Analysis on Variability of Sclerotium rolfsii Causing Cucurbit Collar Rot

By

RITESH KUMAR

PLANT PATHOLOGY
BIHAR AGRICULTURAL UNIVERSITY
SABOUR-813 210, BHAGALPUR

2017

Analysis on Variability of Sclerotium rolfsii Causing Cucurbit Collar Rot

THESIS

Submitted to the
Bihar Agricultural University, Sabour
in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

By

RITESH KUMAR

Reg. No - M/PPLPATH/303/BAC/2015-16

Department of Plant Pathology

Bihar Agricultural University
Sabour, Bhagalpur – 813 210 (Bihar)

(2017)

Dedicated to My Beloved Family

Ritesh Kr.....

BIHAR AGRICULTURAL UNIVERSITY, SABOUR, BHAGALPUR-813 210

Dr. A. P. Bhagat
Chief Scientist cum Univ. Professor
&
Chairman

Deptt. of Plant Pathology
Bihar Agricultural University,
Sabour, Bhagalpur

Dated_____

Certificate-I

This is to certify that the thesis entitled **"Analysis on Variability of *Sclerotium rolfsii* Causing Cucurbit Collar Rot"** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) IN THE SUBJECT OF PLANT PATHOLOGY** of the faculty of Agriculture, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, is genuine record of *bonafide* research work carried out by **Mr. Ritesh Kumar, Regd. No.: M/PPLPATH/303/BAC/2015-16**, under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis has been fully acknowledged.

(A. P. Bhagat)
Chairman,
Advisory Committee

Certificate-II

We, the undersigned members of the Advisory Committee of **Mr. Ritesh Kumar**, **Regd. No.: M/PPLPATH/303/BAC/2015-16** a candidate for the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** have gone through the manuscript of the thesis and agree that the thesis entitled **"Analysis on Variability of *Sclerotium rolfsii* Causing Cucurbit Collar Rot"** may be submitted in partial fulfilment of the requirement for the award of the degree.

A. P. Bhagat
Chairman,
Advisory Committee

Endorsed:

Dr. A.P. Bhagat
Chairman,
Deptt. of Plant Pathology

Members :

1. Dr. Abhijeet Ghatak ……..
Asst. Prof.-cum-Jr. Scientist
Department of Plant Pathology

2. Dr. S. N. Ray ……
University Prof. & Chairman
Department of Entomology

3. Dr. S. N. Singh ……
Univ. Prof.-cum-Chief Scientist & Chairman
Department of SMCA

4. Dr. S. R. Singh ……
Univ. Prof.-cum-Chief Scientist & Chairman
Department of Extension Education
Nominee of DRI-cum-Dean PGS

Certificate – III

This is to certify that the thesis entitled **"Analysis on Variability of *Sclerotium rolfsii* Causing Cucurbit Collar Rot"** submitted by **Mr. Ritesh Kumar, Regd. No.: M/PPLPATH/303/BAC/2015-16** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** of the faculty of Agriculture, Bihar Agricultural University, Sabour, Bhagalpur, Bihar, was examined and approved on ……..

A. P. Bhagat
Chairman
Advisory Committee

Name & Signature of
External Examiner

Endorsed :

Members :

Chairman
Deptt. of Plant Pathology

1. Dr. Abhijeet Ghatak ……..
Asstt. Prof.-cum-Jr. Scientist
Department of Plant Pathology

Associate Dean-cum-Principal
Bihar Agricultural College,
Sabour, Bhagalpur -813 210

2. Dr. S. N. Ray ……..
University Prof. & Chairman,
Department of Entomology

3. Dr. S. N. Singh ……..
Univ. Prof.-cum-Chief Scientist &
Chairman, Department of SMCA

DRI-cum-Dean, PGS
Bihar Agricultural University,
Sabour, Bhagalpur -813210

4. Dr. S. R. Singh ……
Univ. Prof.-cum-Chief Sci. & Chairman,
Department of Extension Education
Nominee of DRI-cum-Dean PGS

Acknowledgment

It's my heart's turn….

Well, where do I begin? Probably I need pages together to thank all those wonderful people around me. I feel so lucky to have gotten them in my life. Interdependence is definitely more valuable than independence. This thesis is the result of two years of work whereby I have been accompanied, supported and guided by many people. I would thus like to thank everyone who, knowingly or otherwise, has provided support, encouragement and assistance along the way.

First and foremost, I would like to give special thanks to Dr. A.K. Singh, Hon'ble Vice-Chancellor, BAU, Sabour for providing all facilities during the course of my investigation.

I am much obliged to my DRI cum Dean PGS Dr. B. C. Saha, for providing necessary facility to carry out the research work in an effective manner.

I am very much thankful to Principal, Bihar Agricultural College, Dr. R. P. Sharma for providing me necessities required during my research programme.

I feel great pleasure to evince my profound sense of veneration and gratitude to my advisor, Dr. A. P. Bhagat, Chief Scientist-cum-University Professor and Chairman, Department of Plant Pathology, B.A.U. Sabour for his constant encouragement, sustained interest, generous assistance at every stage of investigation and his efforts for providing me with the atmosphere and facilities that I needed for completing this work successfully. His enthusiasm, concern, perfection and constructive criticism have always aroused my spirits to do more, to achieve higher. I confess that words are powerless to express my gratitude to him.

I humbly express my profound gratitude to my co-advisor Dr. Abhijeet Ghatak, Assistant professor cum junior scientist, Department of Plant Pathology, B.A.U. Sabour for his constant guidance and valuable support. He contributed his vast knowledge to complete this seemingly never-ending task in a resplendent way. Without him, this all happening was not possible. It has been a rare privilege for me to be associated with him during M.Sc. (Ag.) course.

I express my high regard to Dr. S. N. Ray, University Professor and Chairman, Department of Entomology, B.A.U. Sabour, Dr. S. N. Singh, University Professor-cum-Chief Scientist and Chairman, Department of Statistics, Mathematics and Computer Application, B.A.U. Sabour, Dr. S. R. Singh University Professor-cum-Chief Scientist and Chairman, Department of Extension Education, B.A.U. Sabour of my advisory committee for their kind co-operation and constructive suggestions during my thesis work.

It was a great privilege to have Dr. G. Chand, Associate Professor cum senior scientist, Dr. C. S. Azad, Assistant Professor cum junior scientist, Mr. R. N. Gupta, Assistant Professor cum junior scientist, Dr. A. Kumar, Assistant Professor cum junior scientist, Dr. C. Kushwaha, Assistant Professor cum junior scientist, Dr. M. Ansar, Assistant Professor cum junior scientist, Dr. M. A. Anwer, Assistant Professor cum junior scientist, Mr. S. Sarkhel, Assistant Professor cum junior scientist, Dr. D. P. Singh, Assistant Professor cum junior scientist, Dr. S. Raghwan, Assistant Professor cum junior scientist of Department of Plant Pathology, B.A.U. Sabour. I thank them all for their valuable suggestions, comments and encouragement throughout the period of study.

I express my deepest sense of respect, gratitude and indebtedness to Dr. Anil, Assistant Professor cum junior scientist, Dr. T. N. Goswami, Assistant Professor cum junior scientist, Dr. Ramanuj Vishwakarma, Assistant Professor cum junior scientist, Dr. C. S. Prabhakar, Assistant Professor cum junior scientist of Department of Entomology and Dr. Nintu Mandal, Department of Soil Science and Agricultural Chemistry, B.A.U. Sabour.

I thank non-teaching faculty Mr. Mukesh Ram, Mr. Dileep, Mr. Tribhuvan, Mr. Anirudh Prasad Yadav, Mr. Ramvilas Yadav, Mr. Upendra Yadav, Mr. Sanjay Kumar and Mr. Ram Swaroop Mandal, Department of Plant Pathology, B.A.U. Sabour, for their kind co-operation during my study and research.

If friendship is a blessing, yes, I am really blessed with so many lovable friends. I remember the moments i spent with them with a smile and tears in my eyes. I thank Abhishek, Puja, Priya, Monika, Sujata, Annu, Bhola Nath, Kailash, Jay Prakash, Bipin, Prabhat, Amit, Mukesh, Shivbhawan, Radhe, Maneesh, Devbrat, Sanjay, Samrat, Koushik Goutam my beloved seniors Rishav sir, Ashutosh sir, Kundan sir, Saurav sir, Abhishek sir, Sumitap sir, Amarjeet sir, Ajeet sir, Vikash sir and my juniors Arun and Vinay for all those wonderful moments. I fondly thank them for their friendship, love, help, care, moral support and constant companionship.

My head and heart bow down before my beloved family always backed me with their love and support to whom I am ever grateful. I cannot express how grateful I am for them who kept me enthusiastic throughout my educational career, which enabled me to acquire the present gratification. A special word of thanks to Payal Vihariya for her moral and uncomplaining support with motivational attitude.

Finally, I would also like to thank all those who could not find a separate name but have loved me and always wished for my welfare. Above all, I thank Lord Almighty for giving me the wonderful life and helped to complete this thesis work at proper time.

June, 2017 **Ritesh Kumar** *(Sabour, Bhagalpur)*

Abstract of Thesis

Title of Thesis	**:**	"Analysis on Variability of *Sclerotium rolfsii* Causing Cucurbit Collar Rot"
Name of the student	**:**	Ritesh Kumar
Degree programme	**:**	M. Sc. (Ag.)
Department	**:**	Plant Pathology
Registration No.	**:**	M/PPLPATH/303/BAC/2015-16
Major subject	**:**	Plant Pathology
Minor subject	**:**	Entomology
Major advisor	**:**	A. P. Bhagat
Year	**:**	2017
Name of University	**:**	Bihar Agricultural University, Sabour, Bhagalpur

ABSTRACT

Cultivation of cucurbits is largely affected by several biotic factors. Among diseases, collar rot causes significant damage to cucurbits and ultimately responsible for yield loss. Despite being a dreaded pathogen having wide host range (infects over 270 families of plants); the collar rot dynamics in cucurbits is untouched. Therefore, this study envisaged with collecting the preliminary information regarding to this pathosystem. Collar rot of cucurbits is caused by *Sclerotium rolfsii* Sacc., a soil inhabitant and non-specific facultative parasite that found in almost all types of agro-ecological systems.

At first, the collection of isolates from various hosts and locations of Bihar was made. These isolates were addressed to morphological and biochemical variations. Great variability of isolates from different localities was observed. Additionally, the collected isolates showed variation in melanin production. An isolate, producing highest number of sclerotia (117/plate), was selected for the tests of fungicidal sensitivity and temperature adaptability. The fungicide belonging to sterol biosynthesis inhibition exhibited great potential to reduce the fungal growth. Therefore, new fungicide involving hexaconazole along with the common fungicide used for Sclerotium rot management, carbendazim, should be synthesized. The isolate adapted to high temperature (35°C) after 8th sub-

culturing indicating the adjustment capability of this pathogen towards elevated temperature. Therefore, the problem associated to this fungus may be increased in future, particularly under changing climate. Moreover, biochemical changes in the artificially inoculated plants were determined in different plant organs for the five isolates exhibiting high variability in the morphological observation. High content of total phenol and chitinase was detected at collar region; however, peroxidase estimation rendered non-significant ($p \geq 0.01$) variation amongst the plant organs. To address the varietal response and seasonal impact on collar rot epidemics the pot experiment has been conducted twice in a season (rainy and winter; 4 experiments in total) with cucumber and bitter gourd. Disease incidence (DI) calculated and area under lesion progress curve (AULPC) plotted in order to understand the collar rot dynamics in cucurbit. Additionally, measurement of lesion size was made. The varietal response has been found significant ($p \leq 0.05$) for the two crops. Overall, cucumber shown high susceptibility compared to bitter gourd in both of the seasons. The rainy season exhibited higher DI and AUDPC compared to winter season for both of the crops indicating high temperature is favourable to this pathosystem. Nearly similar trend was observed for lesion length for each condition.

Wide variation among isolates and adaptability of the pathogen to high temperature suggesting to develop improved strategy for management. Development of fungicide with sterol biosynthesis inhibitors can be a reliable option to manage this pathogen. Delay sowing of cucurbits in rainy season would account for lower disease development; therefore, development of late sown varieties is suggested. If agroecosystem, and facility and economical status of the farmer allow the cultivation of cucurbit in summer season should be addressed particularly in areas with sporadic and epidemic history of collar rot.

(A. P. Bhagat) **(Ritesh Kumar)**
Department of Plant Pathology

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

µg	: micro gram
°C	: degree centigrade
AULPC	: Area Under Lesion Progress Curve
BAU Sr	: Bihar Agricultural University *Sclerotium rolfsii*
BOD	: Biological Oxygen Demand
cm	: centi meter
DMRT	: Duncan Multiple Range Test
EC_{50}	: Effective Concentration
et al	: et alia (additional persons are acting in the same)
FCR	: Folin Ceocalteau Reagent
hai	: hours after inoculation
hrs	: hours
kD	: kilo Dalton
L	: litre
mm	: milli meter
N	: Normal
NAG	: N Acetyl Glucosamine
OD	: Optical Density
PDA	: Potato Dextrose Agar
PRP	: Pathogenesis related Protein
rpm	: Revolutions per minute

This segment introduce you about the research programme and the need for conducting this type of research

Chapter- 1

Introduction

INTRODUCTION

Cucurbits belong to family Cucurbitaceae, and include about 118 genera and 825 species. In India, a number of major and minor cucurbits are cultivated, which share a large portion of the total vegetable production (Rai *et al*., 2008). Cucurbit cultivation is adversely affected by a wide range of pests and diseases, including subterranean pests and foliage feeders, leaf spots, stem rot, collar rot, viral diseases, and nematodes (Rai *et al*., 2008).

Sclerotium rolfsii is the fungal pathogen causing collar rot disease having wide host range and causes severe economic loss worldwide (Dandnaik *et al.*, 2006). It causes disease over 270 host genera in the United States alone, and at least 100 families with 500 species of plants are susceptible to this pathogen. *S. rolfsii* produces isolates with great variability in India (Sarma *et al.*, 2002). The significant variation in cultural characteristics, mycelial morphology and pathogenicity amongst test isolates indicated that *S. rolfsii* can be characterized by a combination of cultural characteristics, morphology and virulence on host plants (Chattannavar and Hosagoudar, 2011). The variability among isolates observed, could be further attributed to physio-metabolic differences among isolates arising from different crop production systems, and also determination of biochemical variability will identify its adapting capability to their ecological and environmental conditions. Therefore, studies should be conducted to understand the variability involving cultural morphology, sclerotium characters and other parameters of the pathogen.

Adaptability of *S. rolfsii* to changing environmental conditions such as several chemicals or high temperature regimes is important to study. The chemicals used for control of collar rot disease are fungicides apply through seed priming. Moreover, a few fungicides are available commercially to manage this disease effectively at seedling stage. However, the potentiality of fungicides has been compromised in many occasions under field condition. Therefore, new fungicides with prolong activity should be identified to manage this disease. Apart

from fungicidal issue, the increase in temperature (as a result of climate change) alters the behaviour of both host plant and the pathogen is often recognized. The fungus *S. rolfsii* exhibits invariable change in mycelial growth at 25°C. However, there is a possibility for adaptation of this pathogen to increased temperature regime (35°C). This could be correlated with possibilities to increase in aggressiveness of this pathogen in the coming decades with higher mean global temperature. The impact of increased temperature associated with climate change on plant disease is hazardous to be through both the host plant and the pathogen (Coakley *et al*., 1999; Garrett *et al.*, 2006). This includes adjustments of plant architecture to create more suitable microclimates for pathogen colonization and disease development. An increase of 3°C in the mean annual temperature over the next century is expected (Griefenhagen and Noland, 2003).

Once the plants are diseased, it can cause huge losses in yield of crops, and sometimes, threaten to wipe out an entire species. Considering collar rot disease, the mortality of chickpea seedlings has been estimated as 55-95% (Mahen *et al.*, 1995). Yield loss caused by collar rot disease was found 48-80% in groundnut (Dandnaik *et al.*, 2006) and 10-11% in sunflower (Kolte and Tewari, 1977). Plant disease epidemiologists strive for an understanding of the cause and effects of disease, and develop strategies to intervene in situations where crop losses may occur. The accurate or precise estimation of disease incidence (DI) can be formidable task because of visual or measurement error. The methods of assessment measure the cumulative effect of all the factors operating during the course of epidemic and can be used to estimate different parameters. Among these parameters, area under lesion development curve (AULPC) has been widely used (Mukharjee *et al*., 2009).

No report is found on collar rot disease in vegetables, or particularly, in cucurbit. In addition, the morphological information is lacking for cucurbit collar rot isolates. Investigations on variability within the population in a geographical region carry paramount importance because such documentation would address the temporal changes occurring in the populations. Hence, a part of the present study is formulated to understand the variability in cultural morphology,

biochemical variation such as melanin in the fungus. Additionally, the antimicrobial compounds secreted in the infected region of the plant like chitinase, phenols and peroxidase are also included in this work considering a few isolates collected from different hosts and geographic locations of Bihar. Fungicidal application is used when source of resistance is not identified in a pathosystem. Till date, no work considering fungicides has been conducted involving the isolates of cucurbit origin. This pathogen (*S. rolfsii*) survives as sclerotia in soil in absence of host for more than 10 years and as saprophytic mycelium in crop residues (Nguyen *et al*., 2004). Temperature sensitivity of *S. rolfsii* may also address its survival mechanism in soil and residues. Additionally, the activity of germinating mycelium may be governed by the associating temperature. The increasing trend in temperature due to climate change would determine the severity of this pathogen in future. No work has been done on collar rot disease development in cucurbit, which includes seasonal variation and disease dynamics in different varieties.

Considering all above research gaps this work was designed to identify the morphological variability of this pathogen in Bihar from various hosts. Moreover, sensitivity of cucurbit isolate of *S. rolfsii* to fungicides and temperature was studied. In this connection, the collar rot disease development in different varieties was also investigated. Despite of its economic importance the collar rot pathosystem of cucurbits has not been studied in details. In view of the seriousness of the disease and enormous losses in the yield, the present investigation was conducted with the following objectives:

1) Evaluation of morphological and biochemical variability in isolates of *Sclerotium rolfsii*.
2) Variation in *in-vitro* sensitivity of *Sclerotium rolfsii* to different fungicides.
3) Assessment of cucurbit's varietal response to collar rot epidemic.

Works associate with the present study are hereby reviewed and enlisted in this chapter

Chapter-2

Review of Literature

REVIEW OF LITERATURE

Sclerotium rolfsii Sacc. causing collar rot is a well-known non-target pathogen having wide host range. It is one of the most destructive soil inhabiting pathogens and causing heavy loss in cucurbits, both in rainy as well as winter seasons.

2.1 The pathogen :

Sclerotium rolfsii Sacc. is a well-known and most destructive soil borne fungus. This was first reported by Rolfs in 1892 as a cause of tomato blight in Florida. Later, Saccardo (1911) named the fungus as *Sclerotium rolfsii*. Higgins (1927) worked in detail on the physiology and parasitism of *S. rolfsii*. However, its perfect state was first studied by Curzi (1931) and proposed generic name as *Corticium*. Mundkur (1934) successfully isolated the perfect state of *S. rolfsii*. McClintock (1917) and Butler and Bisby (1931) reported collar rot disease caused by this fungus for the first time from USA and India, respectively. In India, Shaw and Ajrekar (1915) isolated an organism from rotted potatoes and identified as *Rhizoctonia destruens* Tassi. But, later studies revealed that, the fungus involved was *S. rolfsii* (Ramakrishnan, 1930). The fungus infects over 500 plant species, mostly comprised of dicotyledonous and few monocotyledonous plants. It induces symptoms like crown and root rot, collar rot, foot rot, stem rot, stem canker, damping off, southern wilt or blight or southern stem rot (Punja, 1985).

The fungus was placed in genus *Sclerotium* by Saccardo (1913), as it forms differentiated sclerotia and sterile mycelia. Although there are several other sclerotium producing fungi, the fungus is characterized by small tan to dark-brown or black spherical sclerotia with internally differentiated rind, cortex, and medulla, were placed in genus *Sclerotium* (Punja and Rahe, 1992). However, the teleomorphic state was discovered later by Punja (1988) confirming that the fungus is a basidiomycete. The wide host range, prolific growth and ability to produce resting structure i.e. sclerotia contributed to large economic losses by the pathogen. *S. rolfsii* can overwinter as mycelium in infected tissues or plant debris

in the form of sclerotia. Sclerotia of relative uniform size are produced on the mycelium. Mature sclerotia resemble mustard seed. Sclerotia are the means by which a quiescent viable state is maintained by many fungi in the absence of a suitable host or of condition favouring active growth. The sclerotia can survive in the soil for at least three years or more under suitable conditions of temperature and moisture (Cosic *et al*., 2012). These are disseminated by cultural practices (infested soil and contaminated tools), infested transplant seedlings, water (especially through irrigation), wind, and sometimes through seed admixture. In addition, a small percentage of sclerotia may survive in the intestinal passage of sheep and cattle, and thus, could be spread through fertilizers. The fungus occasionally produces basidiospores.

2.2 Distribution and economic importance :

Collar rot disease is wide spread and cause serious losses in Bolivia, China, Egypt, India, Taiwan, Thailand and USA (Bowen *et al.*, 1992). In India, stem rot occurs in all the cucurbits growing states. The stem rot disease occurs regularly in areas wherever cucurbits are grown including states Maharashtra, Gujarat, Madhya Pradesh, Karnataka, Andhra Pradesh, Orissa and Tamil Nadu.

