

Studies on management of  
*Sclerotium rolfsii* Sacc. affecting  
*Zea mays* L. crop

THESIS

Submitted to  
Babasaheb Bhimrao Ambedkar University  
(A Central University)  
Lucknow



In fulfillment for the Award of

**Doctor of Philosophy**

IN  
ENVIRONMENTAL SCIENCE

SUPERVISOR  
**PROF. S.K. DWIVEDI**

SUBMITTED BY  
**GANESH PRASAD**

DEPARTMENT OF ENVIRONMENTAL SCIENCE  
SCHOOL FOR ENVIRONMENTAL SCIENCES  
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY  
(A Central University)  
Vidya Vihar, Raebareli Road  
Lucknow - 226025

Enrollment No.:1175/15

Year - 2020



**DEDICATED  
TO  
MY PARENTS  
AND  
TEACHERS**

## Urkund Analysis Result

**Analysed Document:** thesis\_ganeshat86@gmail.com.docx (D62334665)  
**Submitted:** 1/14/2020 11:17:00 AM  
**Submitted By:** gbl.bbau@gmail.com  
**Significance:** 2 %

### Sources included in the report:

[https://www.researchgate.net/publication/336605140\\_Biological\\_Control\\_of\\_Sclerotium\\_rolfsii\\_by\\_Using\\_Indigenous\\_Trichoderma\\_spp\\_Isolates\\_from\\_Palestine](https://www.researchgate.net/publication/336605140_Biological_Control_of_Sclerotium_rolfsii_by_Using_Indigenous_Trichoderma_spp_Isolates_from_Palestine)  
[https://www.researchgate.net/figure/Mycelial-and-sclerotial-inhibition-of-Sclerotium-rolfsii-by-different-antagonists-Dual\\_tbl1\\_329339762](https://www.researchgate.net/figure/Mycelial-and-sclerotial-inhibition-of-Sclerotium-rolfsii-by-different-antagonists-Dual_tbl1_329339762)  
<https://www.cabi.org/ISC/abstract/20123331604>  
<https://www.semanticscholar.org/paper/Efficacy-of-Trichoderma-against-Sclerotium-rolfsii-Kushwaha-Kumar/d63166c9e36b4106effa56e3655fcb3fdb769c1e>  
[https://www.researchgate.net/publication/274570157\\_In\\_Vitro\\_Antagonism\\_between\\_Phytopathogenic\\_Fungi\\_Sclerotium\\_rolfsii\\_and\\_Trichoderma\\_Strains](https://www.researchgate.net/publication/274570157_In_Vitro_Antagonism_between_Phytopathogenic_Fungi_Sclerotium_rolfsii_and_Trichoderma_Strains)  
<https://bioinfopublication.org/downpdf.php?artid=BIA0004188eb418b08-7b7f-481c-a93a-2dc5127512f1>  
<https://www.ijcmas.com/7-5-2018/Md.%20Shahzaman%20Ahsan,%20et%20al.pdf>

### Instances where selected sources appear:

# DECLARATION

---

I **Ganesh Prasad**, declare that the thesis entitled “**Studies on management of *Sclerotium rolfsii* Sacc. affecting *Zea mays* L. crop**” which is being submitted to Department of Environmental Science, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow in fulfillment of the degree of **Doctor of Philosophy** in Environmental Science has previously not formed the basis for award of any such degree by any university. This is my original research work carried out during 2015-2020 and also declared that the thesis is essentially free from all kinds of plagiarism.

**Date:** 14-02-2020

**Place:** Lucknow

*Ganesh Prasad*

**(Ganesh Prasad)**

Enrollment No. 1175/15

## **CERTIFICATE**

This is certify that the thesis entitled “**Studies on management of *Sclerotium rolfsii* Sacc. affecting *Zea mays* L. crop**” submitted by **Mr. Ganesh Prasad** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations-1999 as an amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

**Date:** 14.02.2020



**Supervisor**



**Head of the Department**

**Head**

Dept. of Environmental Science  
B. B. Ambedkar University  
Rae Bareilly Road, Lucknow-22

## ACKNOWLEDGEMENTS

---

*First of all, 'I praise God,' the almighty for his unparalleled grace, protection and guidance throughout my Ph.D. journey. I want to thank Lord for giving me patience, health, wisdom, strength and resources throughout my research period to complete the research work successfully. I would like to acknowledge all those who helped me to complete this thesis.*

*At this moment of accomplishment of my Ph.D. thesis, the first and foremost person to whom I would like to articulate my deep and utmost gratitude is my supervisor **Prof. S. K. Dwivedi**, Dean, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow, who gave me an opportunity to do research work under his guidance. I owe deepest gratitude for his excellent supervision, invaluable guidance, suggestions, incredible support and ceaseless motivation throughout the course of my Ph.D. During my Ph.D., he always trained me and appreciated each small step that I took during my research work.*

*I express my deepest gratitude to Hon'ble Vice Chancellor **Prof. Sanjay Singh** for encouragement and his blessings.*

*I would also like to thank former Heads of Department **Prof. D. P. Singh** and **Prof. S. K. Dwivedi** and present Head of the Department **Prof. N. K. Arora**, BBA University, Lucknow for providing lab facilities carried out during the research work.*

*I also thank Prof. R. P. Singh for his valuable advices time to time.*

*I would like to thank all faculty members of the Department viz; Mr. N. K. S. More, Dr. Shikha, Dr. Venkatesh Dutta, Dr. Narendra Kumar, Dr. Richa K. Tyagi and Dr. Jiwan Singh for their immense support, invaluable suggestion and coordinational co-operations during the progress of research work.*

*I am also thankful to non-teaching staff of the Department of Environmental Science Mr. A. K. Jain, Mr. Aviral Dixit, Mr. Ranjit Sharma and Mr. Nagesh Tripathi for their official help and support during the research work.*

*I gratefully acknowledge the University Grant Commission (UGC), New Delhi for providing financial support in the form of UGC Non-NET fellowship.*

*I would also like to thank my senior Dr. Sangeeta for her strong moral support and suggestions rendered to me during the research work.*

*I would like to thank my seniors Dr. Deepak Agrahari, Dr. Ram Gopal, Dr. Rima Kumari, Dr. Swadesh Kumar, Dr. Namita Gupta, Dr. Jitendra Mishra, Dr. Shamsad Ahmad, Dr. Dhanaanjay, Dr. Mahesh, Dr. Arya Pandey, Mrs. Neha Gupta for their constant inspiration and motivation.*

*I wish to give my cordial thanks to my friends Pradeep Kumar Majhi, Pawan Kumar, Pradeep Kumar, Miss Nisha Yadav, Mrs. Kanchan Sonkar, Ranjan Singh, Mrs. Shailja Singh,*

*Radhey Shyam Sharma, Mrs. Garima Singh, Mrs. Priya Mishra, Vinay Kumar, Ajay Raj Tripathi, Suresh Kumar, Ashwani Kumar Chaudhary and Priyanka for their co-operation and affection.*

*Finally, I would like to thank all my family members. Words are not enough me to express my love and respect towards them, especially my mother Mrs. Bhanmati. I would like to thank my elder brothers Mr. Omkar Nath Bind and Mr. Diwakar Bind, brothers's wife Mrs. Durgawati and Mrs. Foolpatti for unparalleled love, affection, protection, motivation and financial support. I would also like to thank my wife Mrs. Puja Bind and my lovely daughter Prisha Bind for their co-operation, affection and patience.*

*Lastly, my thanks go to all those who have directly or indirectly supported me to complete my research work.*

*Ganesh Prasad*

**(Ganesh Prasad)**

## PREFACE

---

*Zea mays* is cereal grain, origin of Central America is now worldwide distributed such as India, Thailand, Pakistan and China. United State of America ranked first position and India is at the six<sup>th</sup> position in production of corn. It is most versatile emerging crop have wide adaptive. The crop is growing in alluvial soil (sandy loam) and distributed in Northern India. For the production of maize the pH of alluvial soil ranged 6 to 7. On the basis of size and endosperm composition, there are different types of hybrid maize such as dent corn, flint corn, popcorn and sweet corn. In kharif season, it is sown in March to July; in Rabi season, it is sown in September to December. Maize contains 80% carbohydrate, 10% protein, 3.5% fiber, 2% mineral and remaining 4.5% vitamin. The whole part of the maize has economically important for food and other products. *Zea mays* belongs to Kingdom- Plantae, Division- Magnoliophyta, Class- Liliopsida, Order –Poales, Family –Poaceae, Genus- *Zea*, Species- *mays*.

*Zea mays* is infected from soil borne fungus *Sclerotium rolfsii* causing root-rot disease. *Sclerotium rolfsii* is also known as white mold plant pathogen. The soil-borne plant pathogen causes various plant diseases viz. collar-rot, *Sclerotium* wilt, seedling blight, damping-off of seedlings, stem canker, crown blight, crown, bulb rot, tuber rot, fruit rot, crown rot, stem-rot, charcoal rot, foot-rot, stem blight and root-rot in more than 500 plants species including tomato, chilli, maize, groundnut, sunflower, cucumber, brinjal, soybean, bean and watermelon. *Sclerotium rolfsii* belongs to Kingdom- Fungi, Phylum- Basidiomycota, Class-Agaricomycetes, Order –Agaricales, Family –Typhulaceae, Genus- *Sclerotium*, Species- *rolfsii*.

*Sclerotium rolfsii* is effectively controlled by bioagents (viz. *Aspergillus flavus*, *A. niger*, *Trichoderma viride*, *Penicilium citrinum*, *P. italicum*, *Penicilium* sp.), plant extracts (viz. *Azadirachta indica*, *Ocimum tenuiflorum*, *Syzygium aromaticum*, *Tinospora cordifolia*, *Trachyspermum ammi*) and chemical fungicides (viz. Myclobutanil 10% WP, Carbendazim 50% WP and Conika 50% WP) and composite *Trichoderma viride* metabolite + Myclobutanil10%WP, *Ocimum tenuiflorum* + Myclobutanil10%WP, *Trichoderma viride* metabolite + *Ocimum tenuiflorum* and *Trichoderma viride* metabolite + *Ocimum tenuiflorum* + Myclobutanil10%WP under *in vitro* and *in vivo* condition. All selected bioagents, plant extracts and chemical fungicides significantly suppressed root rot disease incidence in *Zea mays*.

# CONTENTS

---

Declaration .....	I
Certificate.. .....	II
Acknowledgements.....	III
Preface.....	VI
Contents.....	VII
List of Tables .....	X
List of Figures.....	XIII
Abbreviations.....	XVI

---

CHAPTER	DESCRIPTION	PAGE NO.
---------	-------------	----------

---

<b>I.</b>	<b>INTRODUCTION</b>	<b>1-5</b>
-----------	---------------------	------------

---

1.1	Distribution	2
1.2	Economic Importance	2
1.3	<i>Sclerotium rolfsii</i> : overview	3
1.4	Management of root-rot disease in <i>Zea mays</i>	4

---

<b>OBJECTIVES</b>	<b>6</b>
-------------------	----------

---

<b>II.</b>	<b>REVIEW OF LITERATURE</b>	<b>7-25</b>
------------	-----------------------------	-------------

---

2.1.	Factors affecting soil fungal population	8
2.1.1.	Effect of pH on soil microbes	8
2.1.2.	Effect of moisture content on soil microbes	9
2.1.3.	Effect of Temperature on microbes	10
2.1.4	Effect of soil organic matter on microbes	10
2.2.	Effect of Chemicals on <i>Sclerotium rolfsii</i>	10
2.3.	Effect of Bioagent on <i>Sclerotium rolfsii</i>	12
2.4.	Effect of Plant extracts on <i>Sclerotium rolfsii</i>	18
2.5.	Effect of Integrated management on <i>Sclerotium rolfsii</i>	19

---

<b>III.</b>	<b>MATERIALS AND METHODS</b>	<b>26-40</b>
-------------	------------------------------	--------------

---

3.1.	Collection of samples	26
3.2.	Analysis of physico-chemical properties of Soil	26
3.2.1.	Soil texture	26
3.2.2.	pH and Temperature	27
3.2.3.	Percentage of organic carbon in the soil	27

3.2.3.1.	Apparatus	27
3.2.3.2.	Reagents	27
3.2.3.3.	Procedure	28
3.2.4.	Percentage of organic matter in the soil	29
3.2.4.1.	Reagents	29
3.2.4.2.	Procedure	30
3.3.	Isolation and identification of soil mycoflora	30
3.3.1.	Percent occurrence and percent frequency of soil mycoflora	31
3.3.2.	Screening and Maintenance of antagonistic mycoflora	31
3.4.	Isolation and Identification of Pathogen	31
3.5.	Antagonistic activity of some fungal species against <i>Sclerotium rolfsii</i>	32
3.6.	Preparation of metabolite of bioagents	33
3.6.1.	Treatment of <i>Sclerotium rolfsii</i> with metabolite of bioagents	33
3.7.	Effect of chemicals against <i>Sclerotium rolfsii</i>	34
3.8.	Preparation of medicinal plant extracts	34
3.8.1.	Treatment of <i>Sclerotium rolfsii</i> with plant extracts	35
3.9.	Evaluation of integrated management of <i>Sclerotium rolfsii</i> under <i>in vitro</i> condition	36
3.10.	Preparation of Inocula of <i>Sclerotium rolfsii</i> and <i>Trichoderma viride</i>	36
3.11.	Procurement of <i>Zea mays</i> seeds	37
3.12.	Pathogenicity test	37
3.13.	Treatment of <i>Sclerotium rolfsii</i> with bioagent, plant extract and chemical under <i>in vivo</i> condition	38
3.14.	Evaluation of integrated management of root rot disease in <i>Zea mays</i> under <i>in vivo</i> condition	38
3.15.	Histopathological study	39
3.16.	Data analysis	40

---

<b>IV.</b>	<b>RESULTS AND DISCUSSION</b>	<b>41-107</b>
------------	-------------------------------	---------------

---

4.1.	Sample collection	41
4.2.	Analysis of Physico-chemical properties of collected field soil samples	41
4.3.	Isolation and identification of soil mycoflora	43
4.4.	Pathogenicity test	49
4.5.	Evaluation of antagonistic activity of some fungal species against <i>Sclerotium rolfsii</i>	50
4.6.	Treatment of <i>Sclerotium rolfsii</i> with some chemicals	53

4.7.	Effect of plant extracts against <i>Sclerotium rolfsii</i>	60
4.8.	Effect of metabolites of mycoflora against <i>Sclerotium rolfsii</i>	69
4.9.	Evaluation of integrated management of <i>Sclerotium rolfsii</i> under <i>in vitro</i> condition	81
4.10.	<i>In vivo</i> evaluation of selected bioagent, plant extract and chemical against root rot disease in <i>Zea mays</i>	89
4.10.1.	Efficacy of Bioagent against <i>Sclerotium rolfsii</i>	89
4.10.2.	Efficacy of plant extract against <i>Sclerotium rolfsii</i>	90
4.10.3.	Efficacy of chemical fungicide against <i>Sclerotium rolfsii</i>	92
4.11.	Evaluation of integrated management of root rot disease in <i>Zea mays</i> under <i>in vivo</i> condition	97
4.12.	Histopathological study	104

---

<b>V.</b>	<b>SUMMARY</b>	<b>108-112</b>
-----------	----------------	----------------

---

<b>VI.</b>	<b>CONCLUSION</b>	<b>113-116</b>
------------	-------------------	----------------

---

	<b>RECOMMENDATION</b>	<b>117</b>
--	-----------------------	------------

---

	<b>REFERENCES</b>	<b>118-147</b>
--	-------------------	----------------

---

	<b>ANNEXURE - I</b>	<b>148-150</b>
--	---------------------	----------------

---

	<b>ANNEXURE - II</b>	<b>151-166</b>
--	----------------------	----------------

---

# LIST OF TABLES

---

---

TABLE NO.	DESCRIPTION
<b>Table 2.1:</b>	Control of pathogen of some important crops using antagonistic bioagents, botanicals and chemicals
<b>Table 4.1:</b>	Analysis of sand, silt, clay, organic carbon, organic matter, pH and temperature from collected soil samples
<b>Table 4.2:</b>	Percent (%) occurrence and percent frequency of soil mycoflora collected from soil samples at $10^{-3}$ dilution (1/1000) of 1g soil
<b>Table 4.3:</b>	Percent (%) occurrence and percent frequency of soil mycoflora collected from soil samples at $10^{-4}$ dilution (1/10000) of 1g soil
<b>Table 4.4:</b>	Percent (%) occurrence and percent frequency of soil mycoflora collected from soil samples at $10^{-5}$ dilution (1/100000) of 1g soil
<b>Table 4.5:</b>	Antagonistic activity of some bio-agents against mycelial growth (in mm) of <i>Sclerotium rolfii</i> using dual culture technique under <i>in vitro</i> condition
<b>Table 4.6:</b>	Effect of Myclobutanil 10%WP against mycelial growth (in mm) of <i>Sclerotium rolfii</i> at different concentration and incubation period under <i>in vitro</i> condition
<b>Table 4.7:</b>	Effect of Conika 50%WP against mycelial growth (in mm) of <i>Sclerotium rolfii</i> at different concentration and incubation period under <i>in vitro</i> condition
<b>Table 4.8:</b>	Effect of Carbendazim 50%WP against mycelial growth (in mm) of <i>Sclerotium rolfii</i> at different concentration and incubation period under <i>in vitro</i> condition
<b>Table 4.9:</b>	Effect of <i>Azadirachta indica</i> leaf extract at different concentration and incubation period under <i>in vitro</i> condition against mycelial growth (in mm) of <i>Sclerotium rolfii</i> over control
<b>Table 4.10:</b>	Effect of <i>Ocimum tenuiflorum</i> leaf extract at different concentration and incubation period under <i>in vitro</i> condition against mycelial growth (in mm) of <i>Sclerotium rolfii</i> over control

