant Health Prog.* Doi: 10.1094/PHP-2008-0618-01-RS.
- Wyllie, T.D. (1988). Charcoal rot of soybean current status. Pages 203-106 in: Soybean Diseases of the North Central Region. T.D. Wyllie and D.H. Scott, eds. *American Phytopathological Society, St Paul MN.*
- Wyllie, T.D.; Gangopadhyay, S.; Teague, W.R. and Blanchar, R.W. (1984). Germination and production of *Macrophomina phaseolina* microsclerotia as affected by oxygen and carbon dioxide concentration. *Plant and Soil.* **81**(2):195-201.
- Yang, X.B. and Navi, S.S. (2005). First report of charcoal rot epidemics caused by *Macrophomina phaseolina* in soybean in Iowa. *Plant Dis.* **89**(5):526.
- Young, P.A. (1944). Epidemic of charcoal rot of corn and other crops in the East taxes. *Plant Dis. Repr.* **28**: 898-899.

APPENDICES

Analysis of variance of Table-2

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Replication	3	20.237			
(A) Infested and un-infested	1	3.187	3.187	1.199	0.28585
(B) Varieties	3	29.687	9.896	3.725	0.02723
(A X B)	3	3.915	1.305	0.491	0.6922
Error	21	55.795	2.657		
Total	31	112.821			

Analysis of variance of Table-2

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Replication	3	12.75			
(A) Infested and un-infested	1	6.125	6.125	2.208	0.15214
(B) Varieties	3	197.25	65.75	23.704	0
(A X B)	3	1.125	0.375	0.135	0.93794
Error	21	58.25	2.774		
Total	31	275.5			

Analysis of variance of Table-3

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Replication	3	16.75			
(A) Infested and un-infested	1	1.125	1.125	0.559	0.46289
(B) Varieties	3	537	179	88.97	0
(A X B)	3	0.375	0.125	0.062	0.9792
Error	21	42.25	2.012		
Total	31	597.5			

Analysis of variance of Table-3

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Replication	3	29.325			
(A) Infested and un-infested	1	11.838	11.838	2.553	0.125
(B) Varieties	3	1,080.28	360.094	77.67	0
(A X B)	3	1.488	0.496	0.107	0.95506
Error	21	97.36	4.636		
Total	31	1,220.29			

Analysis of variance of Table-4

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Replication	3	0.062			
(A) Infested and un-infested	1	0.252	0.252	4.576	0.04432
(B) Varieties	3	1.759	0.586	10.644	0.00018
(A X B)	3	0.134	0.045	0.81	0.50229
Error	21	1.157	0.055		
Total	31	3.363			

Analysis of variance of Table-4

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Replication	3	0.082			
(A) Infested and un-infested	1	0.353	0.353	4.837	0.0392
(B) Varieties	3	2.222	0.741	10.152	0.00025
(A X B)	3	0.175	0.058	0.801	0.50699
Error	21	1.532	0.073		
Total	31	4.364			

Analysis of variance of Table-5

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Replication	3	20.148			
(A) Infested and un-infested	1	3.445	3.445	0.638	0.4334
(B) Varieties	3	1,037.84	345.945	64.056	0
(A X B)	3	0.273	0.091	0.017	0.99692
Error	21	113.414	5.401		
Total	31	1,175.12			

Analysis of variance of Table-6

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Replication	3	11,812.50			
(A) Infested and un-infested	1	11,400,312.50	11,400,312.50	1,376.79	0
(B) Varieties	3	1,891,837.50	630,612.50	76.158	0
(A X B)	3	1,655,537.50	551,845.83	66.645	0
Error	21	173,887.50	8,280.36		
Total	31	15,133,387.50			

Analysis of variance of Table-7

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Treatment	3	1,995.31	665.104	31.531	0.00001
Error	12	253.125	21.094		
Total	15	2,248.44	S E m± 2.30	CD at 5 % 7.15	

Analysis of variance of Table-7

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Treatment	3	34,089.06	11,363.02	23.026	0.00003
Error	12	5,921.88	493.49		
Total	15	40,010.94	S E m± 11.11	CD at 5% 34.60	

Analysis of variance of Table-7

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Treatment	3	1,551.17	517.057	22.691	0.00003
Error	12	273.438	22.786		
Total	15	1,824.61	S E m± 2.39	CD at 5 % 7.44	