Sclerotium rolfsii is predominantly distributed in tropical and subtropical countries. It is common where high temperature exist during the rainy season. Weber (1931) and Garret (1956) reported that the fungus survived in the soil for many years by producing sclerotial bodies and causing the disease either in the form of stem rot or root rot in addition to leaf blight on several of its hosts. It is estimated that over 5,00,000 ha of fields were infected by this pathogen and yield losses of over 25% have been recorded in several crops (Mayee and Datar, 1988). Reddy *et al.* (1971) carried out survey during 1969-70 in Karnataka and found that, the losses due to collar rot in wheat crop were about 5%. While, Siddaramaiah *et al.* (1979) conducted survey during 1975-76 and 1976-77 in groundnut growing areas of Dharwad district and reported an average of 7.80% infection due to *S. rolfsii* in different varieties resulting in complete mortality. In a survey on foot rot of wheat during *rabi* 1978-79 and *rabi* 1979-80 in Malaprabha,

the maximum disease incidence of 10.20% and 5.20% in rainfed and irrigated fields, respectively (Nargund, 1981). Palalshappa (1986) reported maximum disease incidence of 30% in betel vine in Chikkodi taluka of Belgum district. Harlapur (1988) observed incidence about 9.85% and 4.66% under rainfed and irrigated conditions, respectively of foot rot of wheat caused by *Sclerotium rolfsii*. Sharma and Pathak (1994) conducted a field experiment at Sriganganagar in India during 1982-84 to investigate yield and sucrose losses in the sugarbeet caused by *Sclerotium rolfsii*. They recorded the reduction of yield and sucrose by 46.50% and 62.20%, respectively. Mehan *et al.* (1995) reported that the disease caused by *S. rolfsii* affected groundnut in many countries. The reduction in yield by 10-25% and pod loss of more than 50% in heavily infected fields. Hanumanthegowda (1999) carried out survey on stem rot of groundnut during *kharif* 1998-99 and rabi/summer, 1998-99 in Dharwad, Belgaum and Havier districts. He reported a maximum disease incidence of 12.57% and 8.68% in rained and irrigated fields, respectively. Southern blight of soybean was first reported in Nigeria with yield reduction of about 59% under severe conditions (Aken and Dashiell, 1991). Ansari (2002) reported up to 65% loss under severe epiphytotic conditions in Indore.

2.3 Symptoms :

Symptoms like pre-emergence as well post-emergence death of seedlings was reported by Agrawal and Kotashane (1971). The disease generally develops on isolated plants scattered throughout the field wherever the inoculum is present. The spread to adjacent row can be rapid under favourable conditions. A sudden yellowing, browning and wilting of the entire plant are the first symptoms. Leaves of infected plants turn brown, dry and often cling to the dead stem. The most characteristics sign of the disease is white, fan like mat of fungal mycelium that forms on stem bases, leaf debris and the soil surface around the infected plants. The mycelial mat may extend several centimeters up to the stem above the soil line. Numerous tan to brown, spherical sclerotia of about mustard seed size formed on infected plant material which was found on the soil surface.

The symptoms of stem rot involve mycelium covering the plant stem near the soil surface and produced organic acids, which were toxic to living plant tissue (Wilson, 1953). This followed the necrosis of plant cells. The mycelium invaded the stem, gynophores and also pods and caused rotting of the tissues. The production of abundant white mycelium, and small brown spherical sclerotia on the infected parts were characteristic symptoms of the disease. Beattie (1954) also observed same symptoms on infected plants. Wheeler (1969) reported the symptoms as young plant killed rapidly, but older plant turned yellow and wilted subsequently. The white mycelium often appeared on stem base of drying plants. Siddaramaiah *et al.* (1979) observed foot rot symptoms accompanied by girdling of younger plants and later such plants were succumbed to death. The infection on older plants was also observed on roots as well as pegs at harvest. The rotted pegs broke off and left the pods in the soil, there by resulting in considerable yield losses. The infected seedlings became stunted with chlorotic leaves and ultimately withered and died. Sclerotia developed on the surface of soil and infected stem (Baruah *et al.,* 1980). Nyvall (1989) also described the symptoms as wilting of the plants due to infection of stem at base level. Further, soil level near the infected stem was covered with white mycelium. The wilted plants remained upright. The infected areas of stem shredded and covered with numerous sclerotial bodies. Stem base was covered with elongated and overcrowded brown lesions. These formed reddish colour. During dry whether brown lenticular lesions occurred on stem just below the soil surface. The peg infection caused light to dark brown lesions (0.05–2.00 cm long), which resulted in tissue shredding and pod loss. Lesions on young pods of Spanish peanuts were orange yellowish to light tan in colour. Severely decayed kernels were shriveled and covered with mycelium.

The cortical decay of stem base at ground level and appearance of conspicuous white mycelium which extended into the soil and on organic debris was noticed by Mehrotra and Aneja (1990). The fungus primarily invades host stems, although it may infect any part of a plant under favourable environmental conditions including roots, fruits, petioles, leaves, and flowers. Seedlings are very susceptible and die quickly once they become infected. Invaded tissues are pale

brown and soft, but not watery. The first sign of infection, usually undetectable, are dark-brown lesion on the stem at or just beneath the soil level. The first visible symptoms are progressive yellowing and wilting of the leaves. Following this, the fungus produces abundant white, fluffy mycelium on infected tissues and the soil. The typical symptoms of the disease are rapid wilting and sickly appearance of plants with brownish lesion at the stem base near the soil line which later girdles the stem. White mycelial growth forms over the infected tissue and often radiates over the soil surface. The fungus does not produce asexual spores and perpetuates as sclerotia on plant debris and in soil (Cilliers *et al*., 2000). If the plants lodge and heads touch the soil, seed infection is possible; otherwise seed transmission is negligible (Lakshmidevi *et al*., 1991).

2.4 Pathogenicity :

Differences in morphology and pathogenicity in the isolates of *S. rolfsii* was reported by Edson and Shapvalov (1923) from North Carolina and Arkansas. One of those two isolates was more virulent in potato and seed decay. Sengupta and Das (1970) conducted the cross inoculation studied of isolates of *S. rolfsii* from groundnut, wheat, potato, guava and Bengal gram. They reported that, Bengal gram was the most susceptible host of *S. rolfsii*. But, all the isolates were most virulent to their appropriate hosts. The specialization was not demonstrated conclusively. Datar and Bindu (1974) proved the pathogenicity of *S. rolfsii* on sunflower by soil inoculation method under glass house conditions. The inoculum was prepared by growing the fungus on sterilized maize bran medium and mixed with the sterilized soil one week before sowing. Typical symptoms were produced within a week of germination which was identical to those produced in the field. Mishra and Bais (1987) used 15 days old fungal culture grown on sand corn meal medium for proving pathogenicity of root rot of barley caused by *S. rolfsii* by mixing upper 4-5 cm layer of soil with inoculum at the rate of one flask per pot. Siddaramaiah (1988) confirmed the pathogenicity of *S. rolfsii* on *Desmodium uncimatum* Desv. And *Cotonorisa inesii* Eckl and Zeyh, two important forage legumes of hill zone by similar procedure. Siddaramaiah and Chandrappa (1988)

proved the pathogenicity of *S. rolfsii* on cardamom in pot culture studies by inoculating 25 days old sclerotial cultures which was grown on sand corn meal medium and observed the symptoms a week after inoculation.

2.5 Morphological and Cultural variation in *Sclerotium rolfsii* :

Variation is a rule in most of the root infecting fungi. The variation may arise following change in crop cultivation, genetic modification of hosts, physical or chemical modification of the soil, environment or accidental introduction of new genetic material into a region or local gene pool. It may also be a way of survival of the pathogen under adverse conditions. Plant pathogens exhibit variation in their morphological, biological and pathogenic characters. Morphologic and pathogenic variations are known in many fungal pathogens (Kumar *et al.,* 1955). The extreme variation in morphological characteristics of *S. rolfsii* has been observed in worldwide collection of this pathogen from different hosts and also from the same hosts (Cooper, 1961; Kim, 1974 and Punja, 1985).

Typical characters of *Sclerotium rolfsii* Sacc was described by Subramanian (1971). The fungal mycelium was very floccose, not ropy, producing numerous sclerotia. Sclerotia were pinkish buff to olive brown to clove brown in colour, globuse shape, 82.5 mm in diameter. *S. rolfsii* formed hymenium which was aerolate, putty colored, 30-40 µ thick. Basidia were ovoid, 79 × 4-5 µ, each bearing 2 or 4 parallel or divergent sterigmata and 2.5 × 4-6 µ long. Basidiospores were elliptical to obviate, hyaline, smooth, rounded or apiculate at base measuring 6-7 × 3.5-5 µ. Kim (1974) grouped the isolates of *S. rolfsii* on the basis of difference in morphology, mode of growth in culture and pathogenicity. The mycelium of *S. rolfsii* was very floccose, snow white, thick, cottony and grown rapidly all over the blotting paper during blotter tests while, sclerotia were the resting infective propagules of the fungus and can be seen on the mycelial strands. They were globose to ellipsoid, pinkish, buff to olive brown to clove brown. Young sclerotia appeared white, producing characteristics exudes droplets around them, which slowly become dark as aged and resemble to mustard seeds, 1-2 mm

in diameter when mature. Manjappa (1979) found variation among the isolates of *S. rolfsii* from different crop (sunflower, groundnut, wheat, red gram, tomato, lucerne and tamarind). All the eight isolates showed marked differences in the rate of growth on both solid and liquid media and time taken for sclerotial initiation. The isolates also differed with respect to size, number and weight of sclerotia and the virulence of pathogen. Radwan *et al.* (1987) have also observed variation in mycelial colour and sclerotial production in five isolates collected from different fields. While, Mishra *et al.* (1996) observed morphological differences among *Parthenium* isolates of *S. rolfsii* on the basis of sclerotial size, color and colony type. Prithviraj *et al.* (1996) found two types of sclerotia, *viz.*, 'small' and 'large'. There was significant difference in the surface morphology, both consisting of thick skin, rind, cortex and medulla. The difference in size of sclerotia appeared due to difference in the volume of medullary region. Punja and Damiani (1996) observed differences in morphology (colony characteristics and sclerotial formation) and size of sclerotia in the three different species of *Sclerotium viz.*, *Sclerotium coffeicola* Stahel., *S. rolfsii* and *S. delphinii* from diverse geographical areas, when compared for growth response to different temperature and media. Hernandez and Ysla (1997) evaluated eight isolates of *S. rolfsii* for cultural and morphological characteristics and found variability in their mycelial density, number and diameter of sclerotia, mycelial density, presence of rhizomorphs and duration of sclerotial formation.

Chen *et al.* (1998) classified 50 isolates of *S. rolfsii* on the basis of sclerotial formation, their types, structure fineness, mycelial compatibility grouping and divided them into various groups on the basis of colony morphology. Out of 50 isolates, 16 were smooth type, 7 were rough type and 27 isolates were middle type. They further stated that mature sclerotia are composed of an outer melanized rind of thick 2 to 4 cell layer, an underlying cortical layer and an innermost medullary region comprising loosely inter-woven hyphae. Ansari and Agnihotri (2012) recorded marked variation among 44 isolates of *S. rolfsii* obtained from different fields and location and grouped them into six groups on the basis of differences in sclerotial size.

Almeida *et al.* (2001) reported considerable variability among *S. rolfsii* isolates from Brazil in terms of number, size and location of sclerotia on the medium surface. While, Sarma *et al.* (2002) recorded remarkable variation in colony colour, diameter, sclerotia production (size and colour) among 26 isolates of *S. rolfsii* obtained from different host and geographical regions. Shukla and Pandey (2007) reported diversity in colony morphology, mycelial growth rate, sclerotial formation, size and colour of sclerotia of 32 isolates of *S. rolfsii* isolated from different hosts, soil samples and locations of Central India. While, Adondonen (2000) reported variation in growth rate, sclerotial number and time required for first appearance of sclerotia in *S. rolfsii* isolates collected from different villages in the Oueme valley.

Variability among 26 isolates of *S. rolfsii* were studied for the variation in colony morphology, mycelial growth rate, sclerotia formation, teleomorph production and its size by Sarma *et al*. (2002) in twenty-six isolates of *S. rolfsii* Radial growth, colony morphology, and sclerotial production were evaluated on PDA, whereas basidiocarp induction was observed on *Cyperusrotundus* rhizome meal agar (CRMA) medium. The isolates of *S. rolfsii* varied in all of the characters evaluated, e.g., colony morphology, mycelial growth rate, sclerotial production, basidiocarp induction, sclerotial size, and color. Out of 26 isolates, colonies of 18 isolates were fluffy, whereas 8 were found to be compact. The growth rate of the isolates varied substantially. Nine isolates were fastest growing (31 mm/d), while the isolate from soybean was the slowest (23 mm/d). Others varied from 25 to 30 mm/d. Production of sclerotia among isolates varied significantly. Most of the isolates produced a very large number of sclerotia (300 to 500 sclerotia/ plate), while others produced fewer (80 to 200 sclerotia/ plate).

Variability among 12 isolates of *Sclerotium rolfsii* was collected from various localities of chickpea growing areas of Punjab province was studied by Akram *et al*. in 2008. The isolates of *S. rolfsii* varied in all of the test parameters e.g., colony morphology, mycelial growth rate, colony color, sclerotial production, number and sclerotial size of sclerotia. Out of 12 isolates, colonies of 7 isolates

were fluffy, whereas 5 were compact. The growth rate of the isolates varied substantially, from fast growing (76.7-90 mm diam.) to slow growing (16.0–30.6mm diam.). Others were medium in growth and varied from 40.8 to 61.7 mm diam. Production of sclerotia among isolates varied significantly. Most of the isolates produced a very large number of sclerotia (>300 sclerotia/plate), while some isolates produced fewer (<300 sclerotia/plate). Similarly, the size of sclerotia varied in different isolates. The average size of sclerotia for most of the isolates were >40 µm in diam, whereas some isolates produced small sclerotia of <40 µm in diameter.

Ansari *et al.* (2012) isolated 44 isolates of *S. rolfsii*, the causal organism of collar rot of soybean obtained from different locations in India and categorized into various groups based on morphological characters of sclerotia and its arrangement medium. Observations for various cultural and morphological characteristics of sclerotia i.e. sclerotial color, size and their arrangements were recorded 15 days after incubation. On the basis of sclerotial arrangement, size and color after 15 days of inoculation, they were classified into different groups.

Variation in isolates of *S. rolfsii* has been studied in several locations from southern India. Isolates of *S. rolfsii* were studied by Prasad *et al*. (2012) for morphological and cultural characters in collar rot of sunflower. Twenty-two isolates of *S. rolfsii* causing collar rot were collected from different sunflower growing areas of Andhra Pradesh, Karnataka, Tamilnadu and Maharashtra during 2006-07 and 2007-08 were collected and variations were observed in colony morphology, mycelial growth, sclerotium formation, sclerotial size,colour and number of sclerotia. Some isolates produced more number of sclerotia (>200/plate), while majority of the isolates produced fewer sclerotia (<200 /plate).

Eight isolates of *S. rolfsii* were collected during *Kharif-Rabi*, seasons of 2011-12, maintained and studies their cultural and morphological variations by Kuldhar *et al.* (2014). The radial mycelial growth of the test isolates was ranged from 72.00 mm to 90.00 mm. Colony color of the test isolate was mostly light

white or extra white or buff white. The amount of sclerotia produced by the test isolates varied from fair to excellent and accordingly grouped. The number of sclerotia produced per plate by the test isolates were ranged from 110 to 430. Average size of the sclerotia produced by the test isolates was ranged from 0.8 to 1.8 mm.

Morphological and cultural variability of eight isolates of *S. rolfsii* were studied based on their growth rate, colony color, mycelial dispersion and appearance and sclerotium formation, color, weight and number of sclerotia, arrangement and maturity days of sclerotia by Reddikumar *et al*. (2014). Significant variability with reference to mycelial and sclerotial characters across isolates of *S. rolfsii*, isolated from different locations of Southern zone of Andhra Pradesh was observed. The two isolates were considered to be very fast growing, isolated from RARS farm, Tirupati which covered entire Petriplate (90 mm) within 96 hrs of incubation. Two were considered to be fast growing isolated from western parts of Chittoor district i.e. Kalakadamandal. The remaining 3 isolates were considered to be moderately growing (48-54 mm), which were isolated from Ramatheertham area of Nellore district. On the other hand, the isolate from Kadapa area were recorded to be slow growing (28 mm).

2.6 Biochemical estimation :

Fogarty and Togian (1995) isolated melanin from *Cladosporium* spp. and concluded that melanized fungi possess increased virulence and resistance to microbial attack as well as enhanced survival while under environmental stress. Melanins contain various functional groups which provide an array of multiple nonequivalent binding sites for metal ions.

Suryanarayan *et al*. (2004) first reported melanin in *Phyllosticta* or other foliar endophytes. Melanin in the hyphae of *P. capitalensis* was considered responsible for the success of this fungus as a cosmopolitan endophyte, since melanin is known to enhance the survival capability of fungi in stressful environments.

Analysis of mycelia of *S. rolfsii* grown in broth medium supplemented with garlic (*Allium sativum*) and onion (*Allium cepa*) was carried out to estimate qualitative and quantitative changes in phenolic acids by Pandey *et al.* (2005). Several phenolic acids were detected in varied amounts in mycelial grown. Mycelial mat obtained from potato dextrose broth revealed the presence of 0.14 µg/g chlorogenic acid, 1.01 µg/g ferulic acid and 0.05 µg/g coumaric acid after 10 days of inoculation while 2.23 µg/g gallic acid, 3.20 µg/g ferulic acid, 1.24 µg/g coumaric acid and 0.13 µg/g cinnamic acids after 20 days of inoculation was recorded.

According to Lattanzio *et al.* (2006) plant phenolics are secondary metabolites that encompass several classes structurally diverse of natural products biogenetically arising from the shikimate phenylpropanoids flavonoids pathways. Plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution.

Maurya *et al.* (2007) identified individual phenolic acids of *Sorghum vulgare* after interaction with *Sclerotium rolfsii* and showed the presence of phenolics namely tannic, gallic, ferulic, chlorogenic and cinnamic acids in varying amounts. After 72 h inoculation with *S. rolfsii*, a maximum amount of ferulic acid (166.6 mg/g fresh wt) was present in the collar of inoculated plants, followed by leaves and roots and its level decreased gradually with time. Similarly, the presence of chlorogenic acid was traced after 48 h, while that of cinnamic acid was traced after 72 h of inoculation. Induction of phenolic acids in *S. vulgare* along with the lignin deposition and red pigmentation at collar region is considered a key biomarker in the non-host pathogen interaction in the *S. vulgare*–S. *rolfsii* pathosystem.

Total phenolics were determined by Petkovsek *et al*. (2008) with the Folin-Ciocalteau method from leaf tissues of apple cvs Jonagold and Golden Delicious, healthy and infected by *Venturia inaequalis*. Infection by *V. inequalis* caused an accumulation of phenolic compounds in infected leaves with a 1.4 to 6.2 fold increase of flavonols, a 2 to 6 fold increase of chlorogenic acid and a 1.4 to 2.4 fold increase of the Folin-Ciocalteu values. Rajagopal *et al*. (2011) extracted dark

pigment from *Phomopsis*, a phellophyte of *A. indica* answered positively for melanin. The dark brown pigment from the mycelium could not be extracted with organic solvents such as acetone, chloroform, and ethanol. However, pigment extracted from *Phomopsis* using the alkali procedure of Gadd (1982) responded positively to all the physical and chemical tests.

Total phenols and isozyme were studies by Shilpashree *et al*. (2012) in resistant and susceptible genotypes of cowpea. The study showed that at 60 days after sowing, the diseased leaf showed increased total phenols (0.57 mg/g fresh wt) compared to healthy leaf (0.29 mg/g fresh wt.) in case of susceptible genotype, whereas the total free phenols were more in diseased (0.52 mg/g fresh wt) when compared to healthy (0.40 mg/g fresh wt.) in case of resistant genotype.

The induction of different stress related enzymes like phenyl alanineammonialyase (PAL), chitinase, β-1,3 glucanase, oxidative enzymes like peroxidases (POD), poly phenol oxidases (PPO) and phenolics were examined by Nandi *et al*. (2013) after inoculation of *Sclerotium rolfsii* in collar region of 30 days old cowpea plant. Scanning electron microscopy strengthened the presence of mycelial network in xylem vessel of infected collar region of cowpea at three days after inoculation. Cowpea plants inoculated with *S. rolfsii* isolate showed significantly increased POD, PPO, PAL, chitinase and β-1, 3-glucanase activities at different days after inoculation. In that study they conclude that there was a greater accumulation of total phenol in cowpea plants observed up to five days after inoculation. The highest activity of POD, SOD was found in three days after inoculation and PPO activity was greater in five days after inoculation and thereafter, the activities of such enzymes steadily decreased.

A study was designed by Surekha *et al*., (2014) to understand the role of *Trichoderma viride* in inducing defense enzymes and total phenolic content in black gram exposed to pathogens *Fusarium oxysporum* and *Alternaria alternata.* It was found that the biocontrol agent, *T. viride* induced higher levels of defense enzymes in black gram during pathogenesis by *F. oxysporum* and *A. alteranata*. Therefore, it was concluded that plant defense enzymes play a vital role in mitigating pathogen-induced stress. Zhang *et al*. (2017) stated that *Aureobasidium*

pullulans produced melanin when grown in medium containing low nitrate levels. With high nitrate concentrations, however, the fungus produced a mixture of exopolysaccharides (EPS) without melanin synthesis. At 0.78 g/L N as nitrate, where no melanin synthesis occurred, maximum EPS yields reached 6.92 g/L and then decreased to the final yield of 2.36 g/L. Following melanin addition (0.1 g/L), yields reached 7.02 g/L at 48 h and fell to a final yield of 5.21 g/L.