- Table 4.11:** Effect of *Syzygium aromaticum* oil at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control
- Table 4.12:** Effect of *Tinospora cordifolia* stem extract at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control
- Table 4.13:** Effect of *Trachyspermum ammi* seeds powder at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control
- Table 4.14:** Effect of metabolite of *Aspergillus flavus* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*
- Table 4.15:** Efficacy of metabolite of *Aspergillus niger* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*
- Table 4.16:** Toxicity of culture filtrate of *Trichoderma viride* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*
- Table 4.17:** *In vitro* evaluation of toxicity of culture filtrate of *Penicillium citrinum* at different concentration and incubation period against mycelial growth (in mm) of *Sclerotium rolfsii*
- Table 4.18:** Culture filtrate toxicity of *Penicillium italicum* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*
- Table 4.19:** Culture filtrate toxicity of *Penicillium* sp. at different concentration and incubation period against mycelial growth (in mm) of *Sclerotium rolfsii* under *in vitro* condition
- Table 4.20:** Effect of composite *Trichoderma viride*+Myclobutanil 10% WP at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*
- Table 4.21:** Efficacy of composite *Ocimum tenuiflorum*+Myclobutanil 10% WP at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*
- Table 4.22:** Effect of composite *Ocimum tenuiflorum*+*Trichoderma viride* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

**Table 4.23:** Effect of composite *T.viride*+Myclobutanil 10%WP+ *Ocimum tenuiflorum* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

**Table 4.24:** Effect of *Ocimum tenuiflorum* leaf extract, *Trichoderma viride*, Myclobutanil 10% WP at different concentration against root rot of *Zea mays* and recorded shoot length, root length, fresh weight, dry weight on 50<sup>th</sup> day under *in vivo* condition

**Table 4.25:** Treatment *Sclerotium rolfsii* with *Ocimum tenuiflorum* leaf extract, *Trichoderma viride*, Myclobutanil 10% WP at different concentration under *in vivo* condition and percent disease incidence and percentage of healthy plants on 30<sup>th</sup> and 50<sup>th</sup> day under *in vivo*

**Table 4.26:** Effect of composite *Trichoderma viride*+Myclobutanil 10% WP, *Ocimum tenuiflorum*+ Myclobutanil 10% WP, *Trichoderma viride*+*Ocimum tenuiflorum*, *T. viride*+*Ocimum tenuiflorum*+ Myclobutanil 10% WP at different concentration under *in vivo* condition on shoot length, root length, fresh weight, dry weight of *Zea mays* plant on 50<sup>th</sup> day

**Table 4.27:** Effect of composite *Trichoderma viride*+Myclobutanil 10% WP and *Ocimum tenuiflorum*+ Myclobutanil 10% WP on *Sclerotium rolfsii* at different concentration under *in vivo* condition and percent disease incidence in *Zea mays* plants on 30<sup>th</sup> and 50<sup>th</sup> day

**Table 4.28:** Effectiveness of different treatment on root-rot disease incidence in *Zea mays* due to *Sclerotium rolfsii* at different concentration under *in vivo* condition on 30<sup>th</sup> and 50<sup>th</sup> day



# LIST OF FIGURES

---

---

FIGURE NO.	DESCRIPTION
<b>Fig. 4.1:</b>	The field infected from <i>Sclerotium rolfsii</i> and ‘→’ (arrow) indicate the hyphae expansion in the field of <i>Zea mays</i> crop.
<b>Fig. 4.2:</b>	Different colony of soil mycoflora, screening from soil using serial dilution technique
<b>Fig. 4.3:</b>	Pathogenicity test of <i>Sclerotium rolfsii</i>
<b>Fig. 4.4:</b>	Antagonistic activity of <i>Aspergillus flavus</i> , <i>A. niger</i> , <i>Penicilium citrinum</i> , <i>P. italicum</i> , <i>Penicilium</i> sp. and <i>Trichoderma viride</i> against growth of <i>S. rolfsii</i> on 3 <sup>rd</sup> day
<b>Fig. 4.5:</b>	Percent inhibition of mycelial growth of <i>Sclerotium rolfsii</i> treated with (A) Bioagent (B) Myclobutanil 10% WP under <i>in vitro</i> condition
<b>Fig. 4.6:</b>	Percent inhibition of mycelial growth of <i>Sclerotium rolfsii</i> treated with (A) Conika 50% WP (B) Carbendazim 50% WP under <i>in vitro</i> condition
<b>Fig. 4.7:</b>	Antifungal activity of chemical fungicide (A) Myclobutanil 10% WP (B) Carbendazim 50% WP (C) Conika 50% WP at different concentration against <i>Sclerotium rolfsii</i> using poisoned food technique on 3 <sup>rd</sup> day incubation period
<b>Fig. 4.8:</b>	Percent inhibition of mycelial growth of <i>Sclerotium rolfsii</i> treated with (A) <i>Azadirachta indica</i> leaf extract (B) <i>Ocimum tenuiflorum</i> leaf extract under <i>in vitro</i> condition
<b>Fig. 4.9:</b>	Percent inhibition of mycelial growth of <i>Sclerotium rolfsii</i> treated with (A) <i>Syzygium aromaticum</i> oil (B) <i>Tinospora cordifolia</i> under <i>in vitro</i> condition

- Fig. 4.10:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with *Trachyspermum ammi* seeds powder under *in vitro* condition
- Fig. 4.11:** Antifungal activity of (A) *Azadirachta indica* (B) *Ocimum tenuiflorum* (C) *Tinospora cordifolia* against *Sclerotium rolfsii* using poisoned food technique on 3<sup>rd</sup> day incubation period
- Fig. 4.12:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with culture filtrate of (A) *Aspergillus flavus* (B) *Aspergillus niger* under *in vitro* condition
- Fig. 4.13:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with culture filtrate of (A) *Penicillium citrinum* (B) *Penicillium italicum* under *in vitro* condition
- Fig. 4.14:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with culture filtrate of (A) *Penicillium* sp (B) *Trichoderma viride*
- Fig. 4.15:** Preparation of metabolite of Bioagents using broth culture
- Fig. 4.16:** Treatment of *Sclerotium rolfsii* with metabolite of (A) *Aspergillus flavus* (B) *Aspergillus niger* using food poisoned technique on 3<sup>rd</sup> day incubation period
- Fig. 4.17:** Treatment of *Sclerotium rolfsii* with metabolite of (A) *Penicillium citrinum* (B) *Penicillium italicum* using food poisoned technique on 3<sup>rd</sup> day incubation period
- Fig. 4.18:** Treatment of *Sclerotium rolfsii* with metabolite of (A) *Penicillium* sp. (B) *Trichoderma viride* using food poisoned technique on 3<sup>rd</sup> day incubation period
- Fig. 4.19:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with (A) *T. viride*+Myclobutanil 10%WP (B) *Ocimum tenuiflorum*+Myclobutanil 10%WP under *in vitro* condition
- Fig. 4.20:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with (A) *Ocimum tenuiflorum*+*T.viride* (B) *T.viride*+Myclobutanil 10%WP+*Ocimum tenuiflorum* under *in vitro* condition

- Fig. 4.21:** *Sclerotium rolfsii* treated with composite (A) *Trichoderma viride* + Myclobutanil 10% WP (B) *Ocimum tenuiflorum* + Myclobutanil 10% WP using food poisoned technique under *in vitro* on 3<sup>rd</sup> day incubation period
- Fig. 4.22:** *Sclerotium rolfsii* treated with composite (A) *Trichoderma viride* + *Ocimum tenuiflorum* (B) *Trichoderma viride* + *Ocimum tenuiflorum* + Myclobutanil 10% WP using food poisoned technique under *in vitro* on 3<sup>rd</sup> day incubation period
- Fig. 4.23:** Treatment of root rot disease in *Zea mays* with (A) Myclobutanil 10% WP (B) *Ocimum tenuiflorum* (C) *Trichoderma viride*; As the incubation period was increased the plants became healthier from 15<sup>th</sup> day to 50<sup>th</sup> day
- Fig. 4.24:** Treatment of root rot disease in *Zea mays* with Myclobutanil 10% WP, *Ocimum tenuiflorum* and *Trichoderma viride* on 50<sup>th</sup> day
- Fig. 4.25:** Treatment of root rot disease in *Zea mays* as integrated management with (A) *Trichoderma viride*+Myclobutanil 10% WP (B) *Ocimum tenuiflorum*+ Myclobutanil 10% WP (C) *Trichoderma viride*+ *Ocimum tenuiflorum*; As the incubation period was increased the plants became healthier from 15<sup>th</sup> day to 50<sup>th</sup> day
- Fig. 4.26:** Treatment of root rot disease in *Zea mays* as integrated management with *Trichoderma viride*+Myclobutanil 10% WP, *Ocimum tenuiflorum*+ Myclobutanil 10% WP and *Trichoderma viride*+ *Ocimum tenuiflorum*
- Fig. 4.27:** Pure culture of *Sclerotium rolfsii* and scanning electron microscope (SEM) images at x500 and x1000 magnification
- Fig. 4.28:** T.S. of sclerotia of *Sclerotium rolfsii* in scanning electron microscope (SEM) images at different magnifications
- Fig. 4.29:** T.S. of root of *Zea mays* infected from *Sclerotium rolfsii* in scanning electron microscope (SEM) images at different magnifications
- Fig. 4.30:** T.S. of stem of *Zea mays* infected from *Sclerotium rolfsii* in scanning electron microscope (SEM) images at different magnifications
-

# ABBREVIATIONS

---

%	Percent
@	at
<sup>0</sup> C	Degree Celsius
ANOVA	Analysis of Variance
cm	Centimeter
CV%	Coefficient of Variation
DMRT	Duncan's Multiple Range test
Fig.	Figure
gm	gram
i.e.	that is
Kg	Kilogram
mL	milliliter
mm	millimeter
PDA	Potato Dextrose Agar
pH	Potential of Hydrogen
ppm	parts per million
rpm	Revolutions per minute
SD	Standard Deviation
SEM	Scanning Electron Microscope
sp.	Species
V/V	Volume by Volume
V/W	Volume by Weight
viz.	Namely
W/V	Weight by Volume
W/W	Weight by Weight
WP	Wettable Powder
WG	Water-dispersible granules

---

# CHAPTER - 1

## INTRODUCTION



# INTRODUCTION

---

*Zea mays* is commonly known as Makka in Hindi and corn in English which is short day, large grain crop belonging to family Poaceae. Maize is mostly 2 to 3 meter in height, internodes occurs every 15 to 20 centimeters, each leaf grows 70 to 100 cm in height. Maize is a tall, annual C<sub>4</sub> plant grows in temperate zone and in sandy soil. The corn have many types viz. dent corn, pod corn, flint corn, sweet corn, pop corn, flour corn, normal yellow, white grain, baby corn, waxy corn, high oil corn, high amylase corn and quality protein maize (Kumar et al., 2012). Corn oil is used for cooking and manufacture of soaps; seeds are used in alcohol and stem fiber is used in manufacturing of the paper industry. Corn seed is used in production of ethanol which is used as bio-fuel.

*Zea mays* is infected from bacteria, fungi and pest but fungus disease like root-rot is more dangerous caused by *Sclerotium rolfsii*. *Sclerotium rolfsii* is soil-borne fungus which grows in favorable condition on the maize crop. It was first observed by Rolfs (1892) on tomato plants with 70% losses. The hyphae grew upward on the outside of the infected plant stem, covered with a cottony white mass of mycelium, scattered outside and inside of stem nearby the soil surface. The fungus produced many tiny round white sclerotia of the same size when immature and dark brown on mature stage (Kwon and Park, 2002) and sclerotia diameters ranged 4-8 mm in size which is characteristic feature of *S. rolfsii*. *Sclerotium rolfsii* develops at soil moisture level (70%) of field capacity and temperature ranged between 25<sup>0</sup>C to 30<sup>0</sup>C (Pinheiro et al., 2010).

## 1.1. Distribution

*Zea mays* is cereal grain, origin of Central America but it is now world's wide distributed such as India, Thailand, Pakistan and China. United State of America ranked first position and India is at the six<sup>th</sup> positions in production of corn. Maize is also known as queen of cereals because it contains maximum genetic yield potential (Kumar et al., 2012).

On the basis of colour and taste, the maize can be classified into two groups:

- a. Yellow
- b. White

Yellow maize is used for animal feed. It is grown in northern hemisphere countries.

White maize is generally used for food crop. It is grown in limited countries.

On the basis of size and endosperm composition, there are different types of hybrid maize such as dent corn, flint corn, popcorn, and sweet corn.

In kharif season, it is sown in March to July; in Rabi season, it is sown in September to December. The whole part of the maize has economically important for food and other products (Hareesh, 2016).

## 1.2. Economic Importance

The maize seeds are nutritious and edible that serves as good source of carbohydrates, protein and fat compared to other cereals. It also contain vitamin A, C, K and vitamin B-complex such as B1 (thiamine), B2 (niacin), B3 (riboflavin), B5 (pantothenic acid) and B6 which maintain hair, skin, digestion, heart and brain. Maize contain large amount of betacarotene and selenium that helps in treatment of thyroid gland. Maize syrup is useful in preparing of jams, jellies and other sweets. Saponin, allantoin, alkaloids, sterol, stigmasterol, hordenine and polyphenols are phytochemical metabolites found in maize leaves, seeds and silk. The antioxidant

found in corn seeds removed free radical which is responsible for cellular damage and cancer. Potassium in corn silk is responsible for powerful diuretic and also improves blood pressure and liver function. The corn oils are used for cooking and manufacture of soaps. Corn seeds are used for alcohol production and stem fibres for manufacturing of paper (Kumar and Jhariya, 2013). Maize seeds are used for manufacturing of corn starch, corn syrup, gluten, grain cake, corn oil dextrose, lactic acid and acetone that are used in various industries such as textile and fermentation. Maize contains 80% carbohydrate, 10% protein, 3.5% fiber and 2% mineral (Oladejo and Adetunji, 2012). In India, it covered 85% areas during cultivation season. It is most important cereal crop after wheat and rice. Mostly maize seeds are used in poultry feed industry. All poultry feed industry generally depend on maize for poultry food due to high nutrient quality. The total production of poultry food in India only 50-60% is used for broiler hen and 25-35% for layer hen.

### **1.3. *Sclerotium rolfsii*: overview**

*Sclerotium rolfsii* is also known as white mold and a dreaded soil-borne (Sacchi et al., 2016) plant pathogen, symbiotic, parasite and saprophyte fungus which is responsible for causing various diseases on crops like collar-rot, sclerotium wilt, seedling blight, stem canker, bulb rot, crown blight, tuber rot, fruit rot, crown rot, systemic wilt or blight of whole plant, stem-rot, charcoal rot, foot-rot, stem blight and root-rot in more than 500 plants species including tomato, chilli, maize, groundnut, sunflower, cucumber, brinjal, soybean, bean, watermelon etc (Dwivedi and Prasad, 2016; Gopalakrishnan et al., 2005; Kwon and Park, 2002; Aycock, 1966; Punja, 1985). The infected *Zea mays* crop showed reduced growth and floppy and finally crop died. The white cottony mycelia are formed on the stems and roots of the infected crop at the temperature range in between 25-30<sup>0</sup> C with numerous round

shaped brown sclerotia (Han et al., 2012). *Sclerotium rolfsii* infects many economically important plants at different stages of growth and development. Pathogen firstly infect lower portion of plant and after then it infect whole parts of the plant (Mahadevakumar et al., 2016).

*Sclerotium rolfsii* produces cutinolytic enzymes viz. Pectinases and cellulases which degrade cuticle (Linskines and Haage, 1963). The humid season is favourable for sclerotial germination and mycelial growth; the sclerotia persist in the soil for several years under unfavourable condition. They grow with white mycelium and cover the soil surface and produced mycelial mats. After then large number of sclerotia appeared on the surface of mycelial mats (Paul et al., 2017). Under dry condition, the white mycelium extends around the surface of stem. Infected plant parts become restricted to contact with the soil. The infected vascular bundles of plant root become brownish (Siddique et al., 2018).

#### **1.4. Management of root-rot disease in *Zea mays***

There are many ways to control the diseases by using different practices such as rotation of crop, chemical pesticides and use of resistant varieties. The crop rotation and resistant varieties are best way; however, pesticides has affected physical, chemical and biological property of soil (Rahman et al., 2017). *Sclerotium rolfsii* is controlled through chemicals, biologicals, plant extracts and integrated management as well.

Biological control of plant pathogen is an important method for plant disease management by using bioagents viz. *Aspergillus flavus*, *A. niger*, *Trichoderma viride*, *Penicilium citrinum*, *P. Italicum*, *P. oxalicum* and *Penicilium* sp. These have been reported as strong antagonistic activity than others against mycelial growth of *Sclerotium rolfsii*.