Analysis of variance of Table-7

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
Treatment	3	116,992.19	38,997.40	24.35	0.00002
Error	12	19,218.75	1,601.56		
Total	15	136,210.94	S E m± 20.01	CD at 5 % 62.34	

Analysis of variance of Table-8

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
(A) Infested and un-infested	1	108.166	108.166	83.296	0
(B) Moisture	2	483.984	241.992	186.352	0
(A X B)	2	21.436	10.718	8.254	0.00059
(C) Varieties	3	1,707.75	569.25	438.365	0
(A X C)	3	39.055	13.018	10.025	0.00001
(B X C)	6	99.928	16.655	12.825	0
(A X B X C)	6	17.399	2.9	2.233	0.04946
Error	72	93.497	1.299		
Total	95	2,571.22			

Analysis of variance of Table-9

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
(A) Infested and un-infested	1	386.003	386.003	202.77	0
(B) Moisture	2	1,103.32	551.659	289.791	0
(A X B)	2	37.443	18.721	9.834	0.00017
(C) Varieties	3	507.049	169.016	88.786	0
(A X C)	3	114.216	38.072	20	0
(B X C)	6	141.536	23.589	12.392	0
(A X B X C)	6	41.12	6.853	3.6	0.00354
Error	72	137.063	1.904		
Total	95	2,467.75			

Analysis of variance of Table-10

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
(A) Infested and un-infested	1	0.394	0.394	69.865	0
(B) Moisture	2	2.265	1.132	200.821	0
(A X B)	2	0.19	0.095	16.809	0
(C) Varieties	3	8.898	2.966	525.966	0
(A X C)	3	0.448	0.149	26.495	0
(B X C)	6	1.006	0.168	29.744	0
(A X B X C)	6	0.418	0.07	12.36	0
Error	72	0.406	0.006		
Total	95	14.026			

Analysis of variance of Table-11

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
(A) Infested and un-infested	1	2.591	2.591	688.457	0
(B) Moisture	2	2.787	1.394	370.368	0
(A X B)	2	0.267	0.134	35.48	0
(C) Varieties	3	4.004	1.335	354.702	0
(A X C)	3	1	0.333	88.599	0
(B X C)	6	0.688	0.115	30.473	0
(A X B X C)	6	0.445	0.074	19.689	0
Error	72	0.271	0.004		
Total	95	12.052			

Analysis of variance of Table-12

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
(A) Infested and un-infested	1	26,607,204.17	26,607,204.17	53,362.64	0
(B) Moisture	2	122,233.33	61,116.67	122.574	0
(A X B)	2	122,233.33	61,116.67	122.574	0
(C) Varieties	3	633,179.17	211,059.72	423.295	0
(A X C)	3	633,179.17	211,059.72	423.295	0
(B X C)	6	36,833.33	6,138.89	12.312	0
(A X B X C)	6	36,833.33	6,138.89	12.312	0
Error	72	35,900.00	498.611		
Total	95	28,227,595.83			

Analysis of variance of Table-13

Source of Variance	D.f	S.S	M.S.S	Cal. F	Tab. F.
(A) Infested and un-infested	1	30,397,504.17	30,397,504.17	44,393.92	0
(B) Moisture	2	146,008.33	73,004.17	106.619	0
(A X B)	2	146,008.33	73,004.17	106.619	0
(C) Varieties	3	848,645.83	282,881.94	413.134	0
(A X C)	3	848,645.83	282,881.94	413.134	0
(B X C)	6	44,991.67	7,498.61	10.951	0
(A X B X C)	6	44,991.67	7,498.61	10.951	0
Error	72	49,300.00	684.722		
Total	95	32,526,095.83			

VITA

The author of this manuscript **Mr. Rahul Sharma** S/o Shri Lakhan lal sharma was born on 04 Jan. 1997 at Village Dobra Post- Semra dangi, Tehsil+District Sehore, (M.P.). He completed his High School (10th) in 2012 with 42.33.% marks from Yug nirman Higher Secondary School, and Higher Secondary School (12th) in 2014 with 65.6% marks from Authentic Higher Secondary school dist.- Sehore (M.P.).

Thereafter, he joined AKS University Satna (M.P.) in 2014 and completed B.Sc. (Ag.) Degree in 2018 with 6.84 O.G.P.A.

Then he joined in 2018-19 for the Post Graduation programme in Agriculture (Plant Pathology) R.A.K. College of Agriculture, Sehore (M.P.).

He has completed all required courses and submitting his thesis for M.Sc. (Ag.) degree.