2.7 *In-vitro* sensitivity of *Sclerotium rolfsii* to different fungicides :

Shalaby (1997) studied the effect of benlate, vitavax, Rhizolex T and Tecto TBZ (thiabendazole) on disease severity and as seed treatments on soil fungi causing sesame root rot and concluded that benlate and vitavax were the most effective seed dressings against the *Macrophomina phaseolina* under both laboratory and green house conditions.

Rao *et al.* (1998) reported that Seed treatments consisting of carbendazim WP, carbendazim SD + captan, carbendazim + thiram, captan, thiram, mancozeb, tolclofos-methyl, pyroquilon, TCMTB, carbedazim-Jkstein, quintozene, captafol, carboxin and triadimenol were tested for the management of these fungi under artificial infection of the seed. The seed treatments were found to control the damage due to these fungi. Among the treatments, carbendazim SD (0.05% + captan 0.125%) was the best in reducing seed rot and pre and post-emergence seedling blight and in increasing pod yields significantly in all the three seasons of field experimentation, followed by treatment with captafol (0.25%).

Choudhary *et al.* (2004) observed the effectiveness of four fungicides, *i.e.* Bavistin, Antracol, Indofil M-45 and Ridomil MZ applied at 300, 400, 500 and 1000 ppm, in inhibiting the mycelial growth of *Macrophomina phaseolina*, the causal agent of stem and root rot of sesame, was studied *in vitro* using the poisoned food technique. All fungicides dose-dependently inhibited mycelial growth compared with the untreated control, with Bavistin being the most effective.

Akram *et al*., (2008) studied the sensitivity of *S. rolfsii* to nine fungicides viz., Antracol, Benlate, Captan, Cobox, Dithane M-45, Pentachloro nitro benzene (PCNB), Ridomil, Sancozeb and Trimiltox forte at 10 ppm each using Poisoned Food Technique. Variability among the isolates of *S. rolfsii* was also determined on the basis of their sensitivity to different fungicides. It was observed that there was a significant variability in this regard. All the isolates were sensitive to benlate. It was concluded that Benlate was found to be the most effective in suppressing the growth of the pathogen followed by Ridomil and Sancozeb with respect to efficacy. Captan and PCNB were least effective where all the isolates showed non-sensitivity. Trimiltox forte, Antracol, Dithane M-45 and Copper oxychloride exhibited intermediate response in efficacy.

Khan *et al.* (2008) reported that carbendazim and captan were highly inhibitory in culture of *M. Phaseolina. P. fluorescence* strain PFBC-25 was comparatively less sensitive to carboxin, chlorothalonil and carbendazim. Captan and carboxin were relatively more toxic to strain PFBC-26 .The mycelia growth of *M. phaseolina*was significantly reduced by both the strains of *P. fluorescence*. Ammajamma and Hegde (2009) reported that Carboxin (0.05 and 0.1) and Hexaconazole, Metalaxyl and Triadimefon at 0.1% completely (100%) inhibited the growth of *R. bataticola* and among the non-systemic fungicides tested, Thiram at 0.1, 0.2 and 0.3% concentrations was found effective against *Rhizoctonia bataticola*.

The effects of three fungicides (systemic and nonsystemic) at different concentrations (25, 50 and 100 ppm) of active ingredient were evaluated *in-vitro* against *Sclerotium rolfsii* using poisoned food technique by Gour and Sharma (2010). All the fungicides inhibited the growth of fungus at all the test concentrations of 25, 50 and 100 ppm as compared to control. The per cent growth inhibition was correlated with the increase in concentration of all chemicals tested. Folicur 250 EW was found highly efficacious as it inhibited the growth up to 62.7% even at 50 ppm concentration followed by Tilt 25 EC. Among all these three, contaf 5 EC showed poor inhibition of the fungal growth. Evidently, folicur

250 EW was found to be the most effective against *S. rolfsii* at both the concentrations of 50 and 100 ppm.

Jaiman and Jain (2010) reported that efficacy of fungicides *viz.*, bavistin, raxil, topsin M, captan, indofil M-45 and thiram were tested against *M. phaseolina* causing root rot in cluster bean both *in vitro* and *in vivo*. Maximum inhibition of fungal growth was found with bavistin followed by topsin M. the fungicides checked the disease as compared to control. Tandel *et al*. (2010) observed that for the control of the disease, seven fungicides were tested. Among them Carbendazim + mancozeb (Sixer) was found significantly superior over the rest as it resulted minimum (8.13%) disease intensity. This suggested that leaf blight of mungbean (*Macrophomina phaseolina*) can be controlled very effectively by spraying of Sixer and the huge crop loss can be saved if sprayed at the time of disease initiation. Moradia (2011) reported that poisoned food technique was employed to study the efficacy of different nine systemic fungicides at 250, 500 and 1000 ppm against *M. phaseolina* (groundnut isolate) under *in vitro* conditions. All the fungicides were capable of inhibiting the growth of the fungus at all the concentrations tried. Difenconazole, Carboxin and Saaf were found to be the best, which caused cent per cent inhibition of growth at all the concentrations tried. Yaqub *et al*., (2011) used six fungicides viz., Benomyl, Sancozeb, Thiovit, Dithane M-45, Carbandazim and Topsin-M against *Sclerotium rolfsii* by food poison method at various concentrations of 0.1, 1, 10, 100, 1000 and 10,000 ppm. All the fungicides used during the study showed a gradual decline in growth of *S. rolfsii* with increase in concentration in the medium. Sancozeb was found to be the most effective and gave 60% reduction in growth of *S. rolfsii* when used at 100ppm. Dithane M-45 was the next most effective however its efficacy was not significantly different from that of Sancozeb. It gave >50% reduction in 100 ppm treatment. Benomyl and Carbendazim produced >50% reduction in the growth when used at 1000ppm. Similarly, Thiovit and Topsin-M resulted in >50% reduction in the growth of the pathogen when used at 10,000 ppm. It was concluded that at low concentration, no

fungicide inhibited the growth of *S. rolfsii*, however, at high concentration Dithane M-45 and Sancozeb significantly reduced the growth.

Two chemical fungicides with trade name Triton and Benedict contains active ingredients validamycin and iprobenfos, respectively were evaluated for their ability to control the canola spot disease pathogen, *Alternaria* sp. by Aftab *et al.* (2012). Fungus was grown on growth media incorporated with fungicides by three different methods viz. well diffusion, disc diffusion and food poisoning. Maximum inhibition in fungus growth was recorded by food poisoning method using either of fungicide. In this method, hyphal growth inhibition on the growth medium incorporated with fungicide was determined. All treatments were incubated for 5 days at 25°C±2. Radial fungal growth was measured and inhibition was calculated using the same formula as described for the food poisoning method. The fungus growth was significantly inhibited by 71.29% with maximum zone of inhibition due to Benedict using food poisoning technique as compared to control. Benedict was found to be more effective against *Alternaria* sp. as compared to Triton. Food Poisoning Method was found to be effective technique to assess antifungal activity of chemical fungicides as compared to well diffusion and disc diffusion methods.

Nine fungicides, viz., Bavistin, Brassicol, Captan, Dithane M-45, DM-145, Fytolan, Manzate, Parasan and Sulfex each with ten concentrations i.e., 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% were tested against *Sclerotium rolfsii* in vitro by Chaurasia *et al*. (2014). From the results, it is clear that the radial growth of the pathogen is adversely affected by the fungicides. The effective fungicides, probably may act as antifungal agent and impacts its poisoning effect on metabolic process of pathogen, therefore, the growth of the pathogen might be adversely affected. Amongst the tested fungicides, Brassicol proved to be the most effective against *S. rolfsii* in which 100% inhibition in radial growth was recorded even in a very low concentration, i.e., 0.025%. Next to Brassicol, Manzate has been found to be significantly effective and 100% inhibition in radial growth was recorded above 0.05% i.e., at 0.1 per cent concentration. After Manzate, the Parasan has been found to be the next effective fungicide in which 100% inhibition

in radial growth was recorded at 0.25 per cent concentration. The lower concentration i.e., 0.025 per cent of this fungicide was so significantly found to effective and 72.56% inhibition in radial growth was recorded over control. DM-145 was also found to be toxic like Parasan at 0.25 per cent concentration but at lower concentration i.e., 0.025% of this fungicide have a less toxic effect in comparision to Parasan. The 0.5% concentration of Dithane M-45 and 2.0% concentration of Captan were also found to be most toxic, where 100% inhibition in radial growth was recorded. It was found that Sulfex, Fytolan and Bavistin have poor inhibitory effect on the radial growth of pathogen as compared to other tested fungicides. This may be due to fact that the test pathogen might have developed some resistance towards these fungicides.

In vitro efficacy of fungicides against *S. rolfsii* was evaluated by Reddi Kumar *et al*. (2014) using Poisoned Food Technique. The fungicides used were mixtures of three fungicides (Tricyclazole + Mancozeb, Carbendazim + Mancozeb, Hexaconazole + Zineb), three systemic fungicides (Azoxystrobin, Difenconazole, Hexaconazole) and one nonsystemic fungicide (Blitox). 50ml of double strength PDA was mixed with 50 ml of double concentrated fungicidal solution to obtain final concentrations of 250, 500, 1000 and 1500 ppm. From the results, it is evident that among the three fungicide mixtures, three systemic fungicides and one contact fungicide, Carbendazim + Mancozeb and Hexaconazole + Zineb were found highly effective at low dose i.e. 250 ppm. However, Tricyclazole + Mancozeb and Hexaconazole were effective at 15000 ppm. While the two systemic fungicides, Azoxystrobin and Difenconazole and the contact fungicide, Copper oxychloride were found ineffective even at 1500 ppm.

2.8 Adaptability of pathogen to higher temperature regimes :

Climate change in terms of enhancing global temperature will change the pattern of disease through changes in host distribution and phenology, changes in plant associated microflora and direct biological effects on rapidly adapting pathogens. Atmospheric CO_2, a major greenhouse gas, has increased by nearly 30%and temperature has risen by 0.3 to 0.6°C (Chakraborty *et al*., 1998).

Evaluation of the limited literature in this area suggests that the most likely impact of climate change will be felt in three areas: in losses from plant diseases, in the efficacy of disease management strategies and in the geographical distribution of plant diseases. Change in temperature could have positive, negative or no impact on individual plant diseases.

According to Chakraborty *et al*. (2000) changes in temperature regimes due to climate change may alter the growth stage, development rate and pathogenicity of infectious agents, and the physiology and resistance of the host plant. Increase in temperature has been also reported to cause a shift in the geographical distribution of host pathogens (Mboup *et al*., 2012). Models suggested by Boland *et al*. (2004) predict that expected climate change will significantly affect the occurrence ofplant diseases in agriculture and forestry in the coming years. Direct, multiple effects on the epidemiology of plant diseases are expected, including the survival of primary inoculum, the rate of disease progress during a growing season, and the duration of epidemics. These effects will positively or negatively influence individual pathogens and the diseases they cause. Changes in the spectra of diseases are also anticipated. Abiotic diseases associated withenvironmental extremes are expected to increase, and interactions between biotic and abiotic diseases might represent the most important effects of climate change on plant diseases.

A change in temperature may favour the development of different dormant pathogens, which could induce an epidemic (Mcelrone *et al*., 2005). Increase in temperature with sufficient soil moisture may increase evapotranspiration resulting in humid microclimate in crops and may lead to incidence of diseases favored under these conditions. In North America, needle blight (*Dothistroma septosporum*) is reported to be spreading northwards withincreasing temperature and precipitation (Madden *et al*., 2007). In general,increase in temperature would significantly raise theseverity and spread of plant diseases but quantity of precipitation could act as regulator in deciding theincrease or decrease in disease severity and spread (Woods *et al*., 2005).

According to Gregory *et al*. (2009), temperature can also affect disease resistance in plants, thus affecting the incidence and severity of the diseases. Temperature sensitivity to resistance has been reported for leaf rust (*Puccinia recondita*) in wheat, broomrape (*Orobanche cumana*) in sunflower, black shank (*Phytophthora nicotianae*) in tobacco and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) in rice.

Kudela *et al*., (2009) stated that the temperature is one of the most important factors affecting the occurrence of bacterial diseases such as *Ralstonia solanacearum*, *Acidovorax avenae* and *Burkholderia glumea*. Thus, bacteria could proliferate in areas where temperature-dependent diseases have not been previously observed.

The incidence of most of the virus and other vector-borne diseases will be altered. This is because change in temperature can substantially influence the development and distribution of vectors. (Navas-Castillo *et al*., 2009). Genetic changes in the virus as a result of higher temperature, changes in the vector populations and long-distance transportation of plant material or vector insects due to trade of vegetables and ornamental plants have resulted in the emergence of tomato yellow leaf curl disease, African cassava mosaic disease, diseases caused by bipartite *Begomoviruses* in Latin America, *Ipomovirus* diseases of cucurbits, tomato chlorosis caused by *Criniviruses*, and the torrado-like diseases of tomato.

Dixon *et al*. (2012) reported that in India, for the last decade the disease scenario of chickpea and pigeon pea has changed drastically; dry root rot (*Rhizoctonia bataticola*) of chickpea and *Phytophthora* blight (*Phytophthora drechsleri* f. sp. *cajani*) of pigeon pea have emerged as a potential threat to the production of these pulses25. Higher risk of dry root rot has been reported in *Fusarium* wilt chickpea-resistant varieties in those years when the temperature exceeds 33°C. Mboup *et al*. (2012) stated that a change in temperature could directly affect the spread of infectious diseases and their survival between seasons. There are indications of increased aggressiveness at higher temperatures of stripe rust isolates (*Puccinia striiformis*), suggesting that rust fungi can adapt to and benefit from higher temperatures.

2.9 Disease assessment :

The assessment of disease is vital to our interpretation as to whether disease management practices are successful. Relative magnitudes in success of disease management are judged on a comparative basis by growers and scientist through disease assessment. A slightly different approach to measuring disease incidence reliability was taken by Shokes *et al*. (1987) in the assessment of foliar diseases; however, the approach is equally applicable for collar and root diseases. The goal was to provide a measurement of agreement among several disease evaluators to estimate the importance of disease evaluation.

According to Maddan (1990) area is an important component of disease assessment because it indicates the spatial or geographical scale at which the assessment was made. Campbell and Neher (1994) stated that cost is a factor of primary importance in planning the assessment of any disease. Cost of effort must be balance against the need for a certain amount of information. According to Campbell and Neher (1994) the general goal of disease assessment will remain constant for all types of studies. The goal is to provide reliable estimates of the amount of diseases in an area (plot, field, farm, country, region etc.) based on the specific symptoms and signs, which are known to be the characteristics of the disease at the lowest reasonable cost with known confidence.

Disease assessment must be reliable (Campbell and Neher, 1994). The estimates and measurements of the diseases should be accurate, precise and reproducible. Accuracy refers to the closeness of the sample mean from the assessment to true population mean and is measured as a bias. Mukharjee *et al.*, (2009) attempted to estimate the areaunder the disease progress curve (AUDPC) of rice blast disease caused by *Pyricularia grisea* from two data points on the disease progress curve. Disease incidence was recorded on alternate days beginning from disease initiation until the end of the epidemic. The estimation of AUDPC, and logistic and Gompertz apparent infection rates using either all points (AP) or two-point (TP) methods revealed significant correlations among them. This was also confirmed through regression analysis and factor analysis. Hence, the estimation of

AUDPC from two data points i.e. initial and final of the disease progress curves is recommended as providing information similar to that from all the data points; this should save valuable time, labour and economic resources.

The incidence of finger millet blast was assessed as the percentage of plants with visible symptoms in a field and greenhouse by Gashaw *et al*. (2014). The plants were rated for disease incidence (DI) as the presence or absence of disease (percentage of infected leaves on the plant). Incidence (I) and severity data (S) were used to calculate disease intensity index (DII), DII= (I×S)/4. Blast disease severity and incidence was assessed at, 30 days after inoculating the plant under natural infection. Disease incidence of ratoon stunt disease in sugarcane was calculated by Lee *et al*. (2014). After the survey, result of DI was calculated and come out to be in the range of 0 to 100%.

Methodology of the programme with materials used is discussed in this chapter

Chapter-3

Materials and Method

MATERIALS AND METHODS

3.1 General method of sterilization and preparation of media:

3.1.1 Cleaning and Sterilization of glass wares:

Different types of glassware like petri dishes, flasks, pipettes, test tubes, thistle funnels to be used during experiments were washed and cleaned. After that these were treated with cleaning solution prepared by dissolving 80 g of potassium dichromate ($K_2C_2O_7$) in 400 ml of concentrated H_2SO_4 and diluted in 300 ml of distilled water, again washed thoroughly, first in running tap water and finally with sterilized water as described by Riker and Riker (1936). After normal air drying the glasswares were sterilized at 180°C for 1¼ hours in the hot air oven. The inoculating needle was dipped in spirit and heated over the flame of spirit lamp until red for 2-3 times. Inoculating needle was used for inoculating and transferring inoculum from one culture tube and petriplates to another, whereas 5 mm cork-borer was exclusively used for transferring measured quantity of inoculum either in solid or liquid media wherever required.

3.1.2 Preparation of media:

Potato Dextrose Agar media was used in various cultural and physiological studies. The constituents of media used during investigation were as follows:

Potato (peeled) : 200 g
Dextrose : 20 g
Agar-agar : 20 g
Distilled water : 1 L

3.2 Collection, isolation and purification of various isolates

3.2.1 Symptoms on collar portion:

Sclerotium rolfsii primarily attacks host stems, although infection may be seen at any part of a plant under favourable environmental conditions including roots, fruits, petioles, leaves, and flowers. The first signs of infection, though

usually undetectable, seen were dark-brown lesions on the stem at or just beneath the soil level; the first visible symptoms were progressive yellowing and wilting of the leaves. Following this, the fungus produced abundant white, fluffy mycelium on infected tissues and the soil. *Sclerotia* of relative uniform size were produced on the mycelium: roundish and white when immature then becomes dark brown to black. Mature sclerotia resembled mustard seed. The fungus occasionally produces basidiospores (the sexual stage of reproduction) at the margins of lesions and under humid conditions, though this form is not common. Seedlings were very susceptible and died quickly once they become infected.

3.2.2 Collection of isolates:

Various isolates of *S. rolfsii* causing collar rot were collected from different hosts (cucumber, bitter gourd, brinjal, lentil, pea, cosmos etc.) (fig. 3.1) of various regions of Bihar. Sclerotial bodies or infected samples collected from diseased plant were disinfested, washed, inoculated on PDA medium and incubated at 25±2°C. The isolates were purified by growing single sclerotia or mycelial disc from each colony on PDA slants. After 7 days, pure isolates were obtained and these were maintained at 4°C for further studies.

3.2.3 Isolation and purification of the pathogen:

Isolation were made on PDA Medium from different parts of the diseased plants showing characteristic symptoms of stem and root rots. The specimens were first washed by passing it through running tap water to remove dust or soil particles if any. Diseased parts just touching the healthy portion were chosen and separated with the help of sterilized blade and were cut into smaller pieces (2-5 mm in size). These pieces were washed thoroughly in sterilized water in order to remove surface-contaminates and then surface sterilized with 0.1 per cent mercuric chloride solution for 5 minutes with the help of camel hair brush. These pieces were washed thoroughly in three consecutive changes of sterilized distilled water to remove the residue of $HgCl_2$ completely. Excess moisture was removed by putting the pieces

Fig. 3.1: Symptoms of collar rot caused by *Sclerotium rolfsii* in (A) Brinjal (B) Tomato and (C) Lentil ; (D) Culture of *Sclerotium rolfsii*

pressed in between two folds of sterilized blotting paper. Then it was transferred to PDA slants aseptically in laminar flow. The inoculated PDA slants were incubated in BOD incubator at 25±2°C. As soon as the mycelial growth was visible around the inoculated pieces, growing fungal tips were transferred to the sterilized medium previously poured into sterilized petridishes. After 3-4 days, they were again transferred to a fresh PDA slant to obtain pure culture by hyphal tip isolation method. Regular transfer of hyphal tip pure culture of the pathogen was done during the period of investigation for maintenance of the pathogen.

3.3 Study of variability:

3.3.1 Morphological variability:

Circular disc of 5mm diameter from the margin of 5-7 days old culture was placed in the centre of the plate under aseptic conditions. The plates were incubated at 25±1°C and replicated 4 times. Data were recorded for the study of following morphological parameters :

- Mycelial type
- Growth rate
- Distribution of sclerotia
- Days to formation of sclerotia
- Days to maturation of sclerotia and
- Sclerotial count

3.3.2 Chemical variability :

3.3.2.1 Melanin estimation from the isolates of *S. rolfsii* :

Extraction and estimation of melanin from the culture of *S. rolfsii* was performed by following Gadd, (1982). One gm mycelium was scrapped from one week old culture, boiled for 5 minutes in 5 ml distilled water and further centrifuged at 5000 rpm for 5 minutes at room temperature. The mycelial pellet was then washed,

centrifuged again and the pigment extracted by autoclaving the pellet with 3 ml NaOH (1 N) for 20 minutes at 120°C. It was followed by acidification (pH 2) of the alkaline pigment was extracted with concentrated Hydrochloric Acid (HCl) to precipitate the melanin. Extract was centrifuged again at 5000 rpm for 5 minutes at room temperature. The precipitate was washed thrice in distilled water and dried overnight at 20°C in dehumified condition for further observation. For spectrophotometric assay, dried pellet was solubilized in 1 ml of 1 N NaOH for two hours at 80°C and centrifuged at 12000 rpm for 10 minutes. The supernatant was transferred to fresh tubes and the absorbance was taken at 405 nm. The optical density (OD) value thus obtained was taken as a measure of relative melanisation in different isolates collected of *S. rolfsii*.