Pushpker and saler (2011) studied that leaf extract of *Parthenium hysterophous* and *Calotropis gigantea* were more effective to check the growth of *S. rolfsii* at 50% concentration using food poisoned technique under *in vitro* condition. The leaf extract of plants viz. *Annona squamosa* Linn, *Brassica campestris* Linn and *Ocimum sanctum* Linn were more effective against *Sclerotium rolfsii* under *in vitro* condition at difference concentration (Quadri and Fatima, 2016). Khalequzzaman (2016) studied that lentil seed treatment with Provax 200 significantly controlled root and foot rot caused by *Sclerotium rolfsii*. Some chemicals viz. Index (Myclobutanil 10% WP), Bavastin (Carbendazim 50% WP) and Conika 50% WP (Kasugamycin 5% + Copper oxychloride 45 % WP) were tested at different concentration against mycelial growth of *Sclerotium rolfsii* under *in vitro* condition using poisoned food technique. Fungal bioagent is including *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp. and chemical fungicide including tolclofos methyl, thiram carboxin, fludioxonil, azoxystrobin metalaxyl-M control the mycelial growth of *S. rolfsii* under *in vitro* condition (Akgul et al., 2011).

*Sclerotium rolfsii* was effectively controlled with composite of some bioagent, plant extract and chemical under *in vitro* and *in vivo* condition. *In vitro* experiment was carried out by using two or more bioagent, plant extract and chemical with each other at different concentration against *S. rolfsii* such as bioagent with plant extract, bioagent with chemical and plant extract with chemical. This integrated management was effective against *S. rolfsii*. *In vivo* experiment was carried out in pot. In this experiment, the plant extract, bioagent and chemical were treated separately as well as jointly against *S. rolfsii* causing root-rot disease in *Zea mays*. In case of integrated management the bioagent was most effective against the pathogen.

# OBJECTIVES



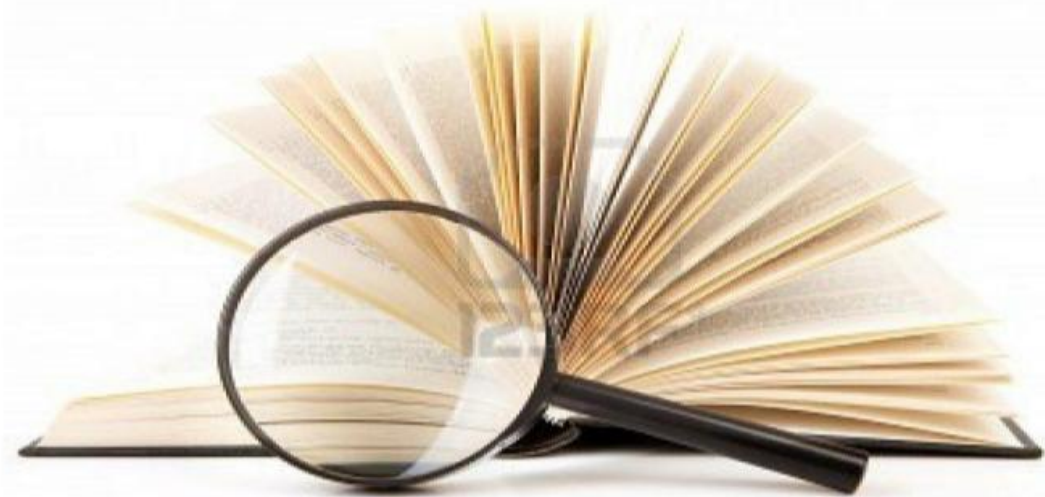
## OBJECTIVES

---

- Collection of soil samples from the non-rhizosphere, rhizosphere and rhizoplane region from the infected as well as healthy crop.
- Analysis of physico-chemical properties of soil (soil texture, organic carbon, organic matter, pH, temperature etc).
- Isolation of general soil mycoflora and causal agent from the infected plant.
- Isolation and selection of antagonistic mycoflora as biocontrol agent.
- Maintenance of pure culture for further studies.
- Study of percentage (%) occurrence and percentage frequency of mycoflora of the samples.
- Study of antagonistic potentiality of biocontrol agent, plant extract, chemicals as well as integrated means at different concentrations against the causal agent under *in vitro* condition.
- Comparative management study of causal agent (*Sclerotium rolfsii*) with biological, chemical, botanical and integrated means under *in vivo* condition.
- Statistical analysis of the data following standard statistical methods.

# CHAPATER - 2

## REVIEW OF LITERATURE



## REVIEW OF LITERATURE

---

Soil-borne fungus *Sclerotium rolfsii* is a facultative parasite and omnipathogenic organism worldwide infects more than 500 plants species (Aycock, 1966; Punja, 1985) including tomato, cucumber, brinjal, soybean, maize, groundnut, bean, watermelon etc. It causes different types of diseases such as *Sclerotium* wilt, collar rot, stem rot, charcoal rot, seedling blight, foot-rot, damping-off, stem blight and root-rot in various economically important crops (Gopalakrishanan et al., 2005). It was first observed by Rolfs (1892) on tomato plants with 70% losses. The hyphae grew upward on the surface of the infected plant with cottony, white mass of mycelium, scattered outside and inside of infected stem nearby the soil surface. Fungus produces many tiny round, white sclerotia of the same size when immature and dark brown at mature stage (Kwon and Park, 2002).

*Sclerotium rolfsii* develops at intermediate soil moisture level (70%) of field capability and at temperature range between 25<sup>0</sup>C to 30<sup>0</sup>C (Pinheiro et al., 2010). *Sclerotium* blight disease caused by *Sclerotium rolfsii* in soybean reduces the crop yield but in certain situation, significantly yield losses can also occur due to the monoculture or short rotation of soybean with another plant susceptible to the pathogen (Pinheiro et al., 2010). It is spread by the wind, water, animal and soil (Kator et al., 2015). Keeping in view the significance of economic maize crop, there is need for management destroyed by *Sclerotium rolfsii*. It can be controlled by using chemical, biological, botanical, solar treatment and integrated means. Out of these except chemicals, other means are safer and ecofriendly.

## **2.1. Factors affecting soil fungal population**

Soil is a rich habitat of all major groups of microorganisms (Hagvar, 1998). It is a complex and dynamic ecosystem surrounded by physico-chemical parameters that contain enough number of living organisms such as bacteria, fungi, yeast, nematodes etc. The soil microbes decompose the dead plants and animals present in the soil and convert into organic matter which influences the physical, chemical and biological properties of the soil (Olsen and Dean, 1965). Among all the soil microbes, the fungi are major groups of organisms which are responsible to decompose the organic compounds. Hawksworth (2001) reported that the fungi are necessary for the survival of other living organisms and they play essential role in ecological process. They are most diverse group of microorganisms which are found in every possible ecosystem and their ability to tolerate low or high temperature that indicates their adaptive mechanisms. Fungal diversity of soil depends upon physical and chemical factors of the soil such as pH, organic carbon, moisture content (Rangaswami and Bagyaraj, 1998).

### **2.1.1. Effect of pH on soil microbes**

The pH and microbial population in soil is correlated with each other (Dwivedi, 1970). It is strongly correlated with bacterial diversity and correlated between soil pH and fungal community. There are some indications that exhibit the soil fungal communities are not related or less strongly related with the pH of soil (Rousk et al., 2010). But we still do not know whether pH directly influence the community structure in changing the environmental factors such as soil moisture content, organic carbon, nutrient availability and vegetation types (Rousk et al., 2010). However, previous studies showed direct connection between the soil pH and fungal community (Beales, 2004).

The soil bacterial communities are strongly influenced by the soil pH. The influence of soil pH on fungal communities has been less explored compared to bacterial communities. Fungal species have been reported to have wide range of optimum pH ranging from 5-9 (Nevarez et al., 2009). Grantina et al. (2011) observed that in acid soils (pH 4.46-5.30), fungal abundance was higher in comparison with natural soil (pH 6.68-7.90) showing that fungi prefer more acidic environment than bacteria.

### **2.1.2. Effect of moisture content on soil microbes**

Soil moisture content is most important environmental factor affecting the disease incidence (Webber, 1931). Sandy soil favours the disease incidence at moisture content of 0.93 % but it has been reported the reduction on fungal growth with increase in soil moisture content (Epps et al., 1951; flados, 1958). Disease development is enhanced by humidity (Cook, 1975). Saprophytic activity of soil fungi was high at 10% soil moisture but at 50 and 70% moisture content the fungus did not survived (Lingaraju, 1977). Ramarao and Raju (1980) reported that fungal diversity in wheat crop increased with the increase in soil moisture content.

Low level of irrigation has been reported to enhance the *Sclerotium rolfsii* population (Curl, 1961). Khati et al. (1983) reported that the endurance of *Sclerotium rolfsii* was maximum at soil moisture levels between 30 and 50% whereas Palakshappa (1986) studied that *Sclerotium rolfsii* survival was better at low soil moisture level than at high soil moisture levels ranged between 20 and 40%. The highest saprophytic activity of the fungus i.e. 86.66% was observed at 40% moisture level where the saprophytic activity of the fungus was 43.33 and 30.00% respectively. John et al. (2006) reported that August to October was the favourable period for the development of fungus pathogens, while May to July was least favourable period.

### **2.1.3. Effect of Temperature on microbes**

Environmental temperature is one of the most important factors affecting the development of soil microbes. The most optimum growth temperature ranged between 20<sup>0</sup> C and 40<sup>0</sup> C. It is the limiting factor for the geographical distribution of the fungus (Higgins, 1927). Hari et al. (1988) reported 26<sup>0</sup> C optimum temperature for growth of *Sclerotium rolfsii* while maximum growth of *Sclerotium rolfsii* was observed at 30<sup>0</sup> C in groundnut field soil.

### **2.1.4 Effect of soil organic matter on microbes**

Soil organic matter is organic material produced by living organisms that goes through the decomposition process in soil (Bot and Benites, 2005). It generally consists of plant residues that contain 60-90% moisture, carbon, oxygen, hydrogen and tiny amount of sulphur, nitrogen, phosphours, potassium, calcium and magnesium (Bot and Benites, 2005). Campbell et al. (1996) studied that the organic matter is an imperative source of plant nutrients and improves the physical and chemical property of soil. Soil microorganisms used organic matter as food and they incorporate more energy from soil organic matter as they assimilate 40-50% carbon during decomposition. It is widely known that many pathogenic microorganisms are directly or indirectly influenced by the organic matter.

## **2.2. Effect of Chemical on *Sclerotium rolfsii***

Banodenil and pentachloronitrobenzene were effective fungicides against the root rot and blight of apple seedling disease (Conway and Tomasino, 1996). The high amount of benzaldehyde and velvet bean inhibits mycelial expansion and sclerotial germination of *Sclerotium rolfsii*. Yaqub and Shahzad (2006) reported that six fungicides viz. sancozeb, benomyl, dithane M-45, thiovit, carbendazim and topsin-M

were effective against *S. rolfsii*. No fungicide could inhibit the growth of *Sclerotium rolfsii* at low concentration while high concentration of dithane M-45 and mancozeb significantly reduced the enlargement of *Sclerotium rolfsii* (Yaquub and Shahzad, 2006). According to Arunasri et al. (2011) Crossandra an important flower plant affected by collar rot disease by *Sclerotium rolfsii* Sacc. was controlled by four fungicides viz. captan 50% WP, propiconazole 25% EC, thiophanate-methyl 70% WP and thiram 75% SD at five different concentration. The fungicide propiconazole, thiram and captan considerably reduced the mycelial expansion of *S. rolfsii*.

The fungicides hexaconazole, propiconazole, difenoconazole, avatar (hexaconazole 4% + zineb 68%), nativo (tebuconazole 50%+ trifloxystrobin 25%) and vitavax powder (thiram 37.5%+carboxin 37.5%) and bioagents *Trichoderma harzianum* inhibits *Sclerotium rolfsii* under field condition (Manu et al., 2012). Tariq et al. (2012) studied that carbendazim, captan, carboxin and metalaxyl were efficient against wilt disease under *in vitro* condition. Taj et al. (2014) tested different fungicides viz. Captan, Carbendazim +Mancozeb, Chlorothalonil, Tebuconazole, Tricyclazole, and Trifloxystoburin at 50, 100, 1000 and 5000 ppm concentration against mycelial expansion of *Sclerotium rolfsii* under *in vitro* condition. Out of these, the Tebuconazole was most efficient fungicide against inhibition of *S. rolfsii*. The two synthetic fungicides bavistin and dithane M-45 contain antifungal efficacy against mycelial growth of *S. rolfsii* but these synthetic fungicides are cost effective, non-economical, create health problem, soil pollution and increase disease resistance.

El-Mohamedy et al. (2014) reported that chemicals viz.  $K_2HPO_4$  (400mM), salicylic acid (100mM), sorbic acid (7.5%) and potassium sorbate (7.5%) were effective against root rot disease of tomato caused by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* and induce positive effect on plant development, yield and fruit quality of tomato grown under field condition during two cropping seasons.

Khan and Javaid (2015) reported that fungicides viz. tegula (tebuconazole), ridomil gold (metalaxyl+mancozeb), thiophanate methyl and mancozeb significantly inhibited the radial enlargement of *S. rolfii* under *in vitro* condition. Besides it, two fungicides thiophanate methyl and mancozeb substantially control the expansion of *S. rolfii* under *in vivo* condition responsible for causing collar rot disease in Chickpea. Wang et al. (2015) reported that fluazinam has potent antifungal activity against *Sclerotium rolfii* which causes stem rot. Soil fumigant such as methyl bromide, methane sodium and chloropicrin inhibited the growth of mycelium of *S. rolfii* (Puri, 2016). Rangarani et al. (2017) reported that Mancozeb, Propiconazole, Thiram, Tricyclazole and herbicides pendimethalin and quizalofop-p-ethyl were effective checked by 100% stem rot disease in groundnut caused by *S. rolfii* under *in vitro* condition. Ahsan et al. (2018) evaluated about five fungicides viz. Hexaconazole, Carbendazim, Carboxin, Propiconazole, and Thiophanate Methyl for seeds treatment @ 2.0 g/kg seed against collar rot disease of chickpea. All the fungicides were significantly effective but Carboxin inhibited (66.70%) collar rot disease in chickpea.

### **2.3. Effect of Bioagent on *Sclerotium rolfii***

*Sclerotium rolfii* form brownish sclerotia that survive in the soil for long period tolerate biological and chemical degradation due to the presence of melanin pigment in the outer membrane of *Sclerotium*. (Chet, 1975) *Pseudomonas* species produce antifungal and antibiotics metabolites like Fe-chelating siderophore, hydrogen cyanide (Babu and Paramageetham, 2013; O'Sullivan and O'Gara, 1992); gluconase, cellulytic and chitinolytic enzymes (Sindhu and Dadarwal, 2001). Biswas and Sen (2000) reported that *Trichoderma harzianum* inhibited stem-rot disease in groundnut caused by *S. rolfii*. Anahosur (2001) studied about fungal antagonists viz. *Gliocladium virens*, *Penicillium* sp., *Paeocilomyces lilacinus*, *Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens*, *Bacillus subtilis* and Mycorrhizae

inhibited foot rot, root rot, wilts and damping-off diseases caused by *Sclerotium rolfsii*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium* sp. and *Pythium debarianum*.

Abd-Allah (2005) reported that *Bacillus subtilis* control *Sclerotium rolfsii* by 92% under greenhouse condition in peanut. *Bacillus subtilis* secrete antifungal substance which is highly antagonistic against *Sclerotium rolfsii* (Nalisa et al., 2006). Radawan et al. (2006) reported *Trichoderma harzianum* and *Trichoderma hamatum* were most effective against *Sclerotium rolfsii* and inhibited the mycelial growth by 79%. *Trichoderma* spp. were used for commercial enzyme productions viz. cellulases, hemicellulases, proteases and  $\beta$ -1, 3-glucanase (Verma et al., 2007). The combination of *Rhizobium* and *T. harzianum* were significantly effective against *S. rolfsii* which caused stem rot disease and promote the plant growth and increase seed production in groundnut.