3.3.2.2 Chitinase estimation:

Colloidal chitin was used as substrate for enzymatic assay and prepared according to the method of Sun *et al*. (2006). Chitinase activity was estimated by the method of Chen and Lee (1995). Chitinase estimation was conducted from artificially inoculated plant of cucumber from three different portions viz. collar, stem and apical foliage. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 µmol of NAG g/fresh wt. Chitinase enzyme solution was Pipette (in milliliters) in the vials. The vials was placed on their sides on a rotary platform at a speed sufficient to keep the chitin in suspension. Test samples with blank were incubated for two hours at 25°C. After two hours, the vials were placed into a boiling water bath for 5 minutes followed by cooling to room temperature. After that the vials were incubated for 30 minutes at 25°C with mixing as before. Centrifugation of the suspensions was done to retain the supernatant liquid. All the containers were placed in a boiling water bath for 5 minutes and allowed to cool to room temperature. The solutions were transferred to suitable cuvettes and spectrophotometric reading was recorded at 540 nm for each of the containers. The calculation for Sample Determination was done as :

$$\text{Units/ml enzyme} = \frac{(\text{mg NAG released}) \times (2.5 + \text{Volume of NAGase})}{(2) \times (1) \times (0.5)}$$

2.5 = Initial reaction volume of assay
2 = Conversion factor for converting 2 hours to 1 hour as per the Unit Definition
1 = Volume (in milliliter) of supernatant used in colorimetric determination
0.5 = Volume (in milliliter) of enzyme used

$$\text{Units/g solid} = \frac{\text{units/ml enzyme}}{\text{g solid/ml enzyme}}$$

3.3.2.3 Peroxidase estimation :

Peroxidase estimation was done from artificially inoculated cucumber plant from three different portions viz. collar, stem and apical foliage. Peroxide enzyme solution was pipette (in milliliters) into suitable cuvettes and mixing was done by inversion and equilibrate to 25°C and Monitoring at 405 nm was done to the until constant, using a suitably thermostatted spectrophotometer. Then immediately peroxide was mixed by inversion and reading was recorded for increase at same wavelength for approximately 2 minutes. Reading was obtained using the maximum linear rate for both the Test and Blank by using the following calculations.

$$\text{units per mg solid} = \frac{(\Delta\text{A405nm/min test} - \Delta\text{A405nm/min blank}) \times (3.05) \times (df)}{(36.8) \times (0.05)}$$

3.05 = Total volume (in milliliters) of assay
df = Dilution factor
36.8 = Millimolar extinction coefficient of oxidized ABTS at 405nm
0.05 = Volume (in milliliter) of enzyme used

$$\text{units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

3.3.2.4 Phenol estimation :

Total phenol estimation was conducted from artificially inoculated plant of cucumber from three different portions viz. collar, stem and apical foliage. Fresh portions of the plant was taken in the moist chamber made by covering petri plates by covering both faces with moistened filter paper from inside. Nearly 0.5 g of bits were taken into pre-chilled pestle and mortar separately from different inoculation hours and crushed separately by adding 0.5 ml of methanol (50%). Further, extracts were put on water bath for 90 minutes at 80 ^{0}C. The extracts were centrifuged at 14000 rpm for 15 minutes. The supernatant was taken for free phenolics determination using Folin-ciocalteau's reagent (FCR). The reaction mixture consisted of 0.1 ml of extract, 0.9 ml distilled water, 0.5 ml of 1N Folin-ciocalteu's reagent and 1ml of 20% sodium carbonate (Na_2CO_3). Absorbance was recorded after 20 minutes at room temperature at 725 nm. The reaction mixture having distilled water in place of extract was taken as blank. Standard curve was drawn using gallic acid as standard and total phenols were expressed as mg/ml of gallic acid.

3.3.3 Maintenance of isolate cultures:

The fungus isolate cultures were sub-cultured on the PDA slants and allowed to grow at 25±2°C. The obtained cultures were stored in refrigerator at 4°C for further studies and were sub cultured periodically once in a month.

3.4 In vitro efficacy of fungicides against *Sclerotium rolfsii*

The following nine fungicides (table 3.1) of different mode of actions were evalulated for their efficacy on mycelial growth of *Sclerotium rolfsii* by food poisoning technique (Schemitz, 1930 ; Chaurasia, 2014b). The radial growth of the fungus was measured by averaging the horizontal measurement and vertical measurement from the petri plate (fig. 3.2)

Figure 3.2 : *In-vitro* radial growth supplemented with Hexaconazole carbendazim mancozeb copper oxychloride propiconazole mycobutanil captan thiophenate methyl vinclozoline at 5 concentrations viz. 0, 10, 50, 100, 150 ppm and measurement of radial growth

Table 3.1 : Fungicides used in the study

Common name	Trade name	Active ingredient	Manufacturer
Cell division inhibitor			
Carbendazim	Bavistin	Carbendazim 50% WP	BASF Chem. Co.
Thiophenate methyl	Kirin	Thiophenate methyl 70% WP	Parijat Ind. pvt. ltd.
Vinclozolin	Apparent	Vinclozolin 50% WP	APDANT pvt. ltd.
Multisite action			
Captan	Captan	Captan 50% WP	Arya laboratories
Copperoxychloride	Blitox	Copper oxychloride 50% WP	RaLIS India ltd.
Mancozeb	Shree	Mancozeb 75% WP	Jaishree Rasayan Udyog ltd.
Sterol inhibitor			
Hexaconazole	Hexa	Hexaconazole 5% EC	Mahabir Bajrang Agrochem pvt. ltd.
Mycobutanil	Index	Mycobutanil 10% WP	Nagarajuna Agrichem ltd.
Propiconazole	Tilt	Propiconazole 25% EC	Syngenta

The effect of all nine fungicides (fig. 3.3) each with four concentrations i.e., 10, 50, 100 and 150 ppm were tried against *Sclerotium rolfsii*. Solutions of measured quantity of fungicides, prepared in sterile distilled water, then was added into sterile melted Potato dextrose agar to get the above concentrations in the medium. Approximately 20 ml of poisoned melted medium was poured into each sterilized petridish. After solidification, each petridish was inoculated aseptically by placing the 5.0 mm diameter of inoculum in the center. The medium without the addition of fungicide was considered as control. Four replications of each treatment was maintained in CRD design and the whole experiment was repeated twice. The growth of pathogen, in each set was measured after 48, 72, 96, 120 and 144 hrs of incubation for radial growth. EC_{50} value for all the used fungicides were calculated on the basis of full plate growth of the mycelium. Duncan's Multiple Range test ($P \leq 0.05$) was done to delineate treatment mean (Steel and Torrie, 1980) for suppression of radial growth by different fungicides and their EC_{50} value was done by using computer software SAS, NY version 9.4.

3.5 Exploration of *Sclerotium rolfsii* adapting high temperature regime in successive generations

The isolate BAU Sr1, which was sampled from infected cucumber root, Agricultural Farm of Bihar Agricultural University, Sabour and selected on the basis of its morphological characteristics and subjected to grow under the two different incubation temperatures i.e. 25°C and 35°C. A disc of actively growing mycelium placed at the center of petri plate and cultivated for 120 h (at 25°C) when the mycelium touched the periphery of the plate (Muthukumar and Venkatesh, 2013). The mycelium was re-isolated for generation to generation upto 20 generations. Four replicates for each temperature regime in a generation were adjusted following a complete randomized design (Gomez and Gomez, 1984) and the whole experiment was repeated twice. The comparison of fungal growth rate was made between 25°C and 35°C for the acclimatization of the pathogen in successive generations in response to climate change and increased temperature.

Cell Division Inhibitor

Multisite action

Sterol Inhibitor

Figure 3.3 : Fungicides used in Food Poison Technique method

3.6 Disease assesment :

The assessment of collar rot disease in two crops viz. cucumber and bittergourd was done after growing in pots (20 cm diameter × 17 cm height) at the Department of Plant Pathology, Bihar Agricultural College, B.A.U., Sabour, Bhagalpur. The garden soil was subjected to double autoclaved in polypropalene bag containing 1.5 kg soil in order to eliminate the effect of any other soil borne microorganism (fig 3.2a). The earthen pots were washed thoroughly with tap water and rinsed with 1% formalin and dried in sun one day before pot filling. Mass multiplication of sclerotia was done (fig 3.2b). Pots were filled-up with the sterilized soil mixed with the sclerotia at the rate of 100 sclerotia per 100 g of soil (Yaqub and Shahjad, 2005). Soil was sterilized twice in a day in an autoclaved at 15 lb/sq. inch pressure for 30 minutes. Filled pots were covered with polythene sheets in order to prevent aerial contamination. Sowing of seeds of 4 different varieties (table 3.2 and fig. 3.5) of each crops, which are commonly used were done. The experiment was done in two seasons i.e. rainy and winter and the whole experiment was performed twice in each season, so the experiment was conducted 4 times. The experiment was conducted in Complete Randomized Design (Gomez and Gomez, 1984) with 5 replications. Three pots were taken for each replications to increase the plant density and 5 plants per pot was maintained (fig. 3.6). The observations were taken at 5 times at weekly interval after commencement of the disease for Disease Incidence and lesion length (cm) was measured after the last observation. Area Under Lesion Progress Curve (AULPC) was made on the basis on number of days of observations and lesion length.

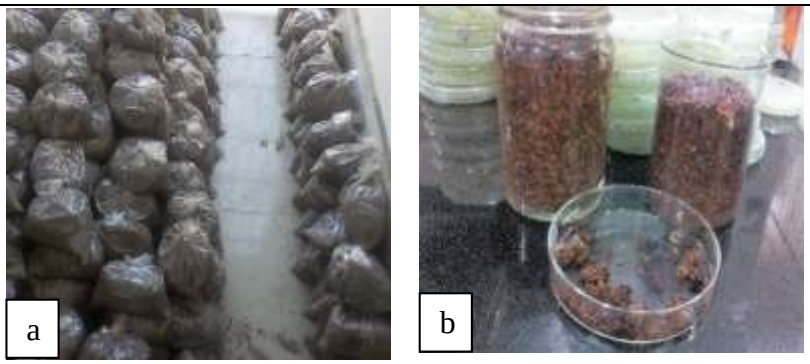

Figure 3.4 a) Autoclved soil and b) mass multiplied sclerotia

Table 3.2 : Varieties used in the study

Crop	**Variety**	**Manufacturer**
Cucumber	Cucumber SS	Ananya seeds
	Cucumber Barsati	Pandey seeds
	Super green (Maharaja)	Doctor seeds Pvt. Ltd.
	Green long	Supreme seeds
Bitter gourd	Parag	Ankur seeds
	Vir	Team seeds Pvt. Ltd.
	Vishwas 228	Shatabdi seeds
	Vivek	Sungro seeds

3.7 Statistical analysis :

The analysis of all the components of objectives were done by statistical software SAS, NY version 9.4. The development of SAS began in 1966 after North Carolina State University re-hired Anthony Barr to program his analysis of variance and regression software so that it would run on IBM System/360 computers. The project was funded by the National Institute of Health and was originally intended to analyze agricultural data to improve crop yields. In 2002, the Text Miner software was introduced. Text Miner analyzes text data like emails for patterns in Business Intelligence applications. In 2004, SAS Version 9.0 was released, which was dubbed "Project Mercury" and was designed to make SAS accessible to a broader range of business users. Version 9.0 added custom user interfaces based on the user's role and established the point-and-click user interface of SAS Enterprise Guide as the software's primary graphical user interface. The Customer Relationship Management features were improved in 2004 with SAS Interaction Management. In 2008 SAS announced Project Unity, designed to integrate data quality, data integration and master data management and most frequently used in agricultural sector.

Cucumber

Bittergourd

Figure 3.5 : Varieties used in the study

Figure 3.6 : Experimental Setup

Experimental results and their consequences are discussed in this chapterare summarized in this chapter

Chapter-4

Results and Discussion

RESULTS AND DISCUSSION

Multi-step series of investigations were carried out on collar rot of cucurbit (cucumber and bitter gourd) caused by *Sclerotium rolfsii*. The results obtained during investigations have been described under following subheads:

4.1 Identification of the isolates:

The 7-day-old colony of suspected fungus was used for observation on mycelial growth and sclerotia production in plate. A mycelial bit of 5 mm was cut at the margin of the colony and placed aseptically at the centre of a fresh 2% PDA-plate. Mycelium of the fungus was confirmed under microscope for fluffy or compact, septate, branched near the distal septum of the fungal cell as mentioned by Sarma *et al*. (2002). Mycelium was observed as white fluffy in the beginning, which changed to black sclerotia with age.

The isolates (table 4.1) were further tested for Koch's postulates. After artificial inoculation in the form of sclerotia, mixed in soil, the inoculated plants were kept for disease development. Regular observations were made for recording gradual development of symptoms. Symptoms appeared about 15 days after inoculation; the incubation period varied between isolates. The collar region showed browning and rotting, which extended to root also and distinguished the infected portion from the uninfected one. The organism was re-isolated from the infected portion of the stem of the inoculated plants by the methods already stated earlier. Then their cultural and morphological characters were compared with those of original one, which was found similar. Thus, satisfying the Koch's postulates and the pathogenicity of the fungus *Sclerotium rolfsii* was established. Every isolates (table 4.1) were tested for making successful infection on cucurbit.

4.2 Variability among isolates of *Sclerotium rolfsii* :

4.2.1 Morphological variability :

The 30 isolates were sampled from 6 different hosts exhibited collar rot symptom. The isolate collection was made from 14 different locations of Bihar. Different morphological characters of 30 isolates of *S. rolfsii* were studied

considering mycelial and sclerotial parameters (table 4.1). Isolates of *S. rolfsii*, collected from different locations of Bihar, and studied for growth parameters i.e. mycelial type, growth rate (mm/day), distribution of sclerotia in the petriplate, days to form sclerotia, days to mature and number of sclerotia/plate. The growth rate varied amongst the isolates. Two types of mycelium types were observed; fluffy and compact. Most of the isolates showed fluffy mycelium; however, isolates namely, BAU Sr7, BAU Sr10, BAU Sr16, BAU Sr22 and BAU Sr27 exhibited compact mycelium type. Similarly, fluffy and compact type of mycelial growth was also reported by Reddikumar *et al.* (2014). In contrary, Kuldhar *et al.* (2014) observed loose and smooth mycelium. Rough and smooth type of mycelium was also recognized in *S. rolfsii* (Venkatesh *et al.*, 2014). Growth rate of the isolates also varied; minimum and maximum growth rate were attained by BAU Sr10 (15 mm/day) and BAU Sr25 (40 mm/day), respectively. Both of these isolates had compact mycelium. Average growth rate of the isolates collected from different locations of Bihar is 26 mm/day. Sarma *et al.* (2002) observed growth rate between 25 and 31 mm/day in Indian isolates of *S. rolfsii*, which is a closure range compared to this study.

Considering different observations under sclerotia and its development; the distribution of sclerotia was two types i.e. (1) regular, meaning distributed uniformly over the petriplate, and (2) periphery, meaning the sclerotia were developed at the margin of the petriplate. Overall, 27 isolates had regular pattern of sclerotia development, and only 3 isolates (BAU Sr10, BAU Sr16 and BAU Sr22) were produced sclerotia at the periphery of the petriplate. Reddikumar *et al.* (2014) reported about isolates showed peripheral and central distribution of sclerotia. In the current study, the sclerotia have begun to develop between 7 and 12 days. A study reveals that isolates took 8 to 10 days of sclerotial development time (Kuldhar *et al.*, 2014). In the present study, sclerotia matured between 12 and 23 days. However, Reddikumar *et al.* (2014) observed rapidly maturing isolates that matured between 10 and 15 days. This study contained a rapidly sclerotia maturing isolate, obtained from cucumber (BAU Sr3), which matures on 12thday after inoculation. A wide range of sclerotia production in a petriplate was

recorded. Both the minimum (43/petriplate) and maximum (117/petriplate) sclerotia producing isolates were collected from cucumber. The mean numeric value is 80.7 sclerotia/petriplate over 30 isolates. Various workers have reported isolates of *S. rolfsii* with different range of sclerotia production in petriplate. Some isolates exhibited 10 to above 51 sclerotia/petriplate (Kuldhar *et al.*, 2014). Prasad *et al.* (2012) reported that the isolates of *S. rolfsii* produced 57 to above 306 sclerotia/petriplate. Likewise, sclerotia production of Indian isolates ranged between 65 and 612 in a petriplate (Sarma *et al.*, 2002).

The mycelium and sclerotia development pattern is shown in fig. 4.1. The isolates produced white cottony mycelium with ropy strands. Thus a variation has been observed between the isolates collected in terms of mycelial growth as well as resting structure.

After observation of all parameters of morphological variability; isolates have been grouped into rapid, medium growing and slow growing (table 4.2). This category was made for two components i.e. growth rate and sclerotia formation. Regarding growth rate, only one isolate (BAU Sr25) found rapid (> 35 mm/day) while the isolates BAU Sr1, BAU Sr9, BAU Sr10 and BAU Sr20 were slow growing (15-20 mm/day). Overall, 25 medium growing isolates have been identified.

In the second category based on time taken in sclerotia formation (table 4.2), the first group consists of 8 isolates of rapid nature, which took 12-16 days for complete sclerotia formation. Likewise, 11 isolates took medium duration of time (17-20 days) for maturation of their sclerotia. Moreover, 21-24 days were taken by 10 isolates and categorized as slow growing.

Variation was recorded as number of sclerotia developed in the petriplates, which varied between 43 (cucumber, Betiah) and 117 (cucumber, Sabour) (table 4.1). In the grouping based of sclerotial count (table 4.3), the isolates were categorized in 8 groups. Most of the isolates (17) produced sclerotia between 71 and 90. The highest sclerotia producer isolates were BAU Sr1 and BAU Sr12,

Table4.1: Variation in morphological characters of isolates of *Sclerotium rolfsii*

Isolate	Isolate code	Host	Geographic origin	Mycelium type	Growth rate (mm/ day)	Sclerotia			
						Distribution	Days to development	Days to maturity	Number per plate
1	BAU Sr1	Cucumber	Sabour	Fluffy	20	Regular	8	15	117
2	BAU Sr2	Cucumber	Sabour	Fluffy	22	Regular	9	14	96
3	BAU Sr3	Cucumber	Sabour	Fluffy	26	Regular	8	12	74
4	BAU Sr4	Cucumber	Sabour	Fluffy	21	Regular	9	13	102
5	BAU Sr5	Bitter gourd	Sabour	Fluffy	24	Regular	8	15	63
6	BAU Sr6	Bitter gourd	Sabour	Fluffy	28	Regular	10	15	79
7	BAU Sr7	Bitter gourd	Sabour	Compact	25	Regular	9	14	54
8	BAU Sr8	Bitter gourd	Sabour	Fluffy	32	Regular	8	14	83
9	BAU Sr9	Lentil	Sabour	Fluffy	20	Regular	12	22	76
10	BAU Sr10	Brinjal	Sabour	Compact	15	Periphery	10	21	48
11	BAU Sr11	Pea	Bhagalpur	Fluffy	26	Regular	9	23	74
12	BAU Sr12	Cucumber	Bhagalpur	Fluffy	29	Regular	7	20	116
13	BAU Sr13	Lentil	Naugachia	Fluffy	23	Regular	8	19	76
14	BAU Sr14	Cucumber	Naugachia	Fluffy	26	Regular	12	18	96
15	BAU Sr15	Pea	Naugachia	Fluffy	24	Regular	9	22	85
16	BAU Sr16	Lentil	Kishanganj	Compact	25	Periphery	8	24	66
17	BAU Sr17	Cucumber	Kishanganj	Fluffy	28	Regular	10	21	93
18	BAU Sr18	Lentil	Motihari	Fluffy	30	Regular	12	16	89
19	BAU Sr19	Lentil	Raxaul	Fluffy	33	Regular	8	19	78
20	BAU Sr20	Chickpea	Adapur	Fluffy	20	Regular	9	23	69
21	BAU Sr21	Cucumber	Sathi	Fluffy	35	Regular	7	19	96
22	BAU Sr22	Cucumber	Betiah	Compact	26	Periphery	12	17	43
23	BAU Sr23	Pea	Sitamarhi	Fluffy	29	Regular	10	21	81
24	BAU Sr24	Cucumber	Sitamarhi	Fluffy	28	Regular	8	23	87
25	BAU Sr25	Cosmos	Sabour	Compact	40	Regular	7	20	82
26	BAU Sr26	Lentil	Purnea	Fluffy	22	Regular	8	20	76
27	BAU Sr27	Cucumber	Patna	Compact	25	Regular	12	22	82
28	BAU Sr28	Lentil	East Champaran	Fluffy	23	Regular	9	23	76
29	BAU Sr29	Cucumber	East Champaran	Fluffy	26	Regular	10	20	77
30	BAU Sr30	Bitter gourd	East Champaran	Fluffy	28	Regular	10	19	88

Isolate 1-5

Isolate 6-10

Isolate 11-15

Isolate 16-20

Isolate 21-25

Isolate 26-30

Figure 4.1 : Left to right: a) mycelial growth of different isolates (7-day old) and b) sclerotia formation in different isolates (20-day old)

Table 4.2 : Grouping of *Sclerotium rolfsii* isolates on the basis of growth rate and time taken in sclerotia formation

		Growth rate		**Sclerotia formation**	
Group	**Category**	**mm/day**	**Isolates**	**Number of days**	**Isolates**
I	Rapid	>35	BAU Sr25	12-16	BAU Sr2, BAU Sr3, BAU Sr4, BAU Sr7, BAU Sr8, BAU Sr1, BAU Sr5, BAU Sr6
II	Medium	21-35	BAU Sr2, BAU Sr3, BAU Sr4, BAU Sr5, BAU Sr6, BAU Sr7, BAU Sr11, BAU Sr12, BAU Sr13, BAU Sr14, BAU Sr15, BAU Sr16, BAU Sr17, BAU Sr18, BAU Sr19, BAU Sr21, BAU Sr22, BAU Sr23, BAU Sr24, BAU Sr26, BAU Sr27, BAU Sr28, BAU Sr29, BAU Sr30	17-20	BAU Sr18, BAU Sr22, BAU Sr12, BAU Sr13, BAU Sr14, BAU Sr19, BAU Sr21, BAU Sr25, BAU Sr26, BAU Sr29, BAU Sr30
III	Slow	15-20	BAU Sr1, BAU Sr9, BAU Sr10, BAU Sr20	21-24	BAU Sr9, BAU Sr10, BAU Sr15, BAU Sr17, BAU Sr23, BAU Sr27, BAU Sr11, BAU Sr16, BAU Sr20, BAU Sr24, BAU Sr28

Table 4.3: Grouping of *Sclerotium rolfsii* isolates based on number of sclerotia formed

Sclerotia/plate	Isolates
40-50	BAU Sr10, BAU Sr22
51-60	BAU S7
61-70	BAU Sr5, BAU S16, BAU Sr20
71-80	BAU Sr3, BAU S6, BAU Sr9, BAU Sr11, BAU Sr13, BAU Sr19, BAU Sr26, BAU Sr28, BAU Sr29
81-90	BAU Sr8, BAU Sr15, BAU Sr18, BAU Sr23, BAU Sr24, BAU Sr25, BAU Sr27, BAU Sr30
91-100	BAU Sr2, BAU Sr14, BAU Sr17, BAU Sr21
101-110	BAU Sr4
111-120	BAU Sr1, BAU Sr12

both were sampled from cucumber. Similar grouping of isolates was also done by Sarma *et al*. (2002). Some isolates collected from different locations of Bihar were found morphologically dissimilar; the variation may be due to differences in ecology, genetic make-up or the nutrient level of the soil (Okereke and Wokocha, 2007). However, consistent production of the teleomorph stage in various isolates of *S. rolfsii* may strengthen the hypothesis that genetic exchange may occur through normal genetic recombination i.e. meiosis discernible in the progeny. Alternatively, the genetic factor responsible for sexual reproduction may be triggered in some isolates. However, according to Nalim *et al.* (1995), nuclear

exchange through anastomosis in hyphae may be responsible for normal genetic recombination in the fungus. Because this pathogen infects various hosts, as indicated in table 4.1, the variation in mycelium type, sclerotia production, and other characters is due to genetic exchange between different isolates of *S. rolfsii*. Since the sexual stage of *S. rolfsii* is rare in nature and its role in the life cycle of the fungus is unknown, the genetic exchange in mycelia of *S. rolfsii* isolates is largely thought to be limited to mycelial compatibility (Nalim*et al.,* 1995; Sarma *et al.,* 2002).

The present results are in agreement with the findings of Yaqub and Shahzad (2005) who reported cultural variation among *S. rolfsii* on different hosts. However, extensive studies are needed with a large set of isolates from diversified agro ecological situations and hosts in order to generate information on pathogenic variation in *S. rolfsii*.

4.2.2 Biochemical variation :

Various chemicals were seen for the variability from various isolates of *S. rolfsii.* Melanin was calculated from all the isolates for their variability. Chitinase, phenols, and peroxidase were calculated from three different portions of plant after artificial inoculations by five most variable isolates among all 30 isolates.

4.2.2.1 Estimation of Melanin pigment from isolates :

Many plant pathogenic fungi produce melanin which is the oxidative polymerization of phenolic compounds, such as glutaminyl-3,4-dihydroxybenzene (GDHB) or catechol or 1,8-dihydroxynaphthalene (DHN) or 3,4-dihydroxyphenylalanine (DOPA). Colouration in fungi is determined by melanin content, and the variation in colour is governed by the derivatives of carotinoids (Issac, 1994). For example, orange colour is appeared due to presence of β-carotene and dark red colour is appeared due to presence of lycopene. The vital role of melanin in the fungal tissue including spores and vegetative hyphae is to provide resistance against damaging environment such as UV radiation. Additionally, it also works as a strengthening component and physically

protectsthe fungal tissue to the enzymatic actions of metabolites produced by other microorganisms.

The pigment extracted from the isolates of *S. rolfsii* showed presence of melanin. The dark brown pigment from the mycelium could not be extracted with organic solvents such as acetone, chloroform, and ethanol. Values of melanin pigment in the isolates were calculated at 405 nm wavelength using spectrophotometer. The isolates rendered variable results for their melanin pigments (fig. 4.2). The tested isolates represented a wide variation in terms of melanin production. The variation ranged between 0.131 OD (BAU Sr27) and 0.404 OD (BAU Sr15). Suryanarayanan *et al*. (2004) obtained isolates of *Phyllosticta capitalensis* exhibited OD value for melanin production from 0.2 to 1.8. The melanin concentration of *Bipolaris sorokiniana* isolates was observed between 0.12 to 2.5mg/g (Bashyal *et al.*, 2010). However, a different work on the same pathogen revealed a relatively wide range in melanin concentration (0.04 to 2.75 mg/g) indicating the same pathogen may serve a wide array in melanin production (Aggarwal *et al.*, 2011). Therefore, the result in this current experiment displays the role of melanin in differentiation of hyphae and sclerotial development in *S. rolfsii*, which may assist the pathogen for adaptability in diverse ecology by increasing its aggressiveness. This may be a paramount character particularly for such pathogens having wide host range like *S. rolfsii*.

In this experiment, grouping of all the collected isolates has been done on the basis of melanin pigment (fig. 4.2). The isolates of *S. rolfsii* were categorized into four groups: poor producer (0.10 to 0.19), average producer (0.20 to 0.29), medium producer (0.30 to 0.39), and strong producer (above 0.40). Compared to sporulating fungi (e.g. *Bipolaris*, *Colletotrichum*) the lower melanin content was detected for *S. rolfsii* in this investigation. This is well known that the sporulating fungi under family Dematiaceae produce coloured spores which are determined by melanin content in the particular fungal genus. Therefore, the isolates of *B. sorokiniana* had high melanin content (Aggarwal *et al.*, 2011). Moreover, the presented findings envisaged that *S. rolfsii* contained melanin, which could relate

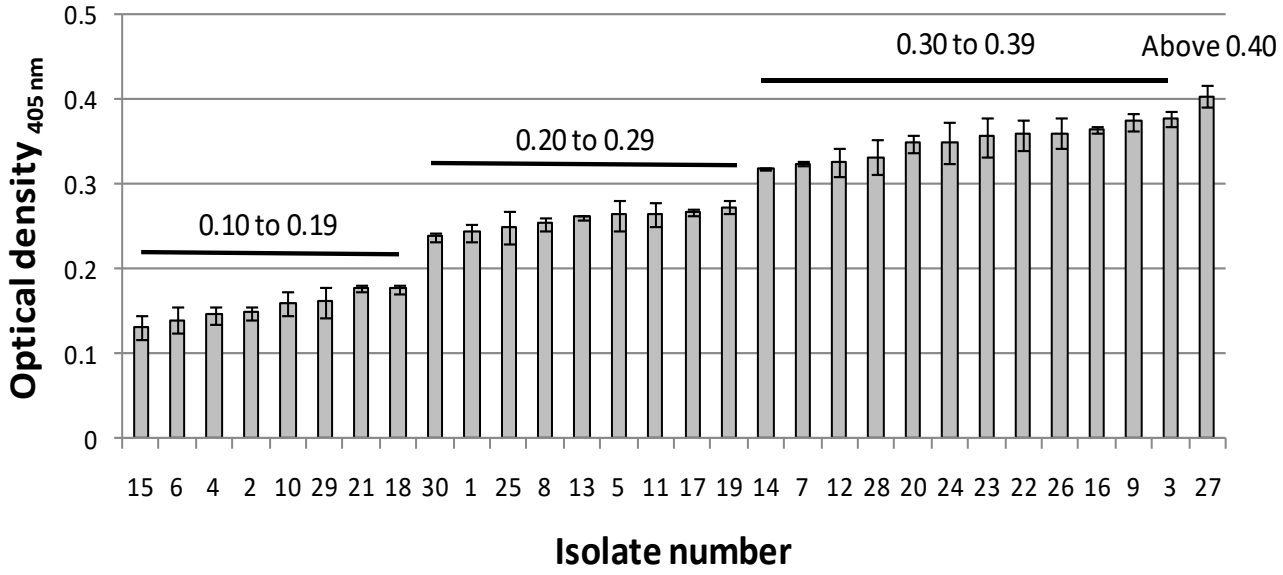

Figure 4.2 : Isolates of *Sclerotium rolfsii* categorized for melanin production on the basis of optical density observed at 405 nm. The details of isolate number are presented in table 4.1.

with its adaptation potentiality involving many-host infection ability and its success rate of survival with competing the other higher fungi and adverse environment. A preliminary work indicated that use of nanoparticle altered the melanin biosynthesis gene expression in *B. sorokiniana* (Mishra and Singh, 2015). Therefore, the current study advocates for conduction of extensive experiments to explore the role of melanin in pathogenesis including the identification of melanin biosynthesis genes involving a wide range of plant pathogenic fungi.

4.2.2.2 Estimation of Chitinase :

Separately five different isolates of *Sclerotium rolfsii* inoculated cucumber plants showed induction in chitinase activity (fig. 4.3). The estimation of chitinase enzyme in the inoculated plants was calculated at 15 DAI in three plant organs of the plant i.e. collar, stem and apical foliage. The maximum content of chitinase was noted in collar followed by stem and apical foliage, respectively. Non-significant difference ($p \leq 0.05$) in chitinase production amongst the isolates was observed for all three plant organs (fig. 4.3). The present study revealed minimum and maximum chitinase production ranged between 0.407 and 2.473 mg/g sample, respectively. A narrow range of chitinase (0.10 to 0.50 mg/g sample) was detected in cowpea infected with *S. rolfsii* (Nandi *et al.*, 2013). Plants have developed a highly sophisticated antioxidative defense system to cope with many biotic and abiotic stresses (Heidari, 2009). Utilization of plant's own defense mechanism against stress is an attractive strategy that enables the plants to thrive well in hostile environment. Plant products play an important role as antifungal, antibacterial and antiviral agents. The products of activated defense genes, which are synthesized *de novo* during the period of pathogenic stress, have been well documented in several host-pathogen interactions but such information is really lacking in cucurbit-*S. rolfsii* pathosystem.

The pathogenesis related protein (PRP)-2 family consists of β-1,3-glucanases, which are able to hydrolyze β-1,3-glucans, a biopolymer found in fungal cell walls (Nandi *et al*., 2013). The PRP-3, 4, 8 and 11 families consist of chitinases belonging to various chitinase classes (I to VII). The substrate of

chitinases is chitin, which is also a major structural component of fungal cell walls. Chitinases hydrolyze the β-1,4-linkage between N-acetylglucosamine (NAG) residues of chitin. In the present study, enhanced activity of chitinase was observed in the infected organ of cucumber inoculated with *S. rolfsii*. Similarly, a correlation in between the systemic induction of chitinase and β-1,3-glucanase and resistance in plants by binucleate *Rhizoctonia* species was demonstrated (Xue *et al.*, 1998)

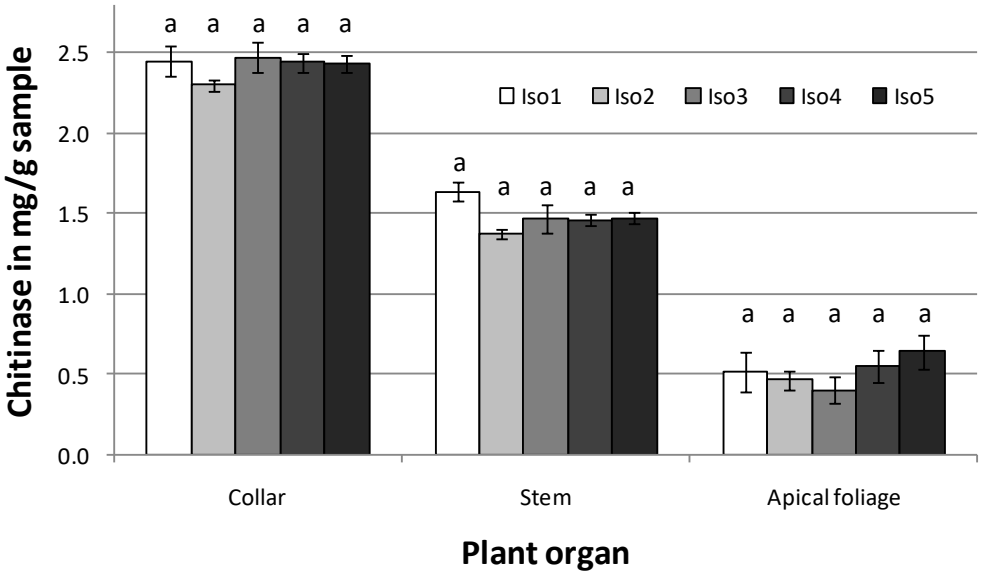

Figure 4.3 : Estimation of chitinase (mg/g) at three different plant organs. Isolates with similar letters within a plant organ are not significantly different ($p \geq 0.05$). The differences were determined using Duncan's multiple range test at 5% error. Error bars represent standard error of the mean. Iso1: BAU Sr1, Iso2: BAU Sr3, Iso3: BAU Sr8, Iso4: BAU Sr12, Iso5: BAU Sr21 (presented in table 4.1).

4.2.2.3 Estimation of total Phenols :

Like chitinase experiment, the same five isolates of *S. rolfsii* were used for this experiment. Two different experiments were carried out for total phenol

estimation at three different organs of cucumber plant (fig. 4.4). The two experiments were performed in two different seasons i.e. rainy and summer. Impact of season was found significant ($p \leq 0.05$) on total phenol content. In each of the experiment collar region was found to be significantly highest in phenol production as compared to stem and apical portion region ($p \leq 0.05$). The phenolic substances secreted in response to different isolates of the pathogen were significantly different ($p \leq 0.05$) for the collar infection. However, the non-significant effect of isolates ($p \geq 0.05$) was determined for infection in stem and apical foliage, which is associated with smaller lesion size at these two organs indicating non-organ preference by *S. rolfsii*. Numerically, Iso4 (BAU Sr12) produced greater total phenol, which was at par with Iso5 (BAU Sr21) in both of the experiments. Iso3 (BAU Sr8) was significantly similar to Iso4 and Iso5 for experiment conducted in rainy season; however, it produced significantly lower phenol in summer-season experiment. Iso1 and Iso2 showed lower phenol production in both of the experiments. The current experiments exhibited total phenols in a range of 65.9 to 612.0 ppm; Nandi *et al.* (2013) detected total phenols between 50 and 243 ppm in cowpea infected with *S. rolfsii*. Petkovsek*et al.* (2008) isolated different fractions of phenols in apple scab pathosystem.

Phenolic compounds may increase the mechanical strength of the plant cell wall producing toxin to the fungus (Pandey *et al.*, 2005). The hyphae of the pathogen surrounded by phenolics substances exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content. The present study showed higher accumulation of phenolics in the plant's collar organ. In many instances, phenols serve as plant defense mechanisms against predation by insects, herbivores and microorganisms. Phenolics that occur constitutively and function as preformed inhibitors are generally referred to as phytoanticipins, and those that are produced in response to infection by the pathogen are called phytoalexins and constitute an active defense response (Nandi *et al.*, 2013). A coordinated defense response system is activated in plants and defense related proteins or pathogenesis related proteins (PR proteins) are produced in the host during the host-pathogen interaction.

Biochemical analyses of various disease responsive components have thrown light on the pivotal role of different enzymes and phenolics in cucurbit and *S. rolfsii* interaction. The suppressed activity of defense arsenals suggests their role in disease development.

4.2.2.4 Estimation of Peroxidase :

Earlier studies suggest that peroxidases are important PR proteins and the plant expresses peroxidase activity during host-pathogen interaction (Saikia *et al.*, 2004). Peroxidase activity and isozymes studies indicate that the suppression of peroxidase, which lead to the weakening of defense mechanisms in plants. This helps in the further spread of the pathogen and eventually severe collar rot symptoms are expressed. So, it is clear that suppression of peroxidase is found to be one of the important factors responsible for the successful pathogenesis in host pathogen interaction. Isomer POD-5 (~20 kD, mf = 0.69) may be associated with the susceptible host-pathogenic interaction in cucurbit *S. rolfsii* system (Passardi *et al.*, 2005).

The present study reveals that the induction in peroxidase activity was significantly different ($p \leq 0.05$) upon inoculation with *S. rolfsii* at three plant organs i.e. collar, stem and apical foliage (fig. 4.5). Inconsistent response of plant organs in peroxidase production was observed for the isolates examined in this experiment. The collar part produced significantly higher peroxidase against the response of Iso2, Iso3 and Iso5 compared to Iso1 and Iso4. But the stem portion produced relatively lower peroxidase for the three isolates mentioned for collar region. However, highest production of peroxidase was determined in apical foliage against only one isolate (Iso3) among the same three isolates responsible for highest peroxidase production in collar region. Therefore, it can be concluded that isolate dependent peroxidase production is operated in different plant organs. The present study observed peroxidase production in a range of 0.337 to 0.940 mg/g sample, which is in a virtual agreement with Nandi *et al.* (2013) who

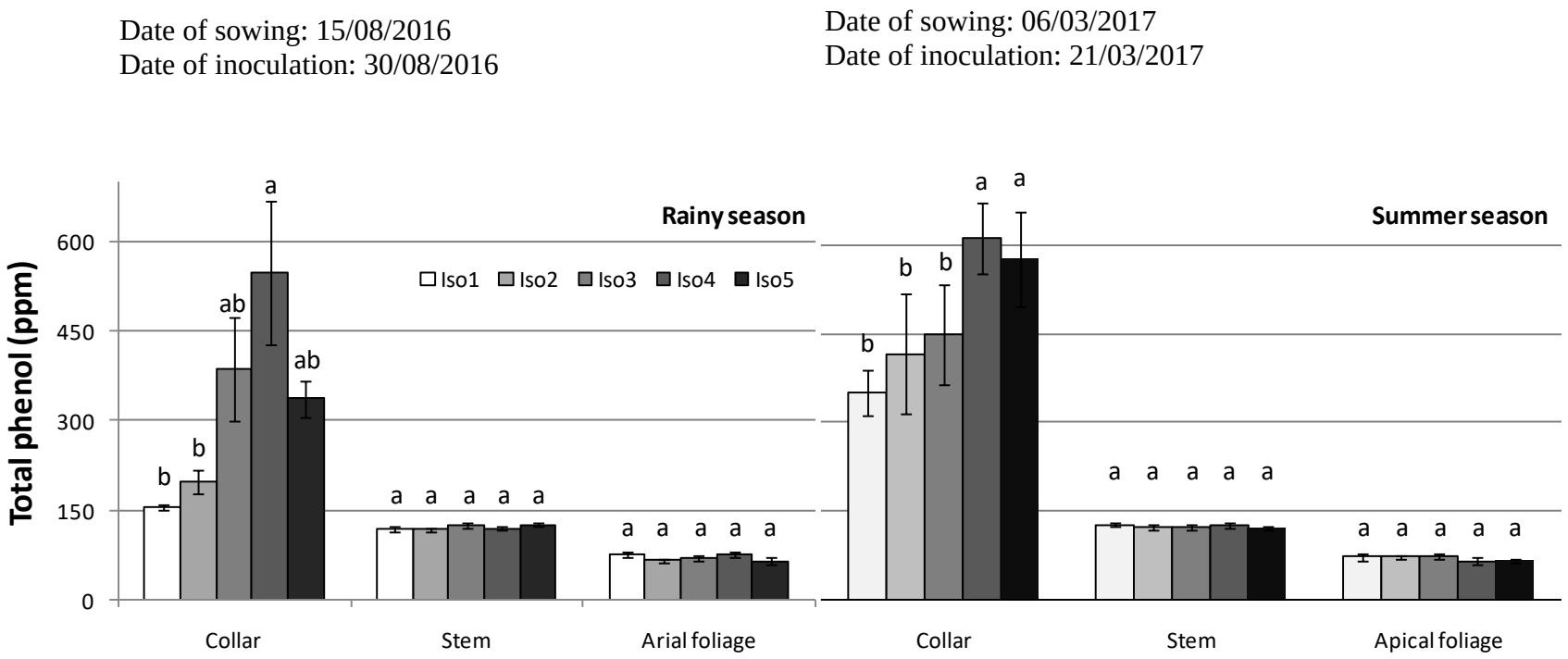

Figure 4.4 : Estimation of total phenol (ppm) at three organs of cucumber plant in two experiments. Isolates with different letter within a plant organ is significantly different ($p \leq 0.05$). The differences were identified using Duncan's multiple range test at 5% error. Error bars represent standard error of the mean. Iso1: BAU Sr1, Iso2: BAU Sr3, Iso3: BAU Sr8, Iso4: BAU Sr12, Iso5: BAU Sr21 (presented in table 4.1).

obtained peroxidase between 0.20 and 0.70 mg/g cowpea sample infected with *S. rolfsii*. Peroxidase has been implicated as the last enzymatic step of lignin biosynthesis i.e. the oxidation of hydroxyl cinnamyl alcohols into free radical inter-mediates, which subsequently are coupled to lignin polymer. Furthermore, peroxidase is involved in the production or modulation of active oxygen species which may play various roles directly or indirectly in reducing pathogen viability and spread (Passardi *et al.*, 2005).