*Trichoderma harzianum* has antagonistic activity against *Sclerotium rolfsii* as it produces antibiotics such as gliotoxin, viridin, glioviridin, dermin and Trichodermin (Eziashi et al, 2007; Ghildiyal and Pandey, 2008). *Trichoderma* sp. has been reported to have antagonistic activity against *S. rolfsii* and *Fusarium cicer* under *in vivo* condition (Anand and Reddy, 2009). *Trichoderma*, *Penicillium* and *Aspergillus* species significantly inhibited the expansion of *S. rolfsii* under *in vitro* condition (Bosah et al., 2010). Pastor et al. (2010) studied that *Pseudomonas* sp. has more potent antagonistic activity against *Sclerotium rolfsii* in groundnut. Several species of *Pseudomonas* produces antifungal and antibiotics viz. 2, 4 diacetyl phloroglucinol, phenazine, oligomycin, pyolyteorin, pyrrolnitrin and pyocyanin which inhibited expansion of *S. rolfsii* (Nielson et al., 1998; Gupta et al., 2001; Muthukumar et al., 2010). *Streptomyces* sp. having biocontrol activity of root and stem rot as it produces antifungal and antibacterial substances which inhibited *Sclerotium rolfsii* and

*Ralstonia solanacearum* in chilli and pepper under *in vivo* condition (Boukaew et al., 2011). Actinomycete produces chitinase enzyme which inhibits growth of *S. rolfsii* under *in vitro* condition and control the stem rot disease and accelerated the growth of Chilli (Pattanapitpaisal and Kamlandham, 2012). *Pseudomonas monteilii* produces diffusible antibiotic, volatile metabolite, hydrogen cyanide and siderophore that inhibits *Sclerotium rolfsii* under *in vitro* condition and accelerate the germination, root length, shoot length, number of leaves and chlorophyll content in groundnut (Paramageetham and Babu, 2012; Rakh et al., 2011). The arbuscular mycorrhizal fungus *Glomus fasciculatum* suppress the harmful effect of stem rot disease caused by *Sclerotium rolfsii*. *Trichoderma harzianum* showed maximum inhibition in the mycelial expansion of *Sclerotium rolfsii* by 83.06% (Bhuiyan et al., 2012). Rekha (2012) reported that *Trichoderma* sp. inhibited mycelial expansion and sclerotial formation of *S. rolfsii*. Samsuzzaman et al. (2012) studied that *Trichoderma harzianum* reduced the mortality of tomato plants when inoculated with *S. rolfsii* in soil and increase height and production of tomato, therefore the biofungicide inhibited expansion of *S. rolfsii* causing collar rot disease in tomato with no risk of environmental pollution.

Arbuscular mycorrhizal fungi (*Glomus fasciculatum*) significantly inhibited *S. rolfsii* which causes stem rot disease in groundnut (Doley and Jite, 2013). *Trichoderma* species acts as biocontrol agent against *S. rolfsii* (Podmaja et al., 2013) causing southern stem blight disease of soybean (Lewis and Papavizas, 1991; Haran et al., 1996a; Hermosa et al., 2000; Elad, 2000; Chandra, 2014). Patro and Madhuri (2013) reported that *T. harzianum* inhibits mycelial expansion of *S. rolfsii* under *in vitro* condition which cause foot rot in finger millet. Eid (2014) isolated four bioagents viz. *Bacillus subtilis*, *Pseudomonas fluorescens*, yeast and *Trichoderma viride* that inhibited damping-off disease in bean caused by *S. rolfsii*. He reported that

*Bacillus subtilis*, *T. viride* and *P. fluorescens* control 88.7%, 83.7% and 86.3% respectively as bioagents against *S. rolfsii* and plants survived by 90.3%, 86.1% and 87.6% respectively. *Trichoderma asperellum* increased chitinase, glucanase and peroxidase activity in bulbs, roots and leaves of onion and also inhibited the enlargement of *Sclerotium rolfsii* (Valle et al., 2013). *Trichoderma viride*, *T.harzianum*, *Pochonia chlamydosporia*, *Pseudomonas fluorescens* and *Bacillus subtilis* have been reported as bioagents against *Sclerotium rolfsii* responsible for causing southern blight disease in *Zea mays* (Hooda et al., 2008; Mohiddin and Khan, 2013). Darvin et al. (2013) studied three *Trichoderma* sp. viz. *Trichoderma viride*, *T. harzianum* and *T. longibrachiatum* against *S. rolfsii*. They reported that *T. viride* and *T. harzianum* inhibited highest radial growth compared to *T. longibrachiatum* using dual culture technique under *in vitro* condition.

*Pseudomonas aeruginosa* inhibited the growth of *Sclerotium rolfsii* by 73.7% under *in vitro* condition (Babu and Paramageetham, 2013). Adhilakshmi et al. (2014) isolated 30 actinomycetes from the rhizospheric soil of groundnut from different area of Tamil Nadu and tested their antagonistic activity against *Sclerotium rolfsii* causing stem rot disease in groundnut using dual culture technique under *in vitro*. They were reported among the various isolates tested *in vitro*, five isolates ie. CBE, ANR, MDU, SA and PDK were found significantly effective to control the mycelial expansion of *S. rolfsii*. Kotasthane et al. (2015) studied about 20 strains of *Trichoderma* and all strains were collected from different geographical region of Chhattisgarh against two soil borne plant pathogen viz. *Sclerotium rolfsii* and *Rhizoctonia solani*. The strains secreted inorganic phosphate, IAA and siderophore which promote growth of cucumber, bitter gourd, bottle gourd and also check the cell wall degrading enzymes cellulose secreted by *S. rolfsii*. *Trichoderma viride* exhibited highest antagonistic activity and plant growth promoting ability compared to other strains.

Mahato and Mondal (2014) reported that *Azotobacter chroococcum*, plaster of Paris, vermicompost, *Pseudomonas*, FYM and *Trichoderma viride* were extremely effective against *S.rolfsii*. *Trichoderma* and *Pseudomonas* showed highest inhibitory activity (68.28% and 74.25% respectively) against *Sclerotium rolfsii* under *in vitro* condition (Muthukumar and Venkatesh, 2014). Sab et al. (2014) studied eight bio-agents against *S. rolfsii* in dual culture plates, all bio-agents inhibited enlargement of *S. rolfsii* which causes collar rot disease in chickpea; but they recorded that *Trichoderma harzianum* inhibited maximum expansion of *S. rolfsii* compared to *Pseudomonas fluorescens* and *Bacillus subtilis*. *Trichoderma viride* and *Trichoderma harzianum* act as biocontrol agent and inhibits 53.8% to 83.1% growth of *Sclerotium rolfsii* and *Rhizoctonia solani* (Majdah et al., 2015). Swathi et al. (2015) reported that *T. harzianum* and *T. virens* were more effective against *S. rolfsii* with 100% inhibition under *in vitro* condition. Khalequzzaman (2015) reported that *Rhizobium* biofertilizer was more efficient to control foot and root-rot of Chickpea. *Trichoderma viride* contain antagonistic activity at optimum temperature 37 °C and at pH six against *Sclerotium rolfsii* (Aanuoluwa et al., 2015). Bacteriocinogenic *Lactococcus lactis* JC10 significantly inhibited the mycelial expansion of *S. rolfsii* under laboratory conditions (Mandal and Jana, 2015).

*Pseudomonas* species act as a biocontrol agent and produce antifungal metabolites like pyoluteorin, pyrrolnitrin, phenazines and 2,4-diacetylphloroglucinol (DAPG) which inhibit the growth of *S. rolfsii* (Jothi and Reddy, 2015). Basumatary et al. (2015) selected six fungal species viz. *Penicillium* sp., *Curvularia* sp. *Aspergillus niger*, *Trichoderma harzianum*, *T. viride* and *Fusarium* sp. as bio-agent against *Sclerotium rolfsii*. The maximum expansion of *Sclerotium rolfsii* was inhibited with *Trichoderma harzianum* (77.39%), *Trichoderma viride* (76.54%) compared to

*Penicillium* sp. (29.05%), *Aspergillus niger* (30.48%), *Curvularia* sp. (13.57%), *Fusarium* sp. (3.02%) under *in vitro* condition. *Trichoderma* sp. produced  $\beta$ -xylosidase, trypsin,  $\alpha$ -glycosidase,  $\beta$ -glucosidase, cellobiohydrolase, chymotrypsin, and N-acetyl  $\beta$ -glucosaminidase and inhibited the mycelial expansion of *Sclerotium rolfsii*.

Elshahawy et al. (2016) reported that ten *Trichoderma* species and seven fungicides were effective against *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina* and *Sclerotium* species. *Trichoderma harzianum* and *T. atroviride* inhibited the growth of *S. rolfsii* but the mutant parent were more efficient compared to parent bio-agent against collar rot disease of chickpea caused by *S. rolfsii*. Mutant strain of *Trichoderma atroviride* and *T. harzianum* successfully inhibited excessive expansion of *S. rolfsii* by 82.9% (Singh et al., 2016). Ramzan et al. (2016) studied 15 bio-agents against *S. rolfsii* and they reported that *Trichoderma harzianum* and *Bacillus subtilis* were more effective compared to *Bacillus cereus*, *T. virens*, *Pseudomonas fluorescens* and *Micrococcus varians*. Karthikeyan et al. (2006) reported that *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* were inhibitory bioagents against stem-rot disease in groundnut caused by *Sclerotium rolfsii*. *Sclerotium rolfsii* causing collar rot disease in tomato was treated with *Trichoderma harzianum*, *Pseudomonas fluorescence*, Carbendazim and composite with each other in pot culture, all treatment enhanced the ability of seed germination, shoot-root length, fresh and dry weight and reduce the collar rot disease (Rajendraprasad et al., 2017). The antagonistic potentiality of *Trichoderma* was studied against stem rot disease caused by *Sclerotium rolfsii* in groundnut. The maximum growth inhibition of *Sclerotium rolfsii* was observed by antagonist *T. virens* NBAII on PDA medium under *in vitro* condition and sclerotia formation was elevated

and reduced interaction with strong antagonist (Hirpara et al., 2017). The fermented biomass of *Trichoderma harzianum* suppressed that collar- rot disease in *Zea mays* caused by *S. rolfsii* (Ahsan et al., 2018). Hirpara et al. (2019) developed heterozygous and homozygous mutants of *Trichoderma fusant*; and protoplast fusion have influential technique for development of multistress tolerant viz. drought, fungicide and salt tolerance of biocontrol activity against *S. rolfsii* causing stem rot in groundnut.

#### **2.4. Effect of Plant extracts on *Sclerotium rolfsii***

Butt et al (2016) reported that the leaf extract of two important indigenous plants like *Alstonia scholaris* and *Azadirachta indica* were more effective against *S. rolfsii* under *in vitro* condition at different concentrations (1%, 2%, 3%, 4% and 5%). Islam and Faruq (2012) reported that clove, garlic, ginger rhizome, allamonda leaf, neem leaf, kalijira seed, turmeric rhizome, katamehedi leaf, bel leaf and onion bulb were effective against damping-off disease in tomato but neem leaf extract was most efficient than other extract. Amin et al. (2013) selected different plant's parts viz. neem leaf, rhizome ginger, tobacco leaf, rhizome of turmeric and cow's urine. All plant extracts inhibited the expansion of *Sclerotium rolfsii* only at higher concentration, while rhizome of turmeric inhibited the growth at a low level. Sab et al. (2014) studied ten botanical extracts against *Sclerotium rolfsii* whereas extract of agave inhibited 90% collar rot in chickpea caused by *S. rolfsii* at 15% concentration. Dania et al. (2014) reported that *Oryza sativa* husk and *Quercus phillyraeoides* extract at 0.5%, 1.0%, 1.5%, 2.5% and 3.5% w/v concentration under *in vitro* and *in vivo* condition were effective against *Sclerotium rolfsii* causing tuber-rot disease in white yam. The extract concentration 3.5% w/v was more effective compared to other concentrations.

Tang et al. (2015) reported that the extract of *Cyperus dofformis* was more resistance against *Sclerotium rolfsii*. *Cymbopogon martini* oil was most efficient and inhibited the mycelial growth of *S. rolfsii* (Guerra et al., 2015). Okigbo et al. (2015) have reported that *Dioscorea bulbifera* extract was most effective against the white yam root rot caused by *S. rolfsii*. The garlic and ginger were found to be most effective plant extract compared to synthetic fungicide with no side effect and inhibited 100% mycelial growth of *S. rolfsii* (Sacchi et al., 2016). Siddiqui et al. (2016) studied leaf, stem and root extracts of *Nerium oleander*, prepared in aqueous, methanol, ethanol, chloroform and acetone, control the growth of *F. oxysporum*, *S.rolfsii* and *Macrophomina phaseolina*. Nugroho et al. (2019) have studied the effectiveness of *Ocimum basilicum* against mycelial expansion of *S. rolfsii* at 25%, 50%, 75% and 100% concentration on 24, 48, 72 and 96 hour under *in vitro* condition. They evaluated sweet basil (*Ocimum basilicum*) extract against *S. rolfsii* causing damping off on tomato seedling under *in vitro* and *in vivo* condition. They were found that the leaf extract of sweet basil was most effective at 100% concentration, and lowest inhibition was recorded at 25% concentration of mycelial growth of *S. rolfsii*.

## **2.5. Effect of Integrated management on *Sclerotium rolfsii***

The combination of nitrogenous compound, ammonium bicarbonate and fungicide carboxin control *S.rolfsii* on turf under field condition (Punja et al., 1982). *Bacillus subtilis*, *Gliocladium virens* and fungicide quintozene were effective against root rot disease in grapevine nurseries caused by *Sclerotium rolfsii* (Keyser and Ferreira, 1988). Adandonon et al. (2006) first reported that the leaf extract of *Moringa* combined with *Trichoderma* act as an integrated biocontrol agent against stem rot and damping-off disease in cowpea under field condition. The antagonistic combination of

*Trichoderma harzianum* and *Pseudomonas fluorescens* can be used for effective management of collar rot (Singh et al., 2006).

Sahani et al. (2008) reported that combination of ZnSO<sub>4</sub> and oxalic acid was 100% effective against collar rot disease in chickpea caused by *S. rolfsii* under greenhouse condition. Banyal et al. (2008) selected ten fungicides viz. carbendazim 50 WP, carbendazim + mancozeb 75 WP, captan 50 WP, chlorothalonil 80 WP, thiabendazole 80 WP, mancozeb 75 WDG, carboxin 75 WP, propineb 70 WP, mancozeb 75 WP and tebuconazole 5 DS; five bioagents *Trichoderma harzianum* (local strain), *Trichoderma viride* (local strain), *Gliocladium virens* (local strain), *Paecilomyces lilacinus* (Bhubaneswar strain) and *T. viride* (Ecoderma). These inhibited *Sclerotium rolfsii* causing collar rot disease in tomato while composite tebuconazole and *T. viride* (local strain) was 100% effective against *Sclerotium rolfsii*. Madhavi and Bhattiprolu (2011) reported that tebuconazole and combination of carbendazim and mancozeb were very efficient against *Sclerotium rolfsii* causing root-rot in chili. The combination of the rhizome of turmeric and cow's urine was more efficient against the growth of *Sclerotium rolfsii* (Amin et al., 2013). *Microbispora* species and fungicide thiram were effective against *Sclerotium rolfsii* (Patil et al., 2014).

The chemical fungicide (indofil M-45, bavistin, blitox, viavax power, kavach, curzet and krilaxyl gold); plant oils (Karanja oil, mahua oil, neem oil and chaulmoogra oil) and leaf extracts (*Mariya exatica* and *Vinca rosea*) inhibited radial expansion of *S.rolfsii* under *in vitro* condition (Mahato et al., 2014). *Trichoderma viride* and fungicide mancozeb and camazeb significantly inhibited expansion of *Sclerotium rolfsii* (Ekundayo et al., 2015). *Trichoderma viride* screened out from ginger soil have been recorded to be very efficient in controlling the development of *Sclerotium rolfsii* while composite of *T. viride* and mancozeb were detrimental to

tomato plant (Ekundayo et al., 2015). In the plant tissues the Oxalic acid combined with calcium to remove the association of pectin compound and cell wall-degrading enzymes cellulose and polygalacturonase in plant cell walls. Aqueous leaf extracts of *Azadirachta indica*, *Carica papaya*, *Chromolaena odorata*, *Trichoderma koningii* and mancozeb contain antifungal properties against *S. rolfsii* and reduced significantly cormel rot in cocoyam cormel; the plant extract and *T. koningii* were more effective for disease control compare to mancozeb (Bartholomew et al., 2017). In an experiment *Sclerotium rolfsii* was inhibited by *T. harzianum* at various levels (Priyadharcini et al., 2018). Siddique et al. (2018) tested some fungicides viz. Bavistin 50 WP, Topgan 50WP, Bordeaux mixture, Dithane M-45, Ridomil Gold MZ 68WG, Neem leaf extract, Poultry manure and bioagent *T. harzianum* against *S. rolfsii* and suggested that chemical fungicide Bavistin 50 WP and bioagent *T. harzianum* were more effective against root and foot rot disease in eggplant caused by *S. rolfsii*. The mycelial expansion of *S. rolfsii* was inhibited by *Bacillus subtilis*, *Trichoderma viride*, Trifloxystrobin+Tebuconazole and Mahua oilcake extract. However, the fungicides and organic amendments have showed 100% inhibition (Latha and Rajeswari, 2019).