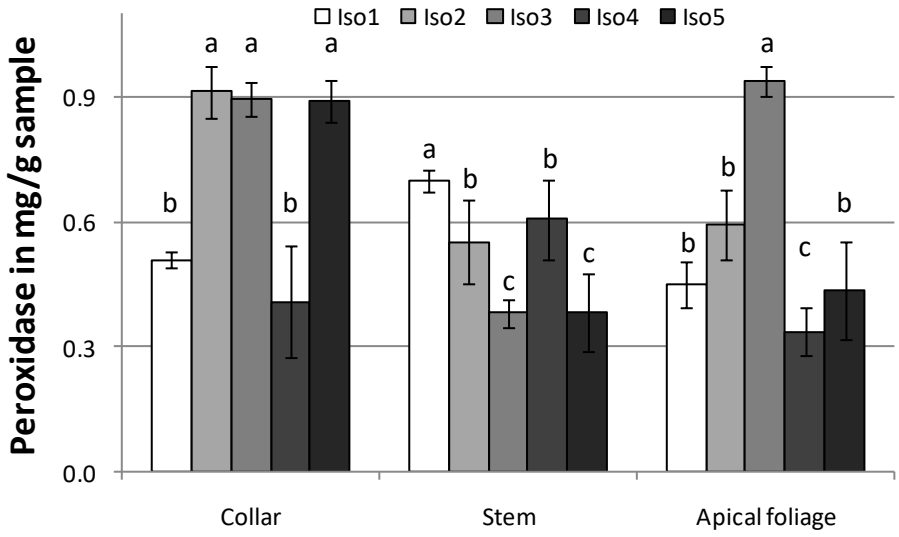

Figure 4.5 : Estimation of peroxidase (mg/g) in three plant organs. Isolates with different letter within a plant organ is significantly different ($p \leq 0.05$). The differences were identified using Duncan's multiple range test at 5% error. Error bars represent standard error of the mean.Iso1: BAU Sr1, Iso2: BAU Sr3, Iso3: BAU Sr8, Iso4: BAU Sr12, Iso5: BAU Sr21 (presented in table 4.1).

4.3 *In-vitro* evaluation of different fungicides against *Sclerotium rolfsii* :

An experiment was conducted to determine the effect of nine fungicides grouped in three different mode of action comprising of three fungicides in each group at different concentrations *viz.*, 0, 10, 50, 100 and 150 ppm by poisoned food technique. The radial growth of the pathogen recorded after 48, 72, 96, 120 and 144 h after inoculation (hai). Effect of experiment on radial growth of *S. rolfsii* was found significant ($p \leq 0.0001$). Therefore, separate analyses were performed to identify the effect of fungicide and concentration, and their interaction on radial growth of the fungus (table 4.4). The various fungicides and concentration affected the fungal radial growth significantly ($p \leq 0.0001$) in both experiments. This could be interpreted that the suppression of radial growth is largely affected by the type of fungicide used and their concentration. The result showed that all the fungicides significantly inhibited radial growth of the pathogen.

Effective concentration (EC_{50}) value was determined for each fungicide (table 4.7). The best fungicide with lowest EC_{50} was hexaconazole, which rendered 41.9 and 40.9 µg/ml EC_{50} for experiment 1 and experiment 2, respectively. The highest EC_{50} was recorded for mycobutanil with recordings of 461.5 and 473.7 µg/ml for experiment 1 and experiment 2, respectively. The observation obtained in this study revealed no relation between fungicide and EC_{50}; the fungicide (mycobutanil) with highest EC_{50} value was the third best fungicide in order to inhibit fungal growth under controlled condition. Overall, hexaconazole at a very low concentration (10 ppm) showed 85.83% suppression of the radial growth of *S. rolfsii* at 144 hai and no growth was observed at 50 ppm. Mycobutanil and propiconazole had poor inhibitory effect at all concentrations as compared to hexaconazole. Out of cell division inhibitor fungicides, carbendazim performed well in fungal growth inhibition followed by vinclozolin and thiophenate methyl, which displayed non-significant difference ($p \geq 0.0001$) between them in order to suppress radial growth of the pathogen. Among multi-site action inhibitors, copper oxychloride followed by captan and mancozeb

proved well for suppression of fungal growth *in vitro*. This result is in agreement with Yang *et al*. (2011).

It was found that analyses for both the experiments were resulted same for a fungicide. However, *S. rolfsii* exhibited different sensitivity against these fungicides in this study. The range of EC_{50} values for the various fungicides were from 40.9 to 473.7 µg/ml. Carbendazim and mancozeb were insignificantly different ($p \geq 0.0001$) for EC_{50} value; however, hexaconazole had lowest EC_{50} value, which inhibited the fungal growth significantly greater when the comparison was made with other fungicides tested.

Significant effect of the interaction of fungicide and concentration indicated that a particular fungicide cold be useful at a specific concentration. Therefore, for effective and judicious use of fungicide, proper concentration of the fungicide should be applied in order to achieve greater impact of management of a pathogen with wide host range like *S. rolfsii* (Ghatak *et al.*, 2017). This research advocated for hexaconazole followed by propiconazole for mycelium suppression of *S. rolfsii* (table 4.6). This result is in agreement with Das *et al.* (2014) who also found hexaconazole as the best fungicide restricting *S. rolfsii* growth. Hexaconazole reported to be the best fungicide restricting *S. rolfsii* infection in brinjal (Chaurasia *et al.*, 2014a). No infection of *S. rolfsii* was observed for the finger millet plants treated with hexaconazole (Manu *et al.*, 2012).

Table 4.4 : Analysis of variances determining the effect of fungicide and concentration on radial growth of *Sclerotium rolfsii*[a]

		Experiment 1			Experiment 2		
Variable	***df***	**MSS**	**F-value**	**Pr > F**	**MSS**	**F-value**	**Pr > F**
Fungicide (F)	8	2668.26	4383.11	<0.0001	2533.62	6438.26	<0.0001
Concentration (C)	4	25522.49	4192.50	<0.0001	27427.47	6969.70	<0.0001
Replication (R)	3	1.13	1.86	0.1411	1.01	2.58	0.0580
F × C	32	853.43	1401.93	<0.0001	550.94	1400.00	<0.0001
Error	96	0.61			0.39		
Total	179	842.60			824.95		

[a] Effect of experiment found significant on radial growth of *S. rolfsii*. Therefore, separate analyses for both experiments were conducted separately.

Table 4.5: Effect of fungicides on radial growth of *Sclerotium rolfsii* and determination of EC_{50} value of the fungicides used in this study

	Mean radial growth (mm)[m]		**EC_{50} value (µg/ml)[n]**	
Fungicide	**Experiment 1[o]**	**Experiment 2**	**Experiment 1**	**Experiment 2**
Cell division inhibitor				
Carbendazim	52.5 d	53.5 d	57.9 b	56.6 b
Mancozeb	50.6 bd	51.8 bd	61.8 b	61.4 b
Copper oxychloride	49.4 bcd	48.1 bcd	146.3 d	145.2 d
Multisite action				
Thiophenatemerthyl	46.8 bcd	47.2 bcd	125.9 c	126.8 c
Vinclozolin	47.5 bcd	46.9 bcd	135.3 c	136.4 c
Captan	44.6 bc	44.8 bc	230.8 e	233.8 e
Sterol inhibitor				
Mycobutanil	42.8 c	43.8 c	461.5 f	473.7 f
Propiconazole	31.7 b	31.0 b	130.4 c	128.6 c
Hexaconazole	19.0 a	18.1 a	41.9 a	40.9 a

[m, n] Values are means over four replication.

[o] Means followed by common letter in a column is not differ significantly ($p \geq 0.0001$) according to Duncan's multiple range test.

4.4 Exploration of *Sclerotium rolfsii* adapting high temperature regime in successive generations :

To estimate the impact of temperature on growth of *S. rolfsii*, the isolate BAU Sr1 was grown on PDA and radial growth of mycelium was observed on 24, 48, 72, 96 and 120 h after inoculation (hai) at two regimes of temperature i.e. 25°C and 35°C. In general, the pathogen grew at 25°C and touched the periphery of the petriplate (90 mm) on 120 h. At 25°C, no mycelial growth was recognized up to 24 h; the visible growth of the fungus was observed on 48 h and completely filled the petriplate on 120 h. A decrease in mycelial growth was observed in *S. rolfsii* at 25°C (fig. 4.6). The fungal growth measured on 72 h incubation was double of the growth taken on 48 h incubation. While the 96 h incubated petriplates rendered 1.5 times increase of mycelial growth over 72 h incubated petriplates. Similarly, the 120 h incubated petriplates had 1.2 times progress of mycelium than 96 h. from the data table it has been clearly seen that a reduction in fungal growth was observed starting from 8^{th} generation at 25°C. Significant difference in the growth reduction was recorded for incubation period 48 h, 72 h and 96 h. However, at 16^{th} generation, a meager reduction in the growth of mycelium was detected; on 120 h the mycelial growth was restricted to 87 mm. This is indicating that the growth of the fungus is invariable to this temperature regime.

Lower fungal growth recorded for high incubation temperature. At 35°C no mycelial progress was observed even after 72 h of incubation (fig. 4.6). Poor growth (< 10 mm) was noted on 96 h incubation. However, greater than double mycelial growth was found on 120 h of incubation compared to 96 h incubation. Up to 4^{th} generation, the fungal growth was approx thrice when comparison was made with the two incubation period. The 5^{th} and 6^{th} generation envisaged with a progress of approximately 2.5 times of fungal growth. At 35°C, an increase trend of mycelial growth was detected on 5^{th} generation and onward. This could interpret with adaptability of *S. rolfsii* to increased temperature regime (35°C). The percent change in mycelial growth of the pathogen was found to be in an increasing order at higher temperature (35°C) and found in a decreasing trend at

lower temperature (25°C). Temperature and generations, both had significant ($p \leq 0.001$) role in the changing of normal growth trend of the pathogen and adaptability to new environment i.e. higher temperature regime (table 4.6). The change was found to be significant ($p \leq 0.001$) after 16th generation at 35°C while almost the same difference level of difference was found after 5th generation at 25°C (fig. 4.6).

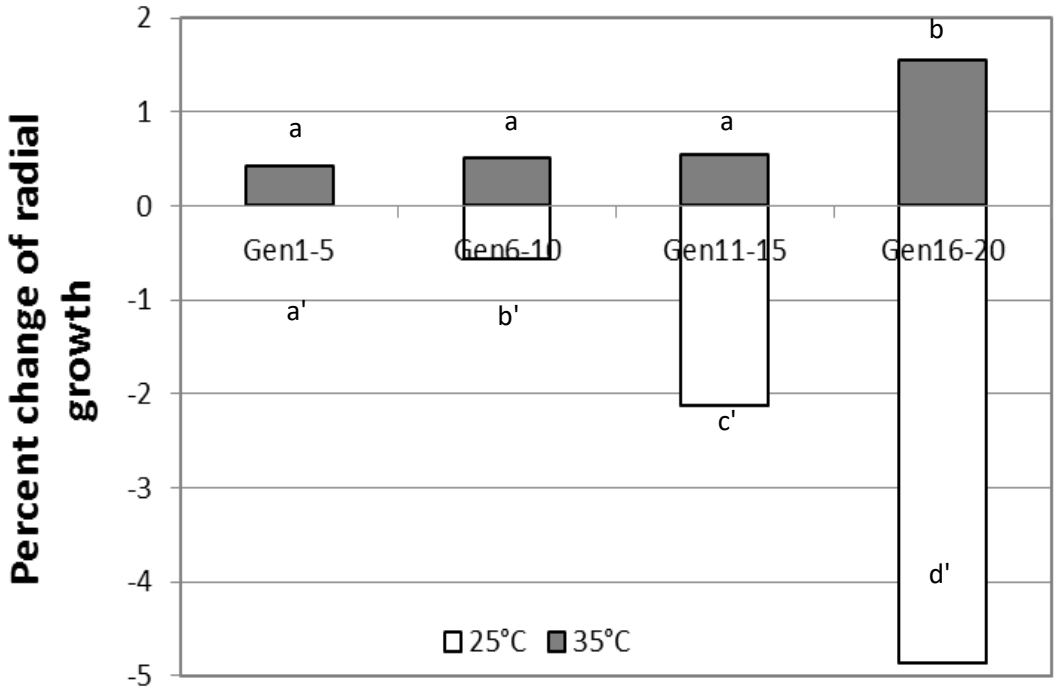

Figure 4.6 : Percent change in radial growth (mm) of *Sclerotium rolfsii* in successive twenty generations. Bars of particular temperature with similar letter are not significantly different according to Duncan's multiple range test at $p \leq 0.001$.

Table 4.6 : Analysis of variances for determining the effect of temperature on growth pattern of *Sclerotium rolfsii* over twenty generation

		96 hrs			120 hrs		
Variable	***df***	**MSS**	**F Value**	**Pr > F**	**MSS**	**F Value**	**Pr > F**
Experiment (E)	1	0.4075	12.30	0.0008	0.140	1.73	0.236
Replication (R)	3	0.0224	0.68	0.5694	0.072	0.74	0.533
Temperature (T)	1	133722.6	4038220	<0.0001	144040.38	1463210	<0.0001
Generation (G)	19	0.7646	23.09	<0.0001	2.7047	27.48	<0.0001
E×T	1	0.0356	1.07	0.3033	0.0318	0.32	0.571
E×G	19	0.0599	1.81	0.0370	0.0991	1.01	0.463
T×G	19	31.2944	945.0	<0.0001	12.1637	123.56	<0.0001
Error	76	0.0331			0.0984		
Total	319						

It can be correlated that the increased global temperature may pose impact on the diseases caused by this fungus of wide host range. Climate change presents considerable challenges for plant disease management (Garrett *et al.*, 2006). While the particular effects of climate change on plant diseases will depend on the plant pathogen system. The impact changes, such as temperature can be predicted for several plant species from empirical studies (Coakley *et al.*, 1999).

Several aspects of the biology of a pathogen can be directly influenced by changing environmental factors (Vati and Ghatak, 2015). Continued period of environmental condition like temperature at its optimum for the development of the pathogen leads increase in severity of epidemics. Thus, with increase in the duration of optimum temperature, many pathogens spread in a new manner and infect the new hosts in the region. It was found that the pathogen *S. rolfsii* is unable to grow at low temperature (15°C), while growth was slower between 20 and 25°C and highest radial growth was observed between 25 and 30°C. Survival in the absence of a host (e.g., over-wintering and over-summering) is also affected by temperature. The current study detected a reverse trend of growth in the exposure of two regimes of temperatures, which is in support of a previous report whereby only 15 generations were monitored (Kumar *et al.*, 2017).

Plant pathogens have three general possibilities related to adapt to a changing environment: (i) exploit the present phenotypic plasticity, (ii) migrate to areas with suitable climate, and/or (iii) evolve new characteristics (Oliva *et al.*, 1993). Temperature in particular has a primary role in the interaction between pathogen and their hosts, affecting epidemic outbursts and co-evolution processes. Plant pathogens can grow and reproduce across a wide range of temperatures and can tolerate large thermal variations in different environments. These thermal adaptations can be achieved through phenotypic plasticity and/or genetic differentiation (Zhang *et al.*, 2011). The potential influence of global warming on a species will depend on its thermal reaction norm and the underlying genetic dissimilarity for temperature sensitivity in the affected populations (Zhang *et al.*, 2011). While in some fungal pathogens adaptation to local temperatures results

mainly from genetic differentiation between populations (Zhang *et al.*, 2011), and the presence of widespread plasticity in response to thermal extremes in other fungal pathogens (Steffanson *et al.*, 2013) and therefore suggests that the lack of genetic variation will not necessarily limit species distribution under climate warming. Adaptive plasticity may also contribute directly to species intrusiveness by allowing rapid colonization of diverse new hosts without the need to undergo local selection (Williams *et al.*, 1995). Finally, individual plasticity may influence the patterns of evolutionary diversification at the population (and ultimately species) level by excluding selective deviation in environmentally distinct sites (Sultan and Spencer, 2002). The present study, at preliminary stage, unveiled a different facet of this fungus. This information opens a scope for further studies considering the temperature adaptability with increased number of generations.

4.5 Disease assessment :

To assess the varietal response of collar rot epidemics in cucurbits, an experiment considering 2 crops (cucumber – *Cucumus sativus* and bitter gourd – *Mimordica charantia*) including 4 varieties in each crop was conducted. Details of crop variety are given in table 3.2. Overall, 8 crop varieties (4 varieties/crop) were adjusted in a completely randomized design. To assure the propagule content of the pathogen and elimination of other microorganism in the soil, the experiments were performed with double autoclaved soil supplemented with sclerotia in the earthen pots (20 cm diameter) according to Sarma *et al.* (2002). Within a season (rainy or summer), the experiment was repeated twice.

Disease incidence (DI) was recorded higher in rainy season; the second attempt in rainy season rendered greater DI for all the varieties (fig. 4.7). Cucumber had significantly higher DI than bitter gourd in experiment 1 ($p \leq 0.05$); however, it was insignificant in experiment 2. Susceptibility of all the four tested varieties for cucumber was similar in experiment 2, whereas Cucumber Barsati and Green Long had significantly lower DI in experiment 1. The level of

susceptibility in bitter gourd varieties was also changed in experiment 2; particularly, Vishwas 228 and Vivek appeared with significantly greater DI ($p \leq 0.05$). A meagre DI obtained in the summer season for all the varieties (fig. 4.8). The range of DI varied between 1.3 and 8.0% for the two experiments conducted in summer. No comparison could be made for DI between the two crops as it showed a non-significant effect on DI ($p \geq 0.05$). However, in both of the experiments, Cucumber SS and Cucumber Barsati had significantly highest DI and Vishwas 228 had significantly lowest DI.

To understand the progress of collar rot epidemics in both of the crops, lesion length was assessed on final observation. With the help of this information area under lesion progress curves (AULPC) were developed for individual variety (McKay *et al.*, 2014). There was no effect of experiments detected on AULPC; therefore, analysis for individual season was done with the pooled observation (fig. 4.9). A wide range of AULPC was observed in the rainy season experiments; the range varied between 23.33 and 186.67 cm-days. Overall, rainy season expressed with greater AULPC than summer season. Cucumber varieties were infected in significantly greater extent. Larger lesion size was assessed in the cucumber varieties; therefore, significantly higher AULPC was obtained for cucumber. The cucumber varieties were insignificantly different for AULPC ($p \geq 0.05$). But the bitter gourd varities *viz.*, Parag, Vir and Vishwas 228 were shown significantly higher AULPC than vivek in the rainy season. AULPC varied between 80.07 and 13.41 cm-days in the summer experiments. In the summer season, larger lesion size was recorded for the cucumber varieties (fig. 4.9). Cucumber SS and Cucumber Barsati produced significantly higher and Green Long had lower AULPC. Intermediate reaction was observed for the cucumber variety Super Green. Lowest AULPC was observed in bitter gourd variety Vishwas 228.

Two most important environmental factors in the development of plant disease epidemics are temperature and moisture (Agrios, 2005). Elevated temperature coupled with high humidity provides favourable condition for the growth of the pathogen (Mboup *et al.*, 2012). This study also supports the above

rule. In the rainy season, significantly greater fungal infection was recorded compared to summer season. The sclerotia-forming pathogens are less effective in temperate regions (Agrios, 2005). Infection is often occurred when temperature and humidity coincide for a pathosystem (Agrios, 2005; Vati and Ghatak, 2015). Resistance or susceptibility of a crop variety is determined with its genetic make-up and also by the action of pathogen in the vicinity. If a virulence pathogen comes in the contact of its host, then the environment would play a critical role for the successful infection of the former. Moisture, in the form of free water or high humidity, is necessary for infection, reproduction, and spread in many plant pathogens, although some pathogens cause disease in dryer conditions. Because environmental conditions favourable to disease development vary greatly among plant pathogens; to its connection, it is vital to understand the environmental requirements of individual plant pathogens before predictions on responses to climate change can be made.