**Table 2.1: Control of pathogen of some important crops using antagonistic bioagents, botanicals and chemicals**

S. No.	Crop	Infected part of crop	Name of disease	Study area	Microbial inoculants/ botanicals/chemicals.	References
1.	Chilli	Root	Root-rot	Aurangabad District	leaf extract of <i>Argemone mexicana</i> Linn, <i>Eucalyptus globulus</i> Labill and <i>Murraya koenigii</i>	Quadri and Fatima (2016)
2.	Soybean	Root and stem	Foot and collar rot	M. P.	<i>Trichoderma harzianum</i>	Bankoliya et al. (2015)
3.	Chickpea	Stem	collar rot	Rawalpindi	Leaf extract and seed oil of Pongame ( <i>Pongamia pinnata</i> )	Akram et al. (2016)
4.	<i>Stevia rebaudiana</i>	Leaves	<i>Sclerotium</i> wilt	Dharwad (karnataka)	<i>T. harzianum</i> , <i>T. viride</i> , <i>Duranta repens</i> , <i>Eupatorium odoratum</i> , <i>Panchagavya</i> , cow urine, <i>Neem oil</i> , <i>Datura stramonium</i> and <i>Jeevamruta</i>	Shwetha and Hegde (2012)
5.	Sugarbeet	Root	Root Rot	Tamil Nadu	<i>Trichoderma sp.</i> , <i>P. fluorescens</i> and <i>Bacillus subtilis</i>	Rasu et al. (2013)
6.	Melon	Melon leaves	Southern blight rot	Wilmar Benih Indonesia	Bamboo rhizospheric Microbe ie. <i>Bacillus subtilis</i>	Darma et al. (2016)
7.	Rice	Stem	Stem rot	Sakaeo campus	Plant extracts <i>H. anthelminthicus</i> (fruits), <i>C. magna</i> (fruits), <i>C. sappan</i> (barks), <i>X. lanceatum</i> (fruits) and <i>C. brachiata</i> (barks)	Jantasorn et al. (2016)

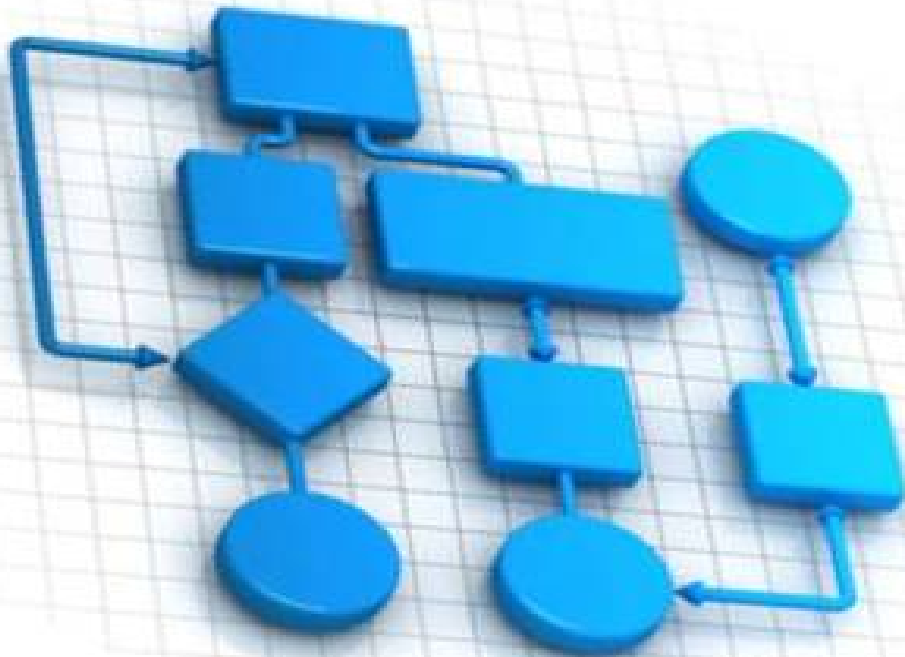
8.	Sunflower	Stem	Collar rot	Sundarbans of West Bengal	<i>T. viride</i> , <i>P. fluorescence</i> , <i>T. harzianum</i> , Cow urine, Neem, ( <i>Azadirachta indica</i> ), Tulsi ( <i>Ocimum sanctum</i> ) and Datura ( <i>Datura stramonium</i> )	Maji and Nath (2016)
9.	Groundnut	Stem	Stem-rot	Madurai	<i>Trichoderma harzianum</i>	Ganesan et al. (2007)
10.	Lentil	Root	foot-rot and root-rot	Pulses Research Centre, Ishurdi, Pabna, Bangladesh	neem leaf extract, neem oil, garlic extract, <i>Trichoderma viride</i> compost, <i>T. harzianum</i> compost, provax 200	Khalequzzaman (2016)
11.	Peanut	Stem	Stem rot	North Lombok District Indonesia	<i>Trichoderma</i> sp.	Suheri et al. (2014)
12.	Groundnut ( <i>Arachis hypogaea</i> )	Stem	Stem rot	Bapatla, Guntur District	<i>Trichoderma</i> spp., <i>Pseudomonas fluorescens</i> and <i>Bacillus subtilis</i>	Darvin and Kumari (2013)
13.	Jute ( <i>Corchorus</i> spp.)	Stem	Anthracnose and Soft-rot	Dhaka	<i>Aspergillus niger</i> and <i>Trichoderma viride</i>	Hosen et al. (2016)
14.	Groundnut	Stem	Stem-rot	Semi-Arid Tropics, Patancher	Actinomycetes	Jacob et al.(2016)

15.	Sunflower	Stem and root	Root and collar-rot	PDBC, Bangalore	<i>Pseudomonas fluorescens</i> , <i>Pseudomonas</i> spp., <i>Alcaligenes odorans</i> , <i>P. putida</i> , <i>Streptomyces</i> spp. and <i>Bacillus pantothenicus</i>	Rangeshwaran and Prasad (2000)
16.	Chilli	Root	Root-rot	Aurangabad, Maharashtra	Leaf extract of <i>Annona squamosa</i> Linn, <i>Brassica campestris</i> Linn. (Sarson) and <i>Ocimum sanctum</i> Linn	Quadri and Fatima (2016)
17.	Peanut	Plant stem	Stem rot	Manisa, Turkey	Fludioxonil, Azoxystrobin+ Fludioxonil + Metalaxyl-M, Fludioxonil+ Metalaxyl-M, Tolclofos-Methyl, Tolclofos-Methyl + Thiram, Carboxin+ Thiram	Akgul, et al. (2011)
18.	Jerusalem artichoke	Stem	Southern stem rot	Khon Kaen, Thailand	<i>Trichoderma harzianum</i> and <i>Glomus clarum</i>	Sennoi,et al.(2013)
19.	Amorphophallus	Lower portion of plant	Collar rot	Thiruvananthapuram, Kerala	<i>Trichoderma harzianum</i> and <i>Trichoderma asperellum</i>	John, et al. (2015)
20.	Groundnut	Whole plant	Collar rot, stem rot, tikka, rust	Junagadh, Gujarat	Mancozeb, Hexaconazole, <i>Trichoderma viride</i>	Kapadiya and Moradiya (2017)

21.	<i>Tomato</i>	Plant stem	Collar rot	West Bengal	Plant extract viz. <i>Andrographis paniculata</i> , <i>Zingiber officinale</i> , <i>Azadirachta indica</i> , <i>Allium sativum</i> , <i>Curcuma longa</i> , <i>Catharanthus roseus</i> , <i>Allium cepa</i> and <i>Ocimum sanctum</i>	Mahato et al. (2018)
22.	Onion	Bulb and root	Bulb rot	Mexico	<i>Trichoderma asperellum</i>	Valle et al. (2013)
23.	Jerusalem artichoke	Stem	Stem rot	Khon Kaen, Thailand	sorghum seed inoculum	Junsopa, et al. (2016)
24.	<i>Cucurbita argyrosperma</i>	Fruit	Fruit rot	Mexico	<i>Trichoderma virens</i> , <i>T. asperellum</i> , metalaxyl, pyraclostrobin, quintozone, cyprodinil + fludioxonil, and prochloraz	Najera, et al. (2018)

# CHAPTER - 3

## MATERIALS & METHODS



## MATERIALS & METHODS

---

The present study was carried out in the department of Environmental science, BBAU, Lucknow. The aim of the present research work was to evaluate the potentiality of some chemical fungicides, biological agents and plants extract to control *Sclerotium rolfsii* causing root rot in *Zea mays* by using different methods.

### 3.1. Collection of samples

The samples were collected from infected agriculture field of maize from district Barabanki, Lucknow and Jaunpur. The infected as well as healthy parts of maize crop and rhizospheric and non-rhizospheric soil were carried out in sterilized polythene bags separately. Some medicinal plants were collected from the BBAU campus and other areas of Lucknow. Some selected bioagents were isolated from collected soil samples. Chemical fungicides were procured from the local market of Lucknow. All collected samples were analyzed in the laboratory.

### 3.2. Analysis of physico-chemical properties of soil

#### 3.2.1. Soil texture

The determination of soil texture is based on CMG GardenNotes #214 given by Whiting et al. (2011) using Jar method. Took a large slender jar and filled one-quarter full of the soil and water was add three-quarters to full it. One teaspoon non-foaming dishwasher detergent was added. The slender jar was tightly fitted to lid and shaken for 10 to 15 minutes. After shaking, the soil was separated into individual mineral particles at different time period according to their size. After one minute, the depth of the sand in the jar was marked. After two hours, the depth of the silt in the jar was

marked. When the water cleared, the depth of clay in the jar was marked. The soil particles settled down according to size namely sand, silt and clay. The percentage of sand, silt and clay was calculated from following formula.

$$\text{Percentage of sand} = \frac{\text{thickness of the sand}}{\text{total thickness}} \times 100$$

$$\text{Percentage of silt} = \frac{\text{thickness of the silt}}{\text{total thickness}} \times 100$$

$$\text{Percentage of clay} = \frac{\text{thickness of the clay}}{\text{total thickness}} \times 100$$

### 3.2.2. pH and Temperature

To estimate the soil pH, 10 gm of soil sample was mixed with 20 mL distilled water in 100 mL beaker and stirred for 30 minutes with sterile glass rod and after that soil pH was measured by using pH electrode. The soil temperature was measured by R-tek wired with probe Aquarium Thermometer during collection of soil samples.

### 3.2.3. Percentage of organic carbon in the soil

Determination of the organic carbon of soil was estimated by following Walkley and Black (1934) Method. In this method, the carbon is oxidized by  $\text{K}_2\text{Cr}_2\text{O}_7$  solution.

#### 3.2.3.1. Apparatus

Hot plate, Heat-resistant sheet, Fume cupboard, 10 mL automatic zero pipette, 20 mL measuring cylinder, 250 mL Erlenmeyer flask, 250 mL beaker, 200 °C thermometer, 50 mL burette, 1000 mL volumetric flask, 100 mL volumetric flask.

#### 3.2.3.2. Reagents

- **1 N Potassium Dichromate:** Took 49.040 gm  $\text{K}_2 \text{Cr}_2 \text{O}_7$  (dried at 105 °C) dissolved with 1 L distilled water in 1 L volumetric flask.

- **Sulphuric Acid 98% w/w**
- **Ferrous Sulphate for 0.4 N:** Dissolved 112 gm  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in the 800 mL distilled water containing 15 mL conc.  $\text{H}_2\text{SO}_4$  and diluted with 1L distilled water and stored in a dark bottle.
- **"Ferroun":** Dissolved 1.485 gm O-phenanthroline monohydrate and 0.695 gm ferrous sulphate in the 80 mL distilled water then diluted with distilled water by 100 mL and stored in the dark bottle.

### 3.2.3.3. Procedure

Took 1 gm dry soil in 250 mL conical flask and added 10 mL 1 N  $\text{K}_2\text{Cr}_2\text{O}_7$ , swirl the flask quietly to scatter soil in the solution. Add 20 mL conc.  $\text{H}_2\text{SO}_4$ , directing the stream into the suspension and swirl the flask until the soil and the reagent are mixed. The flask was kept on a hot plate after putting a thermometer (200 °C) into it awaiting the temperature reaches 135 °C for 30 second. Set to cool gradually on the asbestos sheet in the fume cupboard. Two blanks (without soil) were also run in the similar method. After cooling (20–30 minutes), dilute with 200 mL distilled water and proceed with the  $\text{FeSO}_4$  titration using by the "ferroun" indicator.

**"Ferroun" Titration:** Ferroun indicator (3 to 4 drops) was added to flask and was titrated with 0.4 N  $\text{FeSO}_4$  solution. As the end point is approached, the solution changed to a greenish colour and then changes to dark green. At this point, ferrous sulphate drop-by-drop was added awaiting the colour changes from blue-green to reddish-grey. The percentage of organic carbon in soil was determined by following formula.

$$\text{Percentage of organic carbon in soil} = \frac{3(1 - T/S)}{W}$$

Where:

T = Sample titration

S = Blank titration

W = Dry sample weight

### 3.2.4. Percentage of organic matter in the soil

Determination of organic matter in the soil is based on Walkley and Black (1934) Method. In this method, the carbon is oxidized by the  $K_2Cr_2O_7$  solution.

#### 3.2.4.1. Reagents

- **1 N Potassium Dichromate:** Took 49.040 gm  $K_2Cr_2O_7$  (dried at  $105^\circ C$ ) dissolved with 1 L distilled water in 1 L volumetric flask.
- **Ferrous Ammonium Sulfate  $[Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O]$  for 0.5 N:** Took 800 mL distilled water in 1 L volumetric flask and slowly added 20 mL sulfuric acid. Then 196.1 gm ferrous ammonium sulfate was dissolved and properly mixed.
- **Sulfuric Acid ( $H_2SO_4$ )**
- **Phosphoric Acid ( $H_3PO_4$ )**
- **Sodium Fluoride (NaF)**
- **Diphenylamine ( $C_6H_5NHC_6H_5$ ) Indicator:** Dissolved 0.500 gm diphenylamine in 20 mL distilled water and 100 mL sulfuric acid was slowly added and stirred carefully with a glass stirring rod.

### 3.2.4.2. Procedure

Took 1 gm soil in 500 mL Erlenmeyer flask and add 10 mL 1N potassium dichromate solution. Gently added 20 mL H<sub>2</sub>SO<sub>4</sub> and set for ½ hour then diluted in 200 mL distilled water. After then 10 mL H<sub>3</sub>PO<sub>4</sub>, 0.2 gm NaF and 10 drops C<sub>6</sub>H<sub>5</sub>NHC<sub>6</sub>H<sub>5</sub> indicator was added to it. Then, it was titrated with 0.5N Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O awaiting the color changes dull green to turbid blue. Added the titrating solution drop by drop until the end point is reached when color shifts to brilliant green. Blank titration was also done in the similar method. The percentage of organic matter in soil sample was determined by following formula.

$$\text{Percentage of organic matter in soil} = 10[1(S \div B)] \times 0.67$$

Where:

S = sample titration

B = blank titration

### 3.3. Isolation and identification of soil mycoflora

Soil mycoflora have been isolated from different collected samples using serial dilution technique. In this method, took seven test tubes and filled each with 9 mL distilled water and dissolve 1 gm soil sample in one test tube and transfer 1 mL soil water suspension one by one in test tube. The soil water test tube number 3&4 were selected for isolation of soil mycoflora on PDA plates and incubated at 27<sup>0</sup>C for seven days. A part of growing edge of fungal colony was pulled out by using needle on a fresh slide with cotton blue stain. The excess stain was removed with the help of tissue paper and then the cover slip was placed on the slide and observed under microscope. The fungal culture was identified on the basis of morphology (colour,

shape, appearance of colony, presence of specific reproductive structures, presence of sterile mycelium, spore size, spore bearing structure, structure of conidia, conidiophores, arrangement of spores, microconidia, macro conidia and chlamydospores) and available standard literature (Gilman, 2001; Mukadam, 1997; Barnett and Bary, 1998; Elanchezhian et al., 2018).

### 3.3.1. Percent occurrence and percent frequency of soil mycoflora

The soil samples collected from different fields, the isolation of mycoflora was made using serial dilution technique. The Percent occurrence and percent frequency of mycoflora was calculated by using following formula (Sharma et al., 2001).

$$\text{Percent occurrence} = \frac{\text{Total no. of individual species in each plate}}{\text{Total no. of all species in each plate}} \times 100$$

### 3.3.2. Screening and Maintenance of antagonistic mycoflora

Antagonistic mycoflora were isolated from different soil samples using serial dilution technique under laminar and incubated at  $27 \pm 1^{\circ}$  C in an incubator for seven days. Pure colony was appeared in Petriplate. The pure culture was maintained in PDA slants for further studies (Johnson and Curl, 1972).

### 3.4. Isolation and Identification of Pathogen

*Sclerotium rolfsii* was isolated from infected root of *Zea mays*. The infected root of plant was washed under running tap water to eliminate soil around the plant root, after then washed with sterilized distilled water. The washed infected plant root was cut into small pieces (2-4 mm length) and sterilized with 0.5%  $\text{HgCl}_2$  solution for 30 seconds followed by washing in sterilized distilled water and left dry at room temperature for 5 minutes. Small sections were placed on PDA Petriplates and incubated at  $27 \pm 1^{\circ}$  C in an incubator for seven days. After seven days, the culture was

maintained on PDA slant for further studies (Ekundayo et al., 2015). Morphologically the pathogen bear hyphae grows state forward on the surface of the infected plant stem, covered with a cottony white mass of mycelium, scattered outside and inside of infected stem nearby the soil surface. The old hyphae produce various tiny round, white sclerotia of the same size when immature and dark brown sclerotia on mature stage like mustard seed (Kwon and Park, 2002). The sclerotia diameters ranged 4-8 mm in size which is characteristic feature of *S. rolfsii* (Prasad and Dwivedi, 2017).

### 3.5. Antagonistic activity of some fungal species against *Sclerotium rolfsii*

Antagonistic mycoflora viz. *A. flavus*, *A. niger*, *Trichoderma viride*, *Penicilium citrinum*, *P. italicum* and *Penicilium* sp. were evaluated against the expansion of *S. rolfsii* using dual culture technique under *in vitro* condition (Huang and Hoes, 1976). In dual culture technique, the PDA medium was prepared with standard protocol and autoclaved at 121<sup>0</sup> C on 15 lb pressure for 15 minute. After autoclaving, cooled down PDA was poured in sterilized Petriplates. Five mm diam block of *S. rolfsii* was inoculated one side and antagonist on opposite side of the same Petriplate as a treatment and one block of *S. rolfsii* was inoculated in the center of another Petriplate as control. Treatment and control Petriplates were incubated at 27±1<sup>0</sup>C temperature in an incubator. All treatments and control plates were set in triplicates. After incubation, on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day the mycelial expansion of *S. rolfsii* with control plate and mycelial expansion of *S. rolfsii* in treatment plate was measured. Inhibition (%) of *S. rolfsii* was calculated by following formula.

$$\text{Percent Inhibition} = \frac{\text{Control-Treatment}}{\text{Control}} \times 100$$

### 3.6. Preparation of metabolite of bioagents

The metabolite was extracted in PD broth. In this method firstly three blocks (5mm) of *A. flavus*, *A. niger*, *Trichoderma viride*, *Penicilium citrinum*, *P. italicum* and *Penicilium* sp. cut with 5mm diameter cork borer from the actively growing margin of 5 days old culture was inoculated separately in 500 ml conical flask containing 300 ml of potato dextrose broth (Sennoi et al., 2013). After 15 days of inoculation, the culture was filtered first through Whatman filter paper no 42 and then filtered by Vacuum Seitz filter. Finally the filtrates were stored in sterile conical flask at 4<sup>0</sup>C for further study (Fig.4.15).

#### 3.6.1. Treatment of *Sclerotium rolfsii* with metabolite of bioagents

The filtrates of six bioagents (as mentioned in section 3.6) were tested against the mycelial enlargement of *S. rolfsii* using poisoned food technique (Grover and Moore, 1962). According to this method, the filtrates of bioagent at 10%, 25%, 50%, 75% concentration was added separately in PDA medium and poured into clean Petriplates in laminar flow and left as such for solidification of the medium. After solidification, cut 5 mm block of fresh culture of *S. rolfsii* and inoculated in the centre of each Petriplate. The Petriplates were incubated at 27±1<sup>0</sup> C. For each treatment and control three replicates were maintained. The mycelial enlargement of *S. rolfsii* was recorded on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day. Percent inhibition of mycelial expansion of *S. rolfsii* was calculated by following formula (Vincent, 1947).

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

Where,

I= Inhibition (%) of mycelial expansion of *S. rolfsii*

C= Control Petriplate of mycelial expansion of *S. rolfsii*

T= Treatment Petriplate of mycelial expansion of *S. rolfsii*

### 3.7. Effect of chemicals against *Sclerotium rolfsii*

Efficacy of some selected chemicals viz. Index (Myclobutanil 10% WP), Bavastin (Carbendazim 50% WP) and Conika 50% WP (Kasugamycin 5% + Copper oxychloride 45 % WP) were tested against *S. rolfsii* using poisoned food technique given by Grover and Moore (1962). According to this method, already prepared aqueous stock solution of chemical fungicides at different concentration was added separately in PDA medium and poured into clean Petriplates in laminar flow. After solidification, the 5 mm block of fresh culture of *S. rolfsii* was inoculated in the centre of Petriplate and incubated at  $27\pm 1^{\circ}$  C. For each treatment and control three replicates were maintained. Mycelial expansion of *S. rolfsii* was recorded on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day. Inhibition (%) of mycelial enlargement of *S. rolfsii* was calculated by following formula.

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

Where,

I= Inhibition (%) of mycelial expansion of *S. rolfsii*

C= Control Petriplate of mycelial expansion of *S. rolfsii*

T= Treatment Petriplate of mycelial expansion of *S. rolfsii*

### 3.8. Preparation of medicinal plant extracts

Five medicinal plants viz. *Azadirachta indica*, *Ocimum tenuiflorum*, *Syzygium aromaticum*, *Tinospora cordifolia*, *Trachyspermum ammi* were collected from the

BBAU campus, Lucknow. Took 200 gm of each sample and washed first under tap water and then with sterilized distilled water separately. Samples were placed in 0.2% HgCl<sub>2</sub> solution for few second and washed 3 to 4 times with distilled water to eliminate the HgCl<sub>2</sub> (Dwivedi and Sangeeta, 2015). The washed samples were dried on sterilized tissue paper. The plants part was crushed with motor & pestle and were first filtered through the muslin cloth and centrifuged at 3000 rpm for 5 minutes then filtered through Vacuum Seitz filter. The extract was collected in sterile conical flask for further studies.

### 3.8.1. Treatment of *Sclerotium rolfsii* with plant extracts

The antifungal efficacy of some selected plant extract viz. *Azadirachta indica*, *Ocimum tenuiflorum*, *Syzygium aromaticum*, *Tinospora cordifolia*, *Trachyspermum ammi* were tested against *S. rolfsii* using poisoned food technique (Grover and Moore, 1962). The aqueous plant extract at 10%, 25%, 50% and 75% concentration were added into autoclaved cooled down PDA medium and poured into sterilized Petri plates. After solidification, 5 mm block of fresh culture of *S. rolfsii* was inoculated in the centre of Petriplate. Petriplates were incubated at 27±1<sup>0</sup> C. For each treatment and control three replicates were maintained. The mycelial growth of *S. rolfsii* was recorded on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day. Inhibition (%) of mycelial expansion of *S. rolfsii* was calculated by following formula.

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

Where,

I= Inhibition (%) of mycelial expansion of *S. rolfsii*

C= Control Petriplate of mycelial expansion of *S. rolfsii*

T= Treatment Petriplate of mycelial expansion of *S. rolfsii*

### 3.9. Evaluation of integrated management of *Sclerotium rolfsii* under *in vitro* condition

In this method, *Trichoderma viride* metabolite + Myclobutanil10%WP at 10% + 10 ppm, 25% + 25 ppm, 50% + 50 ppm, 75% + 100 ppm concentrations; *Ocimum tenuiflorum* + Myclobutanil10%WP at 10% + 10 ppm, 25% + 25 ppm, 50% + 50 ppm, 75% + 100 ppm concentrations; *Trichoderma viride* metabolite + *Ocimum tenuiflorum* at 10% + 10%, 25% + 25%, 50% + 50%, 75% + 75% concentrations; *Trichoderma viride* metabolite + *Ocimum tenuiflorum* + Myclobutanil10%WP at 10%+10%+10 ppm, 25%+25%+25 ppm, 50%+50%+50 ppm, 75%+75%+100 ppm concentrations were mixed with autoclaved potato dextrose agar (PDA) medium and poured into sterilized Petri plates. In each PDA containing Petridish, 5mm diameter block of 5 days old culture of *S. rolfsii* was inoculated and plates were incubated at  $27 \pm 1$  °C in an incubator. The treatment and control Petriplates were also maintained in triplicate. The inhibition (%) of mycelial expansion of *S. rolfsii* was measured on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day of incubation period using following formula.

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

Where,

I= Inhibition (%) of mycelial expansion of *S. rolfsii*

C= Control Petriplate of mycelial expansion of *S. rolfsii*

T= Treatment Petriplate of mycelial expansion of *S. rolfsii*

### 3.10. Preparation of Inocula of *Sclerotium rolfsii* and *Trichoderma viride*

*Sclerotium rolfsii* and *Trichoderma viride* were isolated from infected

*Zea mays* plant and rhizospheric soil respectively. The fungi were cultured on PDA plates and incubated at  $27\pm 1$  °C. After 7 days, 5 mm mycelium disk of fungus was cut by cork borer and transferred to conical flask separately containing 300 gm sterilized oat (*Avena sativa*) grains. The flasks were incubated at  $27\pm 1$  °C for 15 days after then oat grains of both the fungus were taken out of the flask and dried at room temperature. The oat grains infested with hyphae of the fungus were grinded separately in electric grinder. The powder was kept in sterilized conical flask at 4 °C in a refrigerator for further studies as inoculum (Sennoi et al., 2013).

### **3.11. Procurement of *Zea mays* seeds**

*Zea mays* seeds variety 'Rasi 4212' was procured from Jay Jawan Jay Kisan Beejbhandar, Bijnore, Lucknow Uttar Pradesh ('Rasi seeds (P) Ltd, 174 Sathyamuthy Road, Ramnagar Coimbatore, Tamilnadu).

### **3.12. Pathogenicity test**

For pathogenicity test earthen pots were sterilized with absolute alcohol. The pots were filled with sterilized soil (4kg/pot)+inoculum (2%w/w) of *Sclerotium rolfsii*. Now surface sterilized (0.2% mercuric chloride solution) seeds of *Zea mays* (Rasi 4212) were sown (05seeds/pot). After the seedling emerges, the pots were watered regularly with sterilized distilled water to maintain the moisture content of the soil. The pots were kept in a greenhouse. Control set was also maintained without adding inoculum of the test fungus. After 30 days, the plants showed root rot symptoms. Such plants were taken out of the pot and washed thoroughly with distilled water. Small sections of infected parts of root was cut and inoculated onto PDA plates. Plate was incubated at  $27\pm 1$  °C for 5 days. The colony grew was identified and

matched with the parent strain of *Sclerotium rolfii* of which the inoculum was prepared (Fig.4.3).

### **3.13. Treatment of *Sclerotium rolfii* with bioagent, plant extract and chemical under *in vivo* condition**

In case of pot experiment, the soil was collected from BBAU campus. The soil was sterilized in an autoclave at 121<sup>0</sup>C (15 lb pressure) for 60 minutes. Took autoclaved soil (4kg/pot) and mixed with 2% w/w inoculum of *S. rolfii* and filled the pots separately. The pots were treated with inoculum of *Trichoderma viride* at 1% w/w, 2% w/w, 4% w/w concentration; leaf extract of *Ocimum tenuiflorum* at 1% v/w, 2% v/w, 3% v/w concentration and fungicide Myclobutanil 10% WP at 200 ppm v/w, 600 ppm v/w, 1000 ppm v/w concentration respectively. The sterilized seeds of *Zea mays* (sterilized with 0.2% HgCl<sub>2</sub> solution) were sown in each pot (10 seeds/pot). On 50<sup>th</sup> day the shoot length, root length, fresh weight, dry weight and crop disease incidence (%) was calculated by given formula:

$$\text{CIP} = \frac{\text{TS} - \text{FS}}{\text{TS}} \times 100$$

Where,

CIP= crop infection percent in each pot

TS= total seedling in each pot

FS= fresh seedling in each pot

### **3.14. Evaluation of integrated management of root rot disease in *Zea mays* under *in vivo* condition**

Soil collected from the BBAU campus, Lucknow, India was sterilized in an autoclave at 121<sup>0</sup>C (15 lb pressure) for 60 minutes. Took autoclaved soil (4 kg/pot)

and thoroughly was mixed with 2% w/w inoculum of the pathogen, was filled in each pot. The pots were treated with *Trichoderma viride* inoculum + Myclobutanil 10% WP at 1% w/w + 200 ppm v/w, 2% w/w + 600 ppm v/w, 4% w/w + 1000 ppm v/w concentration; *Ocimum tenuiflorum* + Myclobutanil 10% WP at 1% v/w + 200 ppm v/w, 2% v/w + 600 ppm v/w, 3% v/w + 1000 ppm v/w concentration; *Trichoderma viride* inoculum + *Ocimum tenuiflorum* at 1% w/w + 1% v/w, 2% w/w + 2% v/w, 4% w/w + 3% v/w concentration; *Trichoderma viride* inoculum + Myclobutanil 10% WP + *Ocimum tenuiflorum* at 1% w/w + 200 ppm v/w + 1% v/w, 2% w/w + 600 ppm v/w + 2% v/w, 4% w/w + 1000 ppm v/w + 3% v/w concentration respectively. Sterilized (0.2% mercuric chloride) *Zea mays* (Rasi 4212) seeds were sown in each pot (10 seeds/pot). On 50<sup>th</sup> day, the shoot length, root length, fresh weight, dry weight followed by crop disease incidence (%) was calculated by given formula:

$$CIP = \frac{TS - FS}{TS} \times 100$$

Where,

CIP= crop infection percent in each pot

TS= total seedling in each pot

FS= fresh seedling in each pot

### 3.15. Histopathological study

The samples were analyzed from USIC BBAU Lucknow. The infected part of *Zea mays* was washed under running water followed by distilled water and then cut T.S. of root & stem. The sections were fixed with 2.5% glutaraldehyde for 2-6 hours. The fixed sections were washed with phosphate buffer for 3-4 times followed by series of dehydration process in 30-100% ethanol and then kept in dry acetone.

Sample was mounted on aluminium stubs with a double sided carbon conductive tape and was coated with a thin layer of palladium under vacuum. Sample was observed and the images were digitally recorded under Scanning Electron Microscope (Jeol JSM-6490 LV).

### **3.16. Data analysis**

The data were expressed as Mean and analyzed statistically using one way ANOVA (analysis of variance), coefficient variance, standard error, standard deviation, DMRT and Least significant difference test ( $p \leq 0.05$ ) were applied to arrive definite conclusion on the results.

# CHAPTER - 4

## RESULTS & DISCUSSION



## RESULTS AND DISCUSSION

---

The present work was carried out to isolate fungal antagonists from rhizospheric soil of healthy *Zea mays* plant on PDA medium using serial dilution technique. The effectiveness of selected chemical fungicides, plants extract and bioagents were evaluated under *in vitro* and *in vivo* condition. All treatments were most effective against root rot causing soil borne fungus *S. rolfsii* under *in vitro* and *in vivo* condition. The results obtained are presented in the following sub-headings.

### 4.1. Sample collection

The samples were collected from three district of Uttar Pradesh viz. Barabanki, Lucknow and Jaunpur. In all three sites, the most infected crop was found in Barabanki district (Fig. 4.1).

### 4.2. Analysis of Physico-chemical properties of collected field soil samples

The soil samples were collected from rhizospheric and non-rhizospheric region of selected sites of Uttar Pradesh. The soil texture divided into sand, silt and clay. The highest sand particle (33.33%) was found in sample no. 4 and minimum sand particle (25%) was recorded in sample no. 2. The highest silt particles (38.09%) was in sample no. 3 and minimum silt particles (25%) was recorded in sample no. 4. The highest clay particle (41.67%) was in sample no. 2&4 and minimum clay particle (33.33%) was recorded in sample no. 3. The highest soil pH (9.34) was in sample no. 2 and lowest soil pH (8.53) was recorded in sample no. 4. The maximum soil temperature (27.15<sup>0</sup> C) was noted in sample no. 2 followed by minimum temperature (24.23<sup>0</sup> C) in sample no. 4 (Table 4.1).



**Fig. 4.1:** The field infected from *Sclerotium rolfsii* and ‘→’ (arrow) indicate the hyphae expansion in the field of *Zea mays* crop.

Junsopa et al. (2016) reported that factors like soil pH, temperature, moisture and humidity are also favorable for development of stem rot disease caused by *S. rolfsii*. The low pH and high temperature affected the inhibition of *S. rolfsii* when treated with *Trichoderma viride* and inhibited the growth by 77% at 37<sup>0</sup> C temperature and at pH 4 (Ayed et al., 2018). *Sclerotium rolfsii* developed at intermediate soil moisture (70%) of field capacity and temperature between 25<sup>0</sup>C to 30<sup>0</sup>C is the main factor for expansion of *S. rolfsii* (Hari et al., 1988; Pinheiro et al., 2010). Lal et al. (2013) reported that most of the fungi develop at temperature range between 25 to 30<sup>0</sup>C and pH range 6.0 to 8.0. These temperature and pH range are suitable for growth and development of fungi as in the present result. In the present study, 30<sup>0</sup> C

temperature was found best for the expansion of *S. rolfsii* which agrees the findings of Taj et al. (2014).

Organic carbon and organic matter are important part of soil for the growth of plants. The maximum organic carbon (2.00%) was recorded in sample no. 1 while minimum organic carbon (1.03%) in sample no. 2. The maximum organic matter (5.79%) was recorded in sample no. 1 followed by minimum organic matter (5.43%) in sample no. 4 (Table 4.1).

**Table 4.1:** Analysis of sand, silt, clay, organic carbon, organic matter, pH and temperature from collected soil samples

Sample No.	Sand (%)	Silt (%)	Clay (%)	Soil pH	Soil Temperature ( <sup>0</sup> C)	Organic carbon (%)	Organic matter (%)
1.	31.81	29.27	40.9	8.92	25.25	2.00	5.79
2.	25	33.33	41.67	9.34	27.15	1.03	5.61
3.	28.57	38.09	33.33	8.64	26.50	1.86	5.25
4.	33.33	25	41.67	8.53	24.23	1.91	5.43

### 4.3. Isolation and identification of soil mycoflora

Twelve soil mycoflora have been isolated from various soil samples using serial dilution technique of 1/1000, 1/10000, 1/100000 dilution. *Aspergillus flavus*, *A. fumigatus*, *A. luchuensis*, *A. niger*, *A. sydowii*, *Alternaria solani*, *Fusarium solani*, *Penicillium citrinum*, *Penicillium italicum*, *Penicillium species*, *Trichoderma viride*, white sterile mycelium were isolated. When dilution increases (1/1000<1/10000<1/100000), the number of fungus colony decreases (Table 4.2, 4.3, 4.4). Out of four collected soil samples, the fungal diversity shown in soil sample number one was highest compared to other soil samples (Table 4.2). The percent frequency and percent occurrence of *Aspergillus flavus* and *Aspergillus luchuensis* was highest compared to other isolated soil mycoflora (Table 4.2, 4.3, 4.4). *Sclerotium rolfsii* was

isolated from infected maize plant on potato dextrose agar (PDA) Petriplate within seven days after inoculation. The isolated fungus produced cottony white mycelia on PDA plate, on the 5<sup>th</sup> day cottony white mycelia changed into light brown and after seven days light brown mycelia changed into dark brown and become hard mustard seed like “sclerotia” are formed which is a characteristic feature of *S. rolfsii* (Chaurasia et al., 2014; Prasad and Dwivedi, 2017). *Sclerotium rolfsii* was identified on the basis of available literature and sclerotial formation.