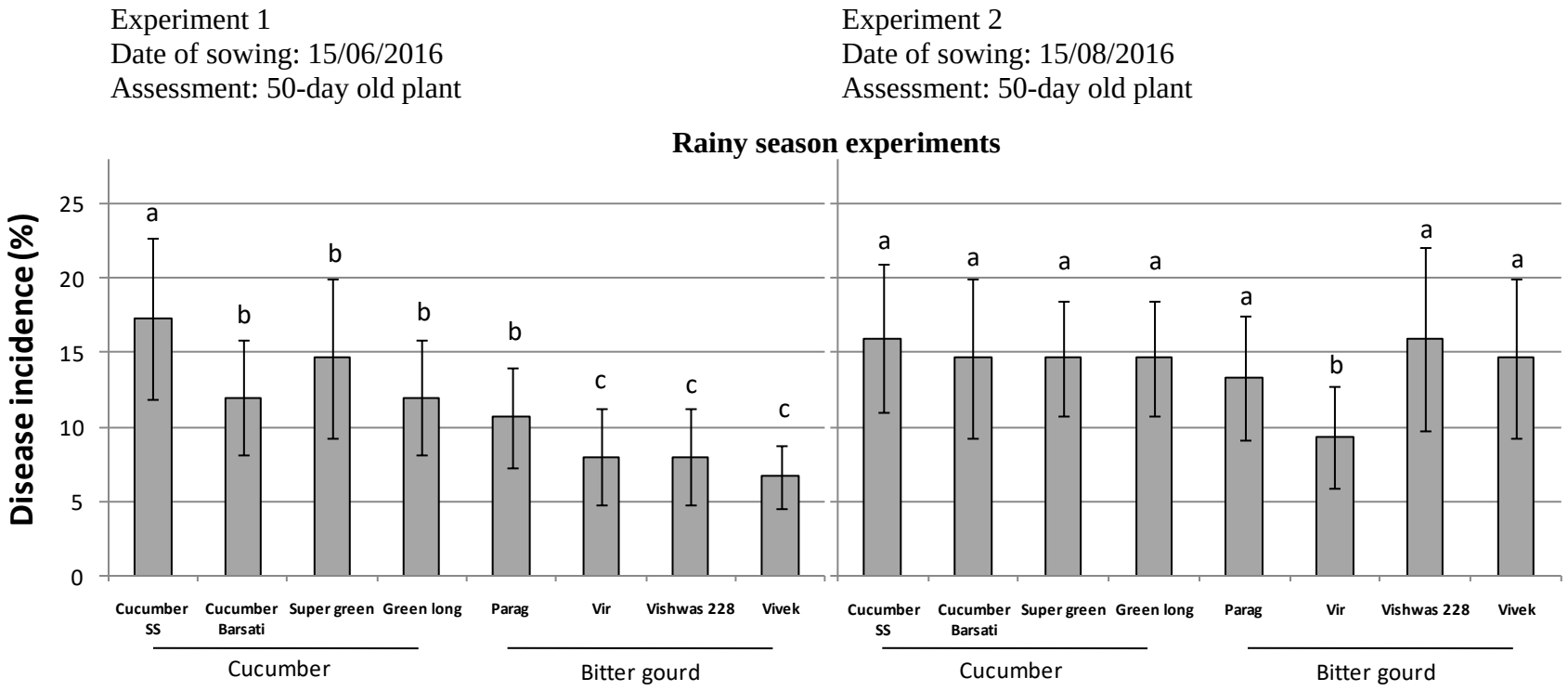

Figure 4.7 : Collar rot incidence estimated in rainy season. Within an experiment, bars with similar letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

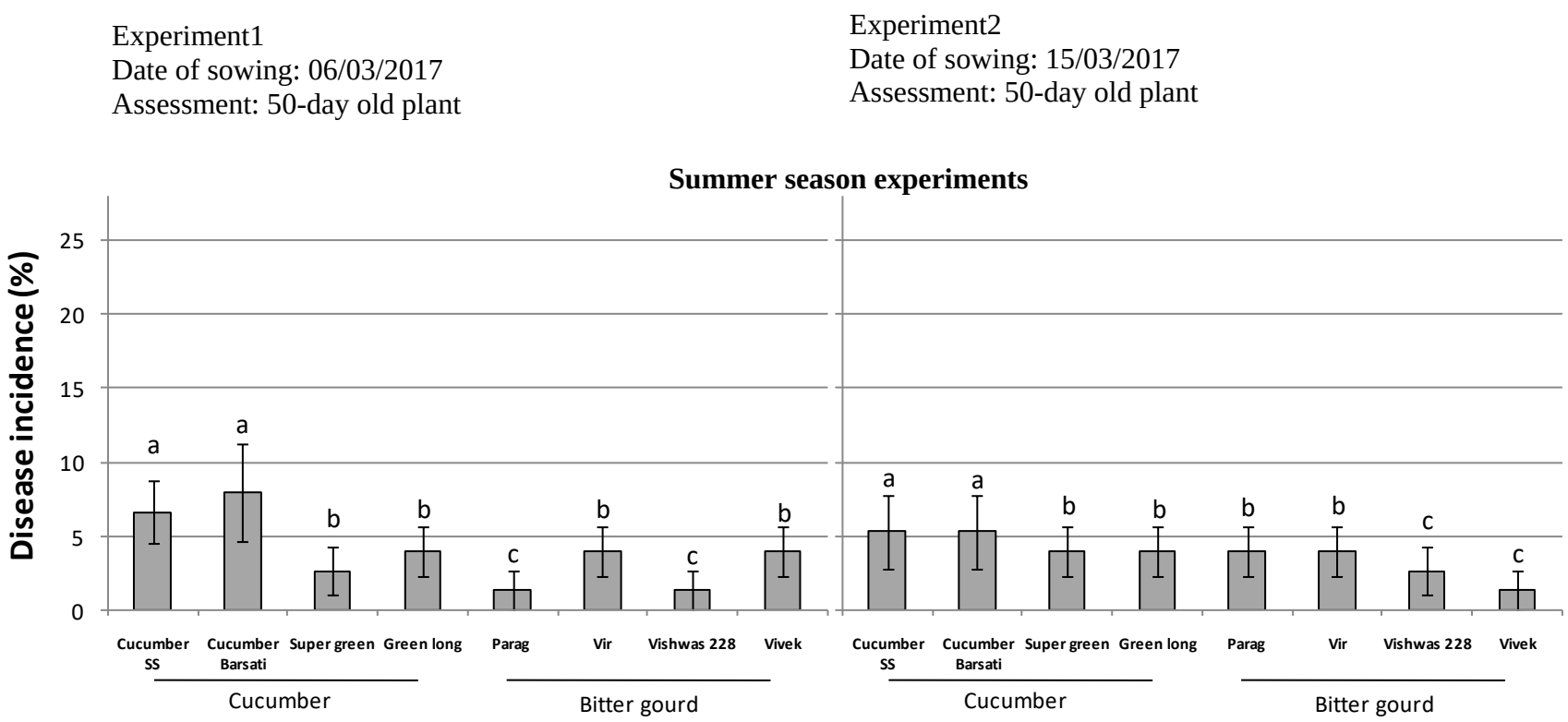

Figure 4.8 : Collar rot incidence estimated in summer season. Within an experiment, bars with similar letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.

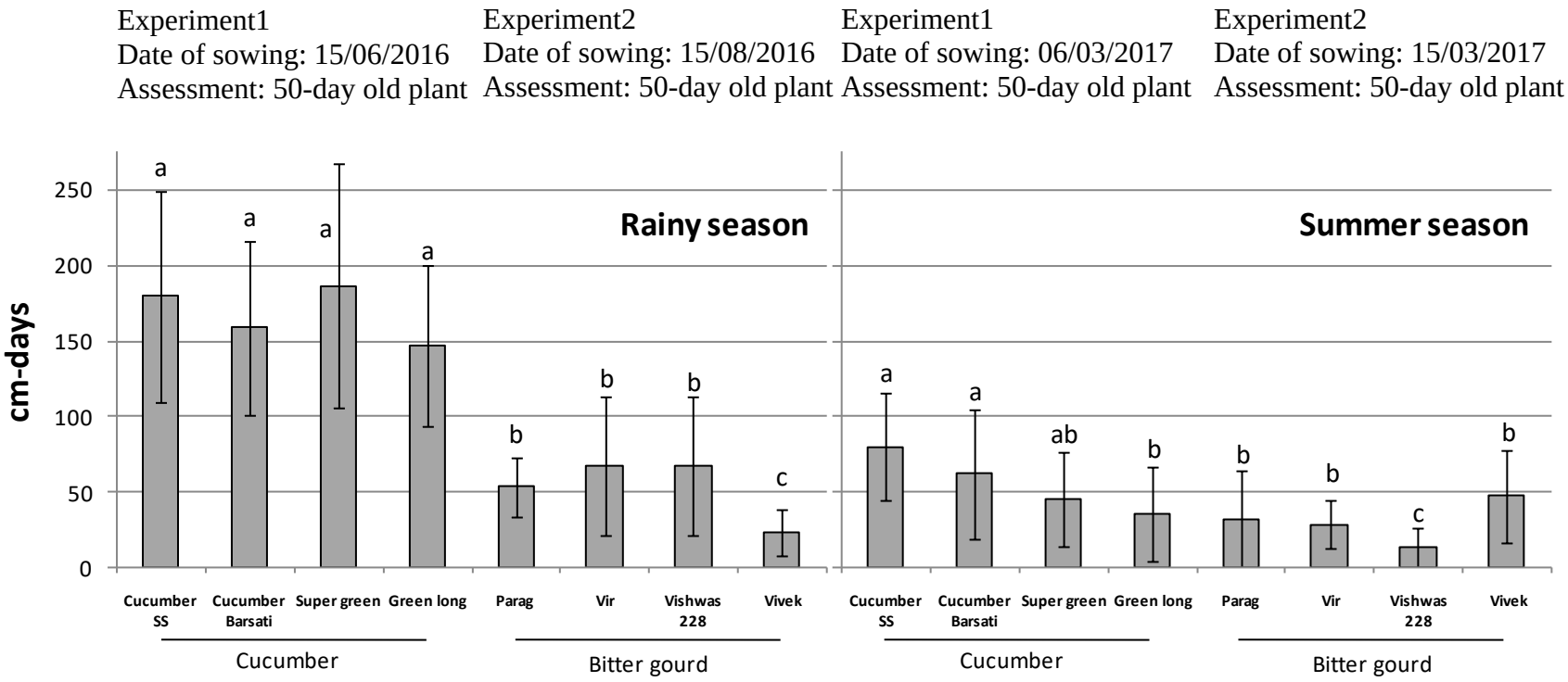

Figure 4.9 : Area under lesion progress curve of collar rot disease estimated in rainy season and summer season. Within a season, the experiment was repeated twice. For a season, bars with similar letter are significantly similar according to Duncan's multiple range test at $p \leq 0.05$.

Results with conclusion and recommendations are incorporated in this chapter

Chapter- 5

Summary

SUMMARY

In India, a number of major and minor cucurbits are cultivated, which share a large contribution in total vegetable production. Low productivity of cucurbits is attributed by many constrains including pathogenic stress. Among them, collar rot is a major problem in cucurbit cultivation. This disease is caused by *Sclerotium rolfsii*. The wide host range, prolific growth and ability to produce persistant sclerotia enable this pathogen for the potential economic loss in cucurbit production system. Therefore, this study was designed with three objectives, which could be summarised as follow:

(1) Variation is a rule in most of the root infecting fungi. Pathogens exhibits variation in terms of morphological, biological, chemical and their pathogenic character Wide variation among isolates of pathogen indicates genetic exchange in mycelia among the isolates is occurred in nature. This hypothesis has a vivid point because the sexual stage of this fungus is rare in nature and its role in the life cycle of the fungus is unknown.

(2) The current study advocates for development of fungicides based on chemical nature identical to hexaconazole, which is supported by mycelium inhibition property at low concentration associating the lowest effective concentration value. The other options to choose a fungicide effectively inhibit this pathogen is also disscued. Further, adaptability of the pathogen to high temperature was recognised, which suggests for development of 'new' modules of management for this pathogen in the field scale under scenario of changing climate associated with elevated temperature. Additionally, the forecasting models of *S. rolfsii* can be developed based on temperature adaptability.

(3) The collar rot epidemics varied between the crops and varieties, and the season; therefore, the growers are advised to select proper

seed material of the crops in order to attain a good harvest. Summer season had low disease incidence and area under lesion progress curve suggesting that an interaction of moisture and temperature is critical if high collar rot epidemics is aimed. The farmers are therefore advised to avoid a period with high temperature and discourage the yield loss due to this disease.

Each pathogen has got its own cardinal temperatue and understanding the temperature of the pathogen will help to standarize the management practices. But the adaptability of the pathogens towards higher temperature in the arena of global warming and climate change will disturb various models of crop protection so from the management side, these needed to be upgraded regularly.

This segment of the thesis contains the list of researchers and their citations

Bibliography

Bibliography

Adondonen, A. (2000). Damping off of cowpea in Benin and South Africa. M.Sc. Thesis. *University of Pretoria, South Africa.*

Aftab, A., Akhtar, N., Nafisa, Shoiab, A. and Malik, U. Z. (2012). *In vitro* screening methods using chemical fungicides against canola black spot pathogen. *Mycopath.,* **10** (2): 63-66.

Aggarwal, R., Banerjee, S., Sharma, S., Gupta, S. and Bashyal, B. M. (2011). Association of melanin content with conidiogenesis and virulence in *Bipolaris sorokiniana*. *J. Wheat Res.* **3** (1) : 29-32.

Agrawal, S. C. and Kotashane, S. R. (1971). Resistance in some of soybean varieties against *Sclerotium rolfsii* Sacc. *Indian Phytopathology,* **24** : 401-403.

Agrios, G. N. (2005). Plant Pathology. *Academic Press*, ISBN: 9780080473789.

Aken, C. N. and Dashiell, K. E. (1991). First report of southern blight caused by *Sclerotium rolfsii* on soybean in Nigeria. *Plant Dis.*, **75** : 537.

Akram, A., Iqbal, M., Qureshi, A. and Rauf, A. (2008). Variability among isolates of *Sclerotium rolfsii* associated with collar rot disease of chickpea in Pakistan. *Pak. J. Bot*., **40** (1): 453-460.

Almeida, A. M. R., Abdelnoor, R. V., Calvo, E. S., Tessnman, D. and Yorinori, J. T. (2001). Genotypic diversity among Brazilian isolates of *Sclerotium rolfsii*. *J. of Phytopathology,* **149** : 493.

Ammajamma, R. and Hegde, Y. R. (2009). Efficacy of fungicides against *Rhizoctonia bataticola* causing wilt of *Coleus forskohlii* (Wild) Briq. *Int. J. Pl. Protec*., **2** (1) : 31-32.

Ansari, M. M. (2012). Control of collar rot of soybean through biological control agents. *J. of Mycol and Plant Pathol*, **32** : 401.

Ansari, M. M. and Agnihotri, S. K. (2012). Morphological, physiological and pathological variations among *Sclerotium rolfsii* isolates of soybean. *Indian Phytopath.*, **53** (1) : 65-67.

Aycock, R. (1966). Stem rot and other diseases caused by *Sclerotium rolfsii*. *N C Agric Exp Stn Tech Bull*, **174** : 202.

Bagwan, N. B. (2010). Screening of *Sclerotium rolfsii* Sacc. isolates for tolerance and sensitivitiveness against commonly used fungicides. *Int jr. of Plant Protection*, **3** (1) : 137-142.

Bashyal, B. M., Chand, R., Kushwaha, C., Sen, D., Prasad, C. and Joshi, A. K. (2010). Association of melanin content with conidiogenesis in *Bipolaris sorokiniana* of barley (*Hordeum vulgare* L.). *World J. Microbiol Biotechnol*., **26** :309–316.

Beattie, J. H. (1954). Growing peanuts United States Department Agriculture. *Farmers Bull*, : 552.

Bell, A. A.and Wheeler, M.H. (1986). Biosynthesis and functions of fungal melanins. *Annual Review of Phytopathol.*, **24** : 411-451.

Boland, G. L., Melzer, M. S., Hopkin, A., Higgins, V. and Nassuth, A. (2004). Climate change and plant diseases in Ontario. *Can. J. Plant Pathol*., **26** : 335-350.

Boltan, M. D., Viviana, R. V., Mendoza, L., Khan, M. F. R. and Secor, G. A. (2012). Efficacy of variable tetraconazole rates against *Cercospora beticola* isolates with differing *in vitro* sensitivities to DMI fungicides. Plant Disease, **96** (12) : 1749-1756.

Bowen, K. L., Hagan, A. K. and Weeks, S. R. (1992). Seven years of *Sclerotium rolfsii* in peanut field; yield losses and means of minimum. *plant dis.*, **76** : 982-985.

Butler, E. J. and Bisby, G. R. 1931. Fungi in India. *Science Monograph, Indian Council of Agric. Res.* New Delhi : 552.

Campbell, C. L. and Neher, D. A. (1994). Estimating Disease Incidence and Severity. *Epidemiology and management of root disease*, **2** : 36-45.

Chakraborty, S. (1998). Potential impact of climate change on plant diseases of economic significance to Australia. *Australas. Plant Pathol*., **27** : 15-35.

Chakraborty, S., Tiedemann, A. V. and Teng, P. S. (2000). Climate change: potential impact on plant diseases. *Environmental Pollution*, **108** : 317-326.

Chattannavar, S. N. and Hosagoudar, G. N. (2011). Morphological characters and virulence studies of *Sclerotium rolfsii* Sacc. on *Bt* and non *Bt* cotton genotypes. *J. Cotton Res. Devp.* **25** (2) : 278-281.

Chauhan, L.S. (1977). In vitro studies on the effect of certain fungicides on *Sclerotium rolfsii*. *J.N.K.V.V. Res. Jour*., **112** (1-4) : 131-132.

Chaurasia, A., Chaurasia, S., Chaurasia, S. and Chaurasia, S. (2014a). Efficacy of antibiotics against *Sclerotium rolfsii* causing foot-rot of brinjal. *Journal of Microbiology*, **3** (1) : 1-4.

Chaurasia, S., Chaurasia, A., Chaurasia, S. and Chaurasia, S. (2014b). Pathological studies of *Sclerotium rolfsii* causing foot rot disease of brinjal (*Solanum melongena* Linn.). *Int. J. of Pharm. Life Sci.*, **5** (1) : 3257- 3264.

Cheit, I. (1975). Ultrastructural basis of *Sclerotium rolfsii* survival in soil. *Microbial Ecology*, **2** : 194-200.

Chen, J. P. and Lee, M. S. (1995). Enhanced production of *Serratia marcescens* chitinase in PEG/ dextran aqueous two-phase system. *Enzyme Microbial Technol.,* **17** : 1021-1027.

Chen, L. C., Yu, R. C., Chen, C. W., Yang, Y. Z. and Young, H. C. (1998). Sclerotial formation types and sclerotial fine structure of *Sclerotium rolfsii* in Taiwan. *Pl. Prot. Bull. Taipei*., **40** (1) : 25-35.

Choudhary C. S., Singh S. N. and Prasad, S. M. (2004). *In vitro* effectiveness of chemicals to control *Macrophomina phaseolina* (Tassi.) Goid, causing stem and root rot of sesame. *J. Appl. Biology*., **14** (1) : 46-47.

Coakley, S. M., Scherm, H. and Chakraborty, S. (1999). Climate change and disease management. *Annual Review of Phytopathology*, **37** : 399-426.

Cooper, W. E. (1961). Strains of *Sclerotium rolfsii* resistance to antagonists. *Phytopathology*, **51** : 113-116.

Cosic, J., Jurkovic, D., Vrandecic, K. and Kaucic, D. (2012). Survival of buried sclerotia in undisturbed soil. *Helia*, **35** (56) : 73-78.

Curzi, M. (1931). Studies on *Sclerotium rolfsii*. *Patol veg roma*, Ser, **2** (11) : 306-373.

Dandnaik, B. P., Dhoke, P. K. and Pensalwar, S.N. (2006). Integrate management of stem rot (*Sclerotium rolfsii*) in groundnut. *J. Pl. Dis. Sci.* **1** (2) : 234-235.

Datar, V. V. and Bindu, K. J. (1974). Collar rot of sunflower, A new host record from India. *Curr.Sci*., **43** : 496.

DeSilva, M. R., Martinelli, J. A., Carlos, L. and Pacheco, T. (2012). Lesion size as a criterion for screening oat genotype for resistance to leaf spot. *European Jornal of Plant Pathology*, **134** (2) : 315-327.

Dixon, G. R. (2012). Climate change – impact on crop growth and food production, and plant pathogens. *Can. J. Plant Pathol*., **34** : 362–379.

Edson, H. A. and Shapvalov, M. (1923). Parasitism of *Sclerotium rolfsii* on Irish potatoes. *J.Agric.Res*., **23** : 4-46.

Ellis, D.H. and Griffiths, D.A. (1974). The location and analysis of melanin's in the cell walls of semi soil fungi. *Canadian Journal of Microbiology*, **20** : 1379-1386.

Ferrandino, F. J. and Elmer, W. H. (1992). Reduction in tomato yield due to Septoria leaf spot. *Plant Disease,* **76** : 208-211.

Fogarty, R. V. and Tobin, J. M. (1995). Fungal melanins and their interaction with metals. *Enzyme and Microbial Technology,* **19** : 311-317.

Gadd, G. M. (1982). Effects of media composition and light on colony differentiation and melanin synthesis in *Microdochium bolleyi* : *Transactions of British Mycological Society,* **78** : 115-122.

Garret, S. D. 1956. Biology of root infecting Fungi, *Cambridge University press,* London, U.K. : 293.

Garrett, K., Dendy, S., Frank, E., Rouse, M. and Travers, S. (2006). Climate change effects on plant disease: genomes to ecosystems. *Annual Review of Phytopathology,* **44** : 489–509.

Gashaw, G., Alemu, T. and Tesfaye, K. (2014). Evaluation of disease incidence and severity and yield loss of finger millet varieties and mycelial growth inhibition of *Pyricularia grisea* isolates using biological antagonists and fungicides in vitro condition. *Journal of Applied Biosciences,* **73** : 5883-5901.

Gautam, H. R., Bhardwaj, M. L. and Kumar, R. (2013). Climate change and its impact on plant diseases. *Current Science,* **105 :** 12.

Ghatak, A., Kushwaha, C., Gupta, R. N., Singh, K. P. and Ansar, M. (2017). Variability in sensitivity among different host origin-*Macrophomina phaseolina* isolates to azoxystrobin fungicide. *International Journal of Plant Protection,* **10** (1) : 26-33.

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research. *John wiley and sons,* NewYork, **2** : 680.

Gour, H. N. and Sharma, P. (2010). Evaluation of fungicides in vitro and in vivo against *Sclerotium rolfsii* Sacc. causing root rot of groundnut. *Indian Phytopath.,* **63** (3) : 352-353.