**Table 4.2:** Percent (%) occurrence and percent frequency of soil mycoflora collected from soil samples at 10<sup>-3</sup> dilution (1/1000) of 1g soil

S.No.	Fungal species	SS No.1		SS No.2		SS No.3		SS No.4		Total	%Occurrence
		Freq	%Freq	Freq	%Freq	Freq	%Freq	Freq	%Freq	Freq	
1.	Af	7	17.07	-	-	-	-	5	20.00	12	12.37
2.	Afm	-	-	-	-	-	-	7	28.00	7	7.22
3.	Al	5	12.19	8	40.00	-	-	6	24.00	19	19.59
4.	An	-	-	-	-	6	54.54	-	-	6	6.18
5.	Asy	-	-	7	35.00	-	-	-	-	7	7.22
6.	As	-	-	-	-	5	45.45	-	-	5	5.15
7.	Fs	4	9.75	-	-	-	-	-	-	4	4.12
8.	Pc	5	12.19	-	-	-	-	-	-	5	5.15
9.	Pi	6	14.63	-	-	-	-	-	-	6	6.18
10.	Psp	-	-	-	-	-	-	7	28.00	7	7.22
11.	Tv	8	19.51	-	-	-	-	-	-	8	8.25
12.	Wsm	6	14.63	5	25.00	-	-	-	-	11	11.34
Total	-	41	-	20	-	11	-	25	-	97	-
% Occurrence		42.26		20.62		11.34		25.77		-	

SS No.= Soil Sample Number, S. No.= Serial Number, Freq= Frequency of Mycoflora, %Freq= Percent Frequency of Mycoflora, Af=*Aspergillus flavus*, Afm=*Aspergillus fumigatus*, Al=*Aspergillus luchuensis*, An=*Aspergillus niger*, Asy=*Aspergillus sydowii*, As=*Alternaria solani*, Fs=*Fusarium solani*, Pc=*Penicillium citrinum*, Pi=*Penicillium italicum*, Psp=*Penicillium species*, Tv=*Trichoderma viride*, Wsm=white sterile mycelium

**Table 4.3:** Percent (%) occurrence and percent frequency of soil mycoflora collected from soil samples at 10<sup>-4</sup> dilution (1/10000) of 1g soil

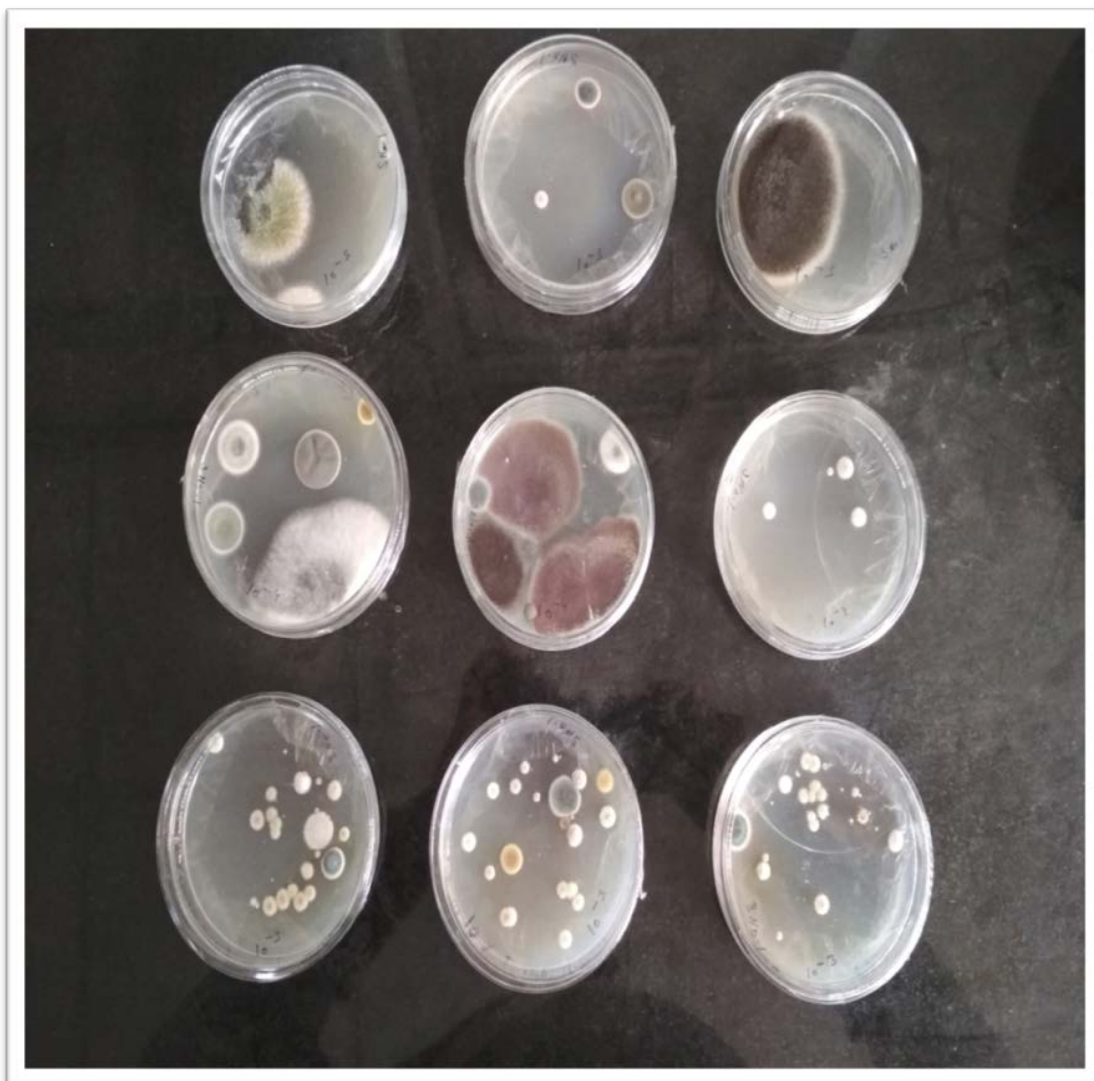
S.No.	Fungal species	SS No.1		SS No.2		SS No.3		SS No.4		Total Freq	%Occurrence
		Freq	%Freq	Freq	%Freq	Freq	%Freq	Freq	%Freq		
1.	Af	4	16.00	-	-	-	-	3	20.00	7	11.86
2.	Afm	-	-	-	-	-	-	4	26.67	4	6.78
3.	Al	3	12.00	5	41.67	-	-	3	20.00	11	18.64
4.	An	-	-	-	-	4	57.14	-	-	4	6.78
5.	Asy	-	-	4	33.33	-	-	-	-	4	6.78
6.	As	-	-	-	-	3	42.86	-	-	3	5.08
7.	Fs	2	8.00	-	-	-	-	-	-	2	3.39
8.	Pc	3	12.00	-	-	-	-	-	-	3	5.08
9.	Pi	4	16.00	-	-	-	-	-	-	4	6.78
10.	Psp	-	-	-	-	-	-	5	33.33	5	8.47
11.	Tv	5	20.00	-	-	-	-	-	-	5	8.47
12.	Wsm	4	16.00	3	25.00	-	-	-	-	7	11.86
Total	-	25	-	12	-	7	-	15	-	59	-
% Occurrence		42.37		20.34		11.86		25.42		-	

SS No.= Soil Sample Number, S. No.= Serial Number, Freq= Frequency of Mycoflora, %Freq= Percent Frequency of Mycoflora, Af=*Aspergillus flavus*, Afm=*Aspergillus fumigatus*, Al=*Aspergillus luchuensis*, An=*Aspergillus niger*, Asy=*Aspergillus sydowii*, As=*Alternaria solani*, Fs=*Fusarium solani*, Pc=*Penicillium citrinum*, Pi=*Penicillium italicum*, Psp=*Penicillium species*, Tv=*Trichoderma viride*, Wsm=white sterile mycelium

**Table 4.4:** Percent (%) occurrence and percent frequency of soil mycoflora collected from soil samples at  $10^{-5}$  dilution (1/100000) of 1g soil

S.No.	Fungal species	SS No.1		SS No.2		SS No.3		SS No.4		Total	%Occurrence
		Freq	%Freq	Freq	%Freq	Freq	%Freq	Freq	%Freq	Freq	
1.	Af	2	14.28	-	-	-	-	1	14.28	3	9.37
2.	Afm	-	-	-	-	-	-	2	28.57	2	6.25
3.	Al	2	14.28	3	50.00	-	-	1	14.28	6	18.75
4.	An	-	-	-	-	3	60.00	-	-	3	9.37
5.	Asy	-	-	2	33.33	-	-	-	-	2	6.25
6.	As	-	-	-	-	2	40.00	-	-	2	6.25
7.	Fs	1	7.14	-	-	-	-	-	-	1	3.12
8.	Pc	1	7.14	-	-	-	-	-	-	1	3.12
9.	Pi	3	21.42	-	-	-	-	-	-	3	9.37
10.	Psp	-	-	-	-	-	-	3	42.86	3	9.37
11.	Tv	3	21.42	-	-	-	-	-	-	3	9.37
12.	Wsm	2	14.28	1	16.67	-	-	-	-	3	9.37
Total	-	14	-	6	-	5	-	7	-	32	-
% Occurrence		43.75		18.75		15.62		21.87		-	

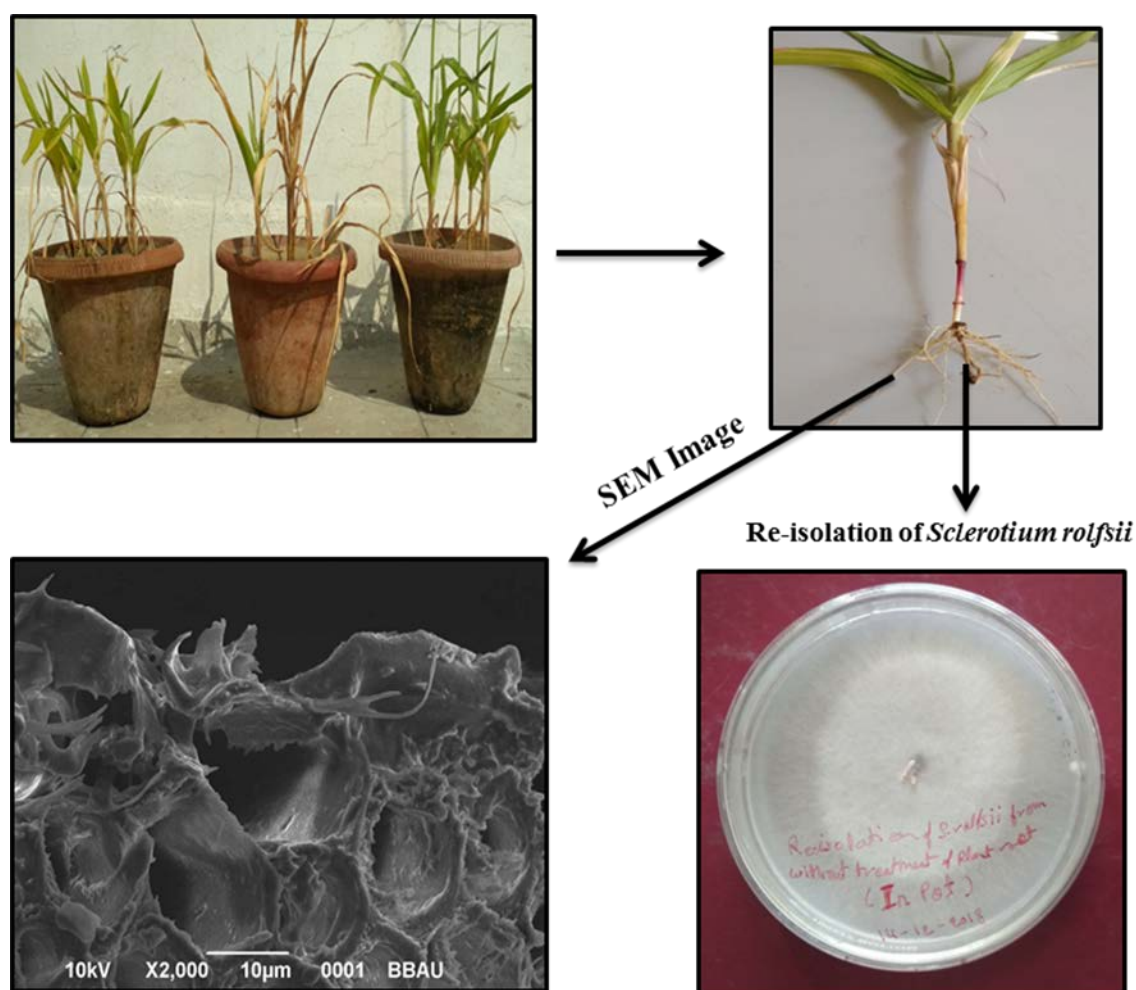
SS No.= Soil Sample Number, S. No.= Serial Number, Freq= Frequency of Mycoflora, %Freq= Percent Frequency of Mycoflora, Af=*Aspergillus flavus*, Afm=*Aspergillus fumigatus*, Al=*Aspergillus luchuensis*, An=*Aspergillus niger*, Asy=*Aspergillus sydowii*, As=*Alternaria solani*, Fs=*Fusarium solani*, Pc=*Penicillium citrinum*, Pi=*Penicillium italicum*, Psp=*Penicillium species*, Tv=*Trichoderma viride*, Wsm=white sterile mycelium



**Fig. 4.2:** Different colony of soil mycoflora, screening from soil using serial dilution technique

#### 4.4. Pathogenicity test

The pathogenicity test of *Sclerotium rolfsii* isolated from the infected root of the *Zea mays* was confirmed following Koch's postulates method given by Koch in 1880. The disease symptom appeared in *Zea mays* plant after 30 days of sowing in infested soil with *S. rolfsii* inoculum. The general morphological symptoms (stunting and yellowing of plant) were observed in *Zea mays*. The sections of infected part was cut and transferred onto PDA plate. The newly re-isolated strain was re-identified and matched with previous isolated strain. T.S. of root was analyzed with the help of SEM which showed ruptured and black discoloration of xylem (Fig. 4.3).



**Fig. 4.3:** Pathogenicity test of *Sclerotium rolfsii*

#### 4.5. Evaluation of antagonistic activity of some fungal species against *Sclerotium rolfsii*

The selected bioagents were significantly ( $p \leq 0.05$ ) (Appendix II–Table 1, 2, 3) effective to check the mycelial expansion of *Sclerotium rolfsii*. The isolated six fungal species viz. *Aspergillus flavus*, *A. niger*, *Trichoderma viride*, *Penicillium citrinum*, *P. italicum* and *Penicillium* sp. were tested under dual culture method to find out their antagonistic activity against *S. rolfsii* under *in vitro* condition (Fig. 4.4). It was observed that among the antagonistic test fungi, *Trichoderma viride* and *Penicillium* sp. were significantly effective bioagent to control *S. rolfsii*. *Trichoderma viride* inhibited the growth of the test fungus by 26.89% compared to *Aspergillus flavus* (18.42%), *A. niger* (22.36%), *Penicillium citrinum* (20.39%), *P. italicum* (17.14%) and *Penicillium* sp. (23.35%) on 3<sup>rd</sup> day under *in vitro* study (Table 4.5, Fig. 4.5A).

Anees et al. (2010) reported that different strain of *Trichoderma* exhibited biocontrol activity against *S. rolfsii*. All the treatments contain inhibitory activity which binds the actively growing mycelial tip of the pathogen and inhibits the mycelial growth of *S. rolfsii*. In a study nine antagonistic microorganisms were selected for their efficacy against *S. rolfsii* using dual culture technique under *in-vitro* condition, out of these, the maximum mycelial expansion of *S. rolfsii* was reduced (63.33%) by *T. viride* (Nagamma and Nagaraja, 2015). Two different species of *Trichoderma* viz. *Trichoderma viride* and *Trichoderma harzianum* were effective against *Sclerotium rolfsii* under *in vitro* and greenhouse conditions (Rajendraprasad et al., 2017). *Trichoderma viride*, *Aspergillus niger* and *Penicillium* species were most effective against *S. rolfsii* causing stem rot in groundnut under *in vitro* condition (Shaigan et al., 2008; Bosah et al., 2010). Lal et al. (2013) tested seven bioagents viz. *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *T. harzianum*, *Trichoderma virens*,

*Pseudomonas fluorescence* and *Pseudomonas* sp. which have antagonistic activities against plant pathogen and they significantly inhibited development of pathogen under *in vitro* study. El-Mougy et al. (2012) reported that *Trichoderma viride*, *T. harzianum* and *T. hamatum* were effective against *Sclerotium rolfsii* causing root rot and damping-off diseases in various vegetable plants like Tomato, Pepper, Cucumber, and Cantaloupe. They also inhibited (58.5%, 61.8% and 56.4%) stem rot disease in sunflower caused by *Sclerotium rolfsii* using dual culture technique under *in vitro* condition (Sumi & Ao, 2015).