Gregory, P. J., Johnson, S. N., Newton, A. C. and Ingram, J. S. I. (2009). Integrating pests and pathogens into the climate change/food security debate. *J. Exp. Bot*., **60** : 2827-2838.

Griefenhagen, S., and Noland, T.L. (Compilors). (2003). A synopsis of known and potential diseases and parasites associated with climate change. *Ontario Forest Research Institute, Ontario Ministry of Natural Resources, Sault Ste Marie, Ont., Forest Research Information,* Paper No. 154.

Hanumanthegowda, B. (1999). Studies on stem rot of groundnut caused by *Sclerotium rolfsii* Sacc. M.Sc. (Agri). Thesis, *Uni. of Agric. Sci,* Dharwad : 37-38.

Harlapur, S. I. (1988). Studies on some aspects of root rot of wheat caused by *Sclerotium rolfsii* Sacc. M.Sc. (Agri). Thesis, *Uni. of Agril Sci,* Dharwad : 64.

Heidari, M. (2009). Antioxidant activity and osmolyte concentration of sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*) genotypes under salinity stress. *Asian. J. Plant Sci*., **8** : 240-244.

Hernandez, M. C. A. and Ysla, L. H. (1997). Variability among *Sclerotium rolfsii* Sacc. isolates in cultural, morphological and pathogenic characteristics. *Fitopathologia*., **32** (3) : 182-186.

Higgins, B. B. (1927). Physiology and parasitism of *Sclerotium rolfsii* Sacc. *Phytopathology*, **17**: 417-448.

Issac, S. (1994). Many fungi are brightly coloured; does pigmentation provide any advantage to those species? *Mycologist*, **8** (4) : 178-179.

Jaimin, R. K. and Jain, S. C. (2010). Effect of fungicides on root rot of cluster bean caused by *Macrophomina phaseolina*. *Environment and Ecology*, **28** (2A) : 1138-1140.

Khan, M. A. and Gangopadhaya, (2008). Efficiency of Pseudomonas fluorescens in controlling Root rot of chickpea caused by *Macrophomina phaseolina. J. Myco. Pl. pathol*., **38** (3) : 580-587.

Kim, K. (1974). Studies on *Sclerotium rolfsii* Sacc. isolated from *Mangolia kabus* in Korea. *Korean J.Pl. Prot.,* **13** : 105-133.

Kolte, S. J. and Tewari, A. N. (1977). Note on effect of planting dates on occurrence and severity of sunflower diseases. *Pant. J. Res*., 2 : 236.

Kudela, V. (2009). Potential impact of climate change on geographic distribution of plant pathogenic bacteria in Central Europe. *Plant Prot. Sci.,* **45** : 27-32.

Kuldhar, D. P., Suryawanshi, A. P. and Wagh, S. S. (2014). Cultural and morphological variability among the isolates of *Sclerotium rolfsii,* causing stem / pod rot of Groundnut. *Trends in Biosciences,* **7** (24) : 4167-4173.

Kumar, R., Ghatak, A., and Bhagat, A. P. (2017). Exploration of *Sclerotium rolfsii* adapting high temperature regime in successive generation. National conference on climate change and agricultural production: Adapting crops to climate variability and uncertainty, April 6-8 : 552-553.

Kumar, S., Thind, T. S. and Mohanchander (1955). Morphogenic and pathogenic variation in *Gleosporium ampelophagum*. *Indian Phytopathology,* **48** : 331-334.

Lattanzio, V., Lattanzio, V. M. T. and Cardinali, A. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochemistry: Advances in Research, ISBN*: 81-308-0034-**9** : 23-67.

Lee, W. F., Shen, K., Huang, Y., Wang, Y., Yin, J., Luo, Z., Zhang, Y.and Shan, H. (2014). Incidence of sugarcane ratoon stunting disease in the major canegrowing regions of China. *Crop Protection*, **60** : 44-47.

Maddan, C. and Lucca, G. B. (1992). Introduction to plant diseases ; identification and management. *Van Nostriend Reinhold,* Newyork : 364.

Madden, L. V., Hughes, G. and Bosch, F. V. (2007). The study of plant disease epidemics. *The American Phytopathological Society Press, St Paul, MN, USA*, **23** : 345-351.

Mahen, V.K., C,D. Mayer, T.B. Brennemen and McDonald, D. (1995). Stem and pod rots of groundnut. *Information Bulletin No. 4*, ICRISAT, India : 28.

Manjappa, B. H. (1979). Studies on the survival and variation in *Sclerotium rolfsii* Sacc. M.Sc. (Agri) Thesis, *Uni. of Agri Sci. Bangalore*, : 72-74.

Manu, T.G., Nagaraja, A., Chetan S. and Janawad, H. V. (2012). Efficacy of fungicides and biocontrol agents against *Sclerotium rolfsii* causing foot rot disease of finger millet, under *in vitro* conditions. *Global J. Biol. Agri. Health Sci.*, **1** (2) : 46 50.

Maurya, S., Singh, R., Singh, D. P., Singh, H. B., Srivastava, J. S. and Singh, U. P. (2007). Phenolic compounds of *Sorghum vulgare* in response to *Sclerotium rolfsii* infection. *Journal of Plant Interactions*, **2** (1) : 25-29.

Mayee, C. D. and Datar (1988). Diseases of groundnut in Tropics. *Rev. Trop. Pl. Path*., **5** : 85-118.

Mboup, M., Bahri, B., Leconte, M., De Vallavieille-Pope, C., Kaltz, O. and Enjalbert, J. (2012). Genetic structure and local adaptation of European wheat yellow rust populations: the role of temperature specific adaptation. *Evol. Appl*., **5** : 341-352.

McClintock, J. A. (1917). Peanut wilt caused by *Sclerotium rolfsii*. *J. of Agric. Res*., **8** : 441-448.

Mcelrone, A. J., Reid, C. D., Hoye, K. A., Hart, E. and Jackson, R. B. (2005). Elevated CO_2 reduces disease incidence and severity of a red maple fungal pathogen via changes in host physiology and leaf chemistry. *Global Change Biol*., **11** : 1828-1836.

McKay, S. F., Shtienberg, D., Sedgley, M. and Scott, E. S. (2014). Anthracnose on almond in Australia : disease progress and inoculum sources of *Colletotrichum acutatum. European Journal of Plant Pathology*, **139** (4) : 773-783.

Mehan, V. K., Mayee, C. D., McDonald, D., Ramakrishanan, N. and Jayanthi, S. (1995). Resistance in groundnut to *Sclerotium rolfsii* caused stem and pod rots. *International J. of Pest Manag*., **41** : 79-82.

Mehrotra, R. S. and Aneja, K. R. (1990). An introduction to Mycology. *Wilen Eastern Ltd*. New Delhi, : 776.

Mishra, B. K. and Bais, B. S. (1987). Studies on seedling blight and foot rot of barley caused by *Sclerotium rolfsii*. *Indian phytopathology,* **40** : 161-167.

Mishra, S., and Singh, H.B. (2015). Silver nanoparticles mediated altered gene expression of melanin biosynthesis genes in *Bipolaris sorokiniana*. Microbiological Research, **172** : 16–18.

Mohan, L., Paranidharan, V. and Prema, S. (2000). New disease of timla fig (*Ficusauriculata*) in India. *Indian Phytopathol.,* **53**: 496.

Moradia, A. M. and Khandar, R. R. (2011). Loss of yield of groundnut (*Arachis hypogaea* L.) due to dry root rot (*Macrophomina phaseolina*) and their management under *in vivo* condition. *Internat. J. agric. Sci.,* **7** (2) : 282-285.

Mukharjee, A., Mohapatra, N. and Nayak, P. (2009). Estimation of area under the disease progress curves in a rice-blast pathosystem from two data points. *Eur J Plant Pathol.,* DOI 10.1007/s10658-009-9568-2.

Mundkur, B. B. (1934). Perfect stage of *Sclerotium rolfsii* Sacc. in culture. *Indian J. Agric. Sci*., **4** : 779-782.

Muthukumar, A. and Venkatesh, A. (2013). Effect of light and aeration on the growth of *Sclerotium rolfsii in vitro*. *African Journal of Biotechnology***, 12** (49) : 6843-6846.

Nalim, F.A., Starr, J., Woodard, K., Segner, S. and Keller, N. (1995). Mycelial compatibility groups in Texas peanut field populations of *Sclerotium rolfsii*. *Phytopathology*, **85** : 1507-1512.

Nandi, S., Dutta, S., Mondal, A., Adhikary, A., Nath, R., Chattopadhyaya, A. and Choudhary, S. (2013). Biochemical responses during the pathogenesis of *Sclerotium rolfsii* on cowpea. *African Journal of Biotechnology*, **12** (25) : 3968-3977.

Nargund, V. B. (1981). Studies on foot rot of wheat caused by *Sclerotium rolfsii* Sacc. in Karnataka. M.Sc. (Agri) Thesis, *Uni. of Agric. Sci*, Bangalore : 78-79.

Navas-Castillo, J., Fiallo-Olive, E. and Sanchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annu. Rev. Phytopathol*., **49** : 219-248.

Nguyen, T. N., Tran, V. M., Nguyen, T. T. and Le, C. N. (2004). Research on groundnut diseases in Quang Binh province. *Natl. Agric. Rural Dev*, **17** : 337-342.

Nyvall, R. F.(1989)). Field crop Diseases Handbook, Second Edition, Published by *Van Nartrand Renhold, New York, U.S.A*, : 817.

Okereke, V. C. and Wokocha, R. C. (2007). In vitro growth of four isolates of *Sclerotium rolfsii* in the humid tropics. *Afric. J. Biotech*., **6**: 1879-1881.

Oliva, G., Martinez, A., Collants.,M. and Dubcovsky, J. (1993. Phenotypic plasticity and contrasting habitat colonization in *Festuca pallascense. Canadian Journal of Botany,* **71** : 970-971.

Palakshappa, M. G. (1986). Studies on foot rot of betelvine caused by *Sclerotium rolfsii* Sacc.in Karnataka. M.Sc. (Agri). Thesis, *Univ. of Agric. Sci*, Bangalore : 62.

Pandey, M. K., Singh, D. P. and Singh, U. P. (2005). Phenolic acid changes in mycelia of *Sclerotium rolfsii* after garlic and onion supplementation in a broth medium. *Mycobiology,* **33** (3) : 137-141.

Passardi, F., Cosio, C., Penel, C. and Dunand, C. (2005). Peroxidases have more functions than a Swiss army knife. *Plant Cell Rep*., **24** (5) : 255-265.

Petkovsek, M. M., Stampar, F. and Veberic, R. (2008). Increased Phenolic content in apple leaves infected with the apple scab pathogen. *Journal of Plant Pathology*, **90** (1) : 49-55.

Prasad, L., Sujatha, K., Naresh, N. and Chander Rao, S. (2012). Variability of *Sclerotium rolfsii* associated with collar rot of sunflower. *Indian Phytopath*., **65** (2) : 161-165.

Prithviraj, B., Singh, U. P., Kobayashi, K., Sugawara, K. M., Nishimura, R. and Sakaiwada, A. (1996). Variability in sclerotial morphology in *Sclerotium rolfsii* : SEM and isoezyme studies. *J. Plant Dis. Prot*., **103** : 495-501.

Punja, Z. K. (1985). The biology, ecology and control of *Sclerotium rolfsii*. *Annu. Rev. Phytopathology*., **23** : 97-127.

Punja, Z. K. (1988). *Sclerotium* (*Athelia*) *rolfsii*, a pathogen of many plant species. In: Sidhu, G. S. eds. *Genetics of plant pathogenic fungi*, **6** : 523–534.

Punja, Z. K. and Gregan, R. G. (1983). Basidiocarp induction, nuclear condition, variability and heterokaryon incompatibility in *Sclerotium rolfsii*. *Phytopathology*, **73** : 1273-1278.

Punja, Z. K. and Rahe, J. E. (1992). *Sclerotium*. : In: Singleton, L. L., Mihail, J. D., Rush, C. M. eds. Methods for research on soilborne phytopathogenic fungi. St. Paul : APS Press : 166–170.

Radwan, I. A., El-Wakil, A. A., Satour, M. M. and Zayed, M. A. (1987). Comparative morphological and physiological studies on certain isolates of *Sclerotium rolfsii*. *Egypt Res. Rev*., **63** : 59-69.

Rai. M., Ratan, V. and Srivastava, S. S. L. (2008). Management of root rot of urd bean (*Vigna mungo*) caused by *Macrophomina phaseolina*. *Farm Sci. J*., **14** (1) : 89.

Rajagopal, K., Kathiravan, G. and Karthikeyan, S. (2011). *African Journal of Microbiology*, **5** (1) : 145-151.

Ramakrishanan, T. S. (1930). A wilt of Zinnia caused by *Sclerotium rolfsii*. *Madras Agric. J*., **16** : 511-519.

Rao, V. V. R., Rao, K. C., Shantaram, M. V. and Reddy, M. S. (1998). Management of seed and seedling diseases of groundnut (*Arachis hypogaea L*.) through seed treatment. *Indian J. Pl. Protec*., **26** (1) : 1-8.

Ravishankar, J.P., Muruganandam, V. and Suryanaryanan, T.S. (1995). Isolation and characterization of melanin from a marine fungus. *Botanica Marina*, **38** : 413-416.

Reddikumar, M., Madhavisanthoshi, M. V., Giridharakrishna, T. and Rajareddy, K. (2014). Cultural and morphological variability of *Sclerotium rolfsii* isolates infecting groundnut and its reaction to some fungicidal. *International Journal of Current Microbiology and Applied Sciences*, **3** (10) : 553-561.

Reddy, M. R., Fazalnoor, K. and Hedge, R. K. (1971). Screening of commercial varieties and genetic stocks of wheat against foot rot caused by *Sclerotium rolfsii* Sacc. *Mysore J. of Agric. Sci*., **5** : 252-256.

Richhariya, R.B. (1984). Phsiological and enzymological studies on *Sclerotum rolfsii* (Sacc.) causing foot rot of Tomato (*Lycopersicon esculentum* Mill.). *M.Sc. Thesis, A.P.S. University Rewa M.P., India.*

Riker, A. J. and Riker, R. S. (1936). Introduction to Research on Plant Disease. *John S. Swift Co. No.4, New York, U.S.A.*

Rolfs, P. A. (1892). Tomato blight some hints. *Bulletin of Florida Agricultural Experiment Station*, U.S.A. : 18.

Saccardo, P. A. (1911). Notae Mycologiae. *Annual Mycology*, **9**: 249-257.

Saccardo, P. A. (1913). Sclerotium rolfsii. Sylloge Fungorum XXII. Pavia, Italy : 1500.

Saikia, R., Singh, B. P., Kumar, R., Arora, D. K. (2004). Detection of pathogenesis-related proteins -chitinase and β -1,3-glucanase in induced chickpea. *Curr Sci*., **89** (4) : 659-663.

Sarma, B. K., Singh, U. P. and Singh, K. P. (2002). Variability in Indian isolates of *Sclerotium rolfsii. Mycologia,* **94** (6) : 1051–1058.

Schemitz, H. (1930). Poisoned food technique. *Indust. Engin. Chem. Analyst*, 361.

Sengupta, P. K. and Das, C. R. (1970). Studies on some isolates of *Sclerotium rolfsii*. Z. *pflackranth P. Fl Schutz*, **77**: 582-584.

Shalby, S. I. M. (1997). Effect of fungicidal treatment of sesame seeds on root rot infection, plant growth and chemical components. *Bulletin of Faculty of Agril. Univ. of Cairo*, **48** (2) : 397-411.

Sharma, B. S. and Pathak, V. N. (1994). Yield and sucrose losses in sugarbeet due to root rot. *Indian Phytopathology*, **47** : 408-411.

Shaw, F. J. P. and Ajrekar, S. L. (1915). The genus *Rhizoctonia* in India. Memories of department of agriculture in India. *Botanical Series*, **7** : 177-194.

Shilpashree, K., Patil, M. S. and Uma, M. S. (2013). Estimation of total phenols and peroxidase isozyme in plants infected with blackeye cowpea mosaic disease. *Karnataka J. Agric. Sci.,* **26** (2) : 318-319.

Shokes, F. M., Berger, R. D., Smith, D. S. and Rasp, J. M. (1987). Reliability of disease assessment procedure ; a case study with leaf spot of peanut. *Oliageneaux,* **42** : 245-251.

Shukla, R. and Pandey, A. K. (2007). Diversity in isolates of *S.rolfsii* from Central India. *J. Mycol. Plant. Pathol.,* **37** : 3.

Siddaramaiah, A. L. (1988). Stem, sheath and leaf rot disease of cardamom caused by *Sclerotium rolfsii* from India. *Curr. Res*., **16** : 82.

Siddaramaiah, A. L. and Chandrappa, H. M. (1988). New collar rot disease on *Desmodium uncimatum* and *Lutononis bainesii* from India. *Curr. Res*., **16** : 83.

Singh, U. P. and Pavgi, M. S. (1965). Spotted leaf rot of plants - a new sclerotial disease. *Pl Dis Reptr,* **49** : 58-59.

Stefansson, T. S., Mc Donald, B. and Willi, Y.(2013). Local adaptation and evolutionary potential along a temperature gradient in the fungal pathogen *Rhynchosporium commune*. *Evolutionary Applications,* **6** : 524-34.

Subramanian, C. V. (1971). Hyphomycetes, An account of Indian species, except *Cercospora*. ICAR, New Delhi.

Sultan, S. E. and Spencer, H. G. (2002). Metapopulation structure favors plasticity over local adaptation. *American Naturalist,* **160** : 271-283.

Sun, M. H., Gao, L., Shi, Y. X., Li, B. J., Liu, X. Z. (2006). Fungi and actinomycetes associated with *Meloidogyne spp*. eggs and females in China and their bio-control potential. *J. Invertebr Pathol*., **93** (1) : 22-28.

Surekha, C., Neelapu, N., Prasad, B. S. and Ganesh, P. S. (2014). Induction of defence enzymes and phenolic content by *Trichoderma viridae* in Vigna mungo infested with *Fusarium oxysporum* and *Alternaria alternata*. *International Journal of Agricultural Science and Research,* **4** (4) : 31-40.

Suryanarayan, T. S., Jagadesa, P., Govindan, V. and Thokur, S. (2004). Characterization of the melanin pigment of a cosmopolitan fungal endophyte. *Mycol. Res*., **108** (8) : 974-978.

Tandel, D. H., Sabalpara, A. N., Pandya, H. V. and Naik, R. M. (2010). Effect of leaf blight (*Macrophomina phaseolina*) on growth parameters and yield of greengram and its chemical control. *Int. J. Pl Protect*., **3** (2) : 329-331.

Thomas, M. (1955). Melanins. In (K.Patch and M.V.Tracey,Eds): Modern Methods of plant Analysis. *Springer – Verlag, Berlin,* **4** : 661-675.

Vati, L., and Ghatak, A. (2015). Phytopathosystem modification in response to climate change. In: Climate Dynamics in Horticultural Science: Impact, Adaptation, and Mitigation; Choudhary, M. L., Patel, V. B., Siddiqui, M. W., Verma. R. B., Eds.; CRC Press: Florida : 161–178.

Venkatesh, T., Adiver, S. S., Kenchangoaudar, P. V., Virupakshaprabhu, H. and Math, G. (2012). Morphological characters and biochemical composition of sclerotia of *Sclerotium rolfsii* Sacc. isolated from groundnut. *Karnataka J. Agric. Sci..*, **27** (2) : 242-244.

Weber, G. F. (1931). Blights of carrot caused by *Sclerotium rolfsii* with geographic distribution and host range of the Fungi. *Phytopathol*., **21** : 103-109.

Wheeler, B. E. J. (1969). An introduction to plant diseases. Published by the *English language Book Society and John Wiley and Sons Ltd., London,* : 374.

William, D. J., Mack, R. N. and Black, R. A. (1995). Ecophysiology of introduced *Pennisetum setaceum* on Hawaii: the role of phenotypic plasticity. *Ecology*, **76** : 1569-80.

Wilson, C. (1953). Preventing the Diseases of Peanut. *United state Department of Agriculture Year Book,* : 448-454.

Woods, A., Coates, K. D. and Hamann, A. (2005). Is an unprecedented Dothistroma needle blight epidemic related to climate change? *BioScience*, **55** : 761-769.

Xue, L.,Charest, P.M. and Jabaji-Hare, S.H. (1998). Systemic induction of peroxidases, 1,3-β-glucanases, chitinases and resistance in bean plants by binucleate *Rhizoctonia* Species. *Biol Control*, **88** (4) : 359-365.

Yang, C., Hamel, C., Vujanovic, V. and Gan, Y. (2011). Fungicide:modes of action and possible impact on nontarget microorganisms. *ISRN Ecology*, doi:10.5402/2011/130289.

Yaqub, F. and Shahzad, S. (2005). Pathogencity of *Sclerotium rolfsii* on different crops and effect of inoculum density on colonization of mungbean and sunflower roots. *Pak. J. Bot*, **37** : 175-180.

Yaqub, F. and Shahzad, S. (2011). Efficacy and persistance of microbial antagonists against *Sclerotium rolfsii* under field condition. *Pak. J. Bot*., **43** (5) : 2627-2634.

Zhang, J. and Mc Donald, B. (2011). Thermal adaptation in fungal pathogen *Mycospharela graminicola*. *Molecular Ecology*, **20** : 1689-1701.

Zheng, W., Bradley, S. C., McDaugall, B. and Seviour, R. J. (2017). Effects of melanin on the accumulation of exopolysaccharides by *Aureobasidium pullulans* grown on nitrate. *Bioresources technology*, **99** (16) : 7480-7486.