Ekundayo et al. (2015) observed similar result in root-rot disease in *Zea mays* when treated with *Aspergillus flavus*, *A. niger*, *T. viride* and *Penicillium italicum* against mycelium growth of *S. rolfsii* under *in vitro* condition. The antagonistic effect of *Trichoderma viride* was most significantly effective against test pathogen *Sclerotium rolfsii* which agrees the results of Jegathambigai et al. (2010). Rekha et al., (2012) selected forty four *Trichoderma* strain (Tri-1 to Tri-44) for their efficiency against mycelial expansion and formation of sclerotia in *Sclerotium rolfsii* under *in vitro* condition; out of these 44 strain, only 10 strains viz. Tri-8, Tri- 13, Tri- 15, Tri- 16, Tri- 19, Tri-23, Tri-27, Tri- 29, Tri- 41 and Tri- 44 were found to be effective in tumbling both mycelial expansion and formation of sclerotia whereas the metabolites of two strains viz. Tri-13 and Tri-29 were effective against mycelial enlargement of *S. rolfsii*. Rao and Kulkarni (2003) reported that *Trichoderma harzianum* and *T. viride* were very efficient in reducing the mycelial expansion of *S. rolfsii*. Karthikeyan et al. (2006) isolated three bioagents viz; *T. viride*, *T. harzianum* and *Pseudomonas fluorescens* and studied their effectiveness against growth of *S. rolfsii*. Out of these three bioagents, only *T. viride* inhibited 69.40% mycelial development and 62.50% sclerotial germination of *S. rolfsii*. The bioagents *Trichoderma viride*, *T. lignorum* and

*Gladiolus virens* were most efficient against collar rot disease caused by *S. rolfii* (HaralPatil and Raut 2008). Madhavi and Bhattiprolu (2011) tested two *Trichoderma* sp. viz. *T. viride*, *T. harzianum* against *S. rolfii* using dual culture technique and reported the growth inhibition by 55.8%, 57.5% respectively. The percent inhibition of mycelial expansion of *S. rolfii* by five *Trichoderma* species viz *T. hamanatum*, *T. koningii*, *T. harzianum*, *T. ressei* and *T. viride*, indicated as enhanced antagonists in sustainable agricultural and environmental protection (Bagwan, 2011). *Trichoderma viride* contained antagonistic activity against mycelial expansion of *S. rolfii* under *in vitro* condition (Zape et al., 2014). *In vitro* efficacy *Trichoderma harzianum* contained antagonistic activity against mycelial growth of *S. rolfii* (Wavare et al., 2017).

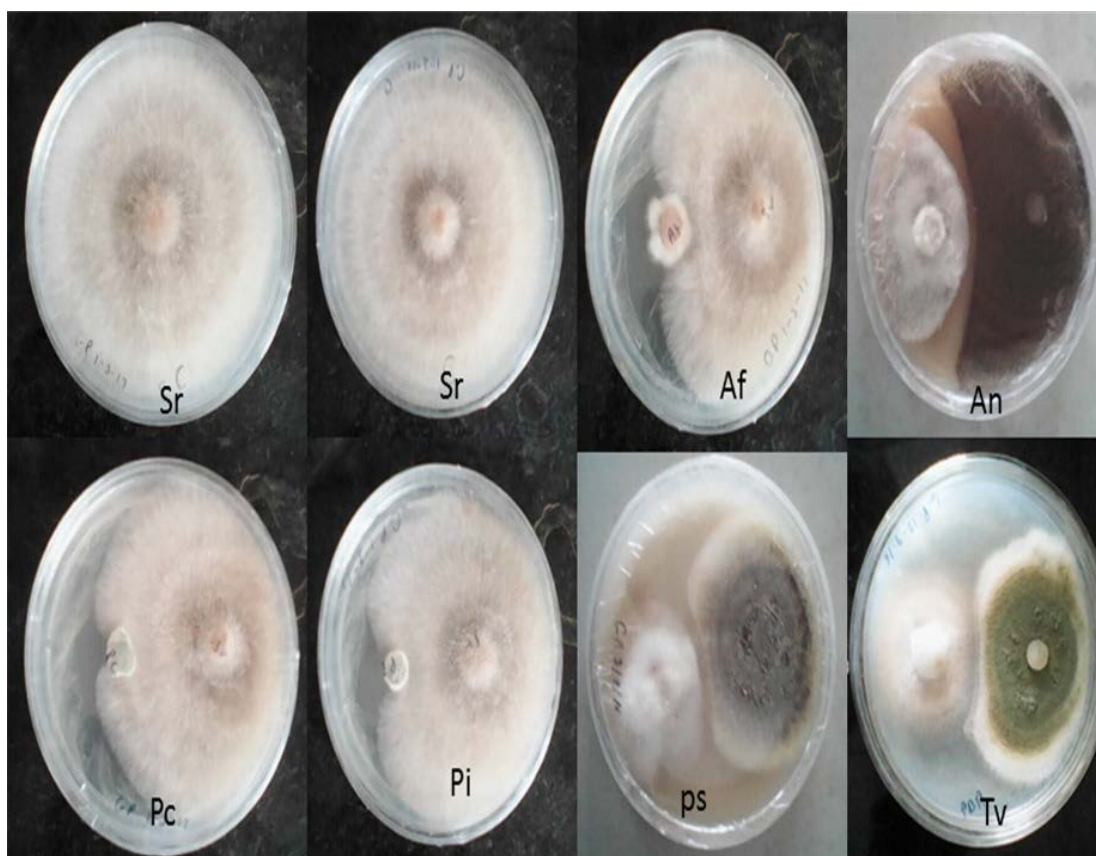
**Table 4.5:** Antagonistic activity of some bio-agents against mycelial growth (in mm) of *Sclerotium rolfii* using dual culture technique under *in vitro* condition

Treatment	1st day	2nd day	3rd day	CV%
Control	32.58±1.24	63.08±0.79	84.58±0.67	36.03
<i>Aspergillus flavus</i>	31.25±0.75	57±5.47	69±12.38	33.73
<i>Aspergillus niger</i>	30.33±0.98	59.58±7.56	65.67±11.79	33.76
<i>Penicillium citrinum</i>	31.33±1.07	58.33±3.20	67.33±7.64	30.95
<i>Penicillium italicum</i>	32.17±1.03	60.25±4.05	70.08±9.43	31.9
<i>Penicillium sp.</i>	31.42±0.67	56.25±5.82	64.83±12.28	32.01
<i>Trichoderma viride</i>	30.5±1.24	55.5±6.04	61.83±10.60	31.13
<b>F-cal</b>	7.608*	3.148*	6.499*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05



**Fig. 4.4:** Antagonistic activity of *Aspergillus flavus*, *A. niger*, *Penicillium citrinum*, *P. italicum*, *Penicillium* sp. and *Trichoderma viride* against growth of *S. rolfsii* on 3<sup>rd</sup> day

#### 4.6. Treatment of *Sclerotium rolfsii* with some chemicals

Three chemicals viz. Index (Myclobutanil 10% WP), Bavastin (Carbendazim 50% WP) and Conika 50% WP (Kasugamycin 5% + Copper oxychloride 45 % WP) were tested against *S. rolfsii* under *in vitro* condition and they were significantly ( $p \leq 0.05$ ) (Appendix II–Table 4–12) effective against mycelial expansion of *S. rolfsii* (Fig.4.7). Myclobutanil 10% WP inhibited maximum (77.16%) mycelial growth of *S. rolfsii* at 100 ppm (v/v) concentration followed by 10 ppm (26.28%), 25 ppm (41.57%), 50 ppm (64.03%) concentration on 3<sup>rd</sup> day (Table 4.6; Fig. 4.5B). At 600 ppm concentration, Conika 50% WP inhibited the growth by 70.57% compared to 100 ppm (1.99%) 200 ppm (7.99%) 400 ppm (57.86%) concentration (v/v) against

mycelial expansion of *S. rolfsii* on 3<sup>rd</sup> day of incubation period (Table 4.7; Fig.4.6A). Carbendazim 50% WP was most effective and inhibited the growth of *S. rolfsii* by 84.19% at 6 ppm (v/v) concentration whereas at 1 ppm, 2 ppm, 4 ppm (v/v) concentration the growth of *S. rolfsii* was inhibited by 17.08%, 75.20%, 81.38% respectively on 3<sup>rd</sup> day of incubation under *in vitro* condition (Table 4.8; Fig.4.6B). Amongst these chemical fungicides, the Carbendazim 50% WP was most effective compared to Myclobutanil 10% WP and Conika 50% WP against mycelial growth of *S. rolfsii* at different concentration on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day of incubation period.

Chaurasia et al. (2014) reported nine chemical fungicides viz. Bavistin, Brassicol, Captan, Dithane M-45, DM-145, Fytolan, Manzate, Parasan, Sulfex against *S. rolfsii* under *in vitro* condition and reported that all fungicides had adverse effect on the expansion of *S. rolfsii* and inhibited the expansion by 100% at 0.1% concentration. In a study, carbendazim maximum inhibited the expansion of *S. rolfsii* by 93.7% at 0.2% concentration under *in vitro* condition (Madhavi and Bhattiprolu, 2011). The chemical fungicide Metalaxyl + Mancozeb (0.1%), Propiconazole (0.05%), Difenconazole (0.05%), Metiram (0.1%) and Hexaconazole (0.05%) were efficient in controlling collar rot disease caused by *S. rolfsii* (HaralPatil and Raut, 2008). Bavistin, Provax-200, Dithane M-45, Tilt, Ridomil and Rovral 50 WP were tested at 100, 200 and 400 ppm concentration against radial expansion of *S. rolfsii*. All chemical fungicides completely inhibited radial enlargement of *S. rolfsii* (Bhuiyan et al., 2012). Taj et al. (2014) studied seven fungicides viz. Captan, Carbendazim, Chlorothalonil, Mancozeb, Tebuconazole, Tricyclazole and Trifloxystoburin against *Sclerotium rolfsii* and effectively inhibited mycelial expansion of *Sclerotium rolfsii* at 1000 ppm concentration. Hexaconazole was most effective compared to Carbendazim, difenoconazole, mencozeb, chlorothalonil at 125 ppm, 250 ppm, 500

ppm concentration against *S. rolfii* under *in vitro* condition (Sharma and dhruj, 2018). Wavare et al. (2017) reported that Carbendazim inhibited 100% mycelial expansion of *S. rolfii*. Five fungicides viz. Bavistin, Propiconazole, Topsin M, Hexaconazole, and Vitavax at 100, 250 and 500 ppm concentration were tested for their efficacy against *S. rolfii* under *in vitro* condition. The Vitavax, Hexaconazole and Propiconazole completely inhibited expansion of *S. rolfii* while Topsin M and Bavistin showed 71.78% and 79.52 growth embarrassment respectively at 500 ppm concentration agrees with the finding of Ahsan et al. (2018).

**Table 4.6:** Effect of Myclobutanil 10%WP against mycelial growth (in mm) of *Sclerotium rolfii* at different concentration and incubation period under *in vitro* condition

Treatment	1st day	2nd day	3rd day	CV%
<b>Control</b>	29.92±0.79	61.58±1.56	89.42±0.90	40.91
<b>10ppm</b>	22.25±0.86	42.75±0.86	65.92±1.73	41.53
<b>25ppm</b>	22.33±0.88	37.33±2.87	52.25±1.65	33.59
<b>50ppm</b>	18.42±0.90	21.42±1.08	32.16±1.19	25.29
<b>100ppm</b>	14.42±0.66	17.33±0.88	20.42±0.79	14.9
<b>F-cal</b>	577.232*	1413.568*	5202.041*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.7:** Effect of Conika 50% WP against mycelial growth (in mm) of *Sclerotium rolfsii* at different concentration and incubation period under *in vitro* condition

Treatment	1st day	2nd day	3rd day	CV%
Control	30.08±0.90	65.42±0.51	91.75±0.62	41.06
100ppm	29.58±0.51	65.33±0.49	89.92±0.51	40.77
200ppm	27.92±0.51	60.83±2.69	84.42±0.90	40.8
400ppm	22.5±0.67	30.58±0.90	38.66±1.07	22.07
600ppm	19.16±1.40	27±0.60	27±0.60	15.79
F-cal	366.596*	2514.522*	19355.34*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

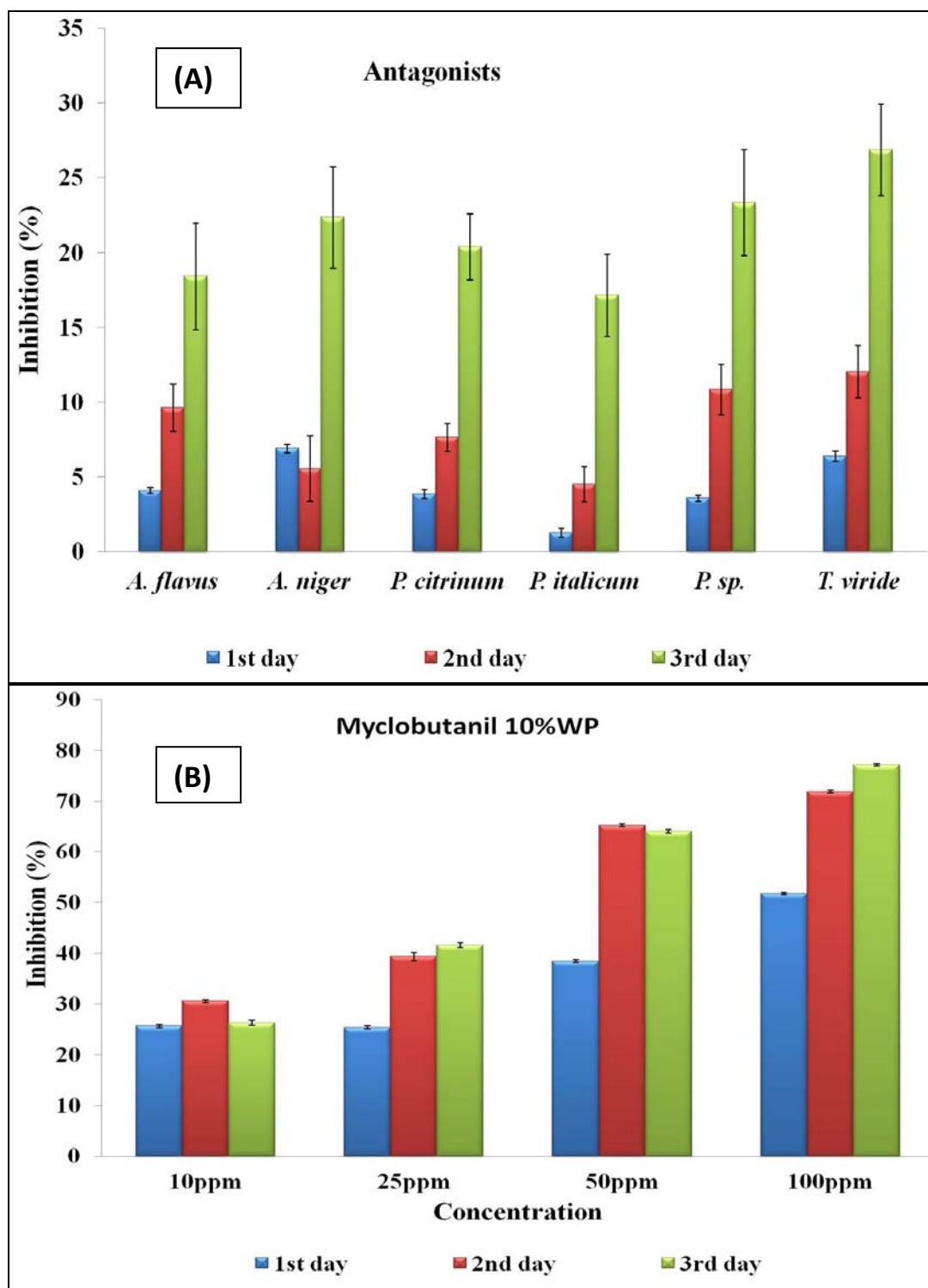
**Table 4.8:** Effect of Carbendazim 50% WP against mycelial growth (in mm) of *Sclerotium rolfsii* at different concentration and incubation period under *in vitro* condition

Treatment	1st day	2nd day	3rd day	CV%
Control	30.08±0.90	65.42±0.51	91.75±0.62	41.06
1ppm	26.42±0.79	51.58±0.67	76.08±0.79	40.05
2ppm	18.92±0.67	22.75±0.75	22.75±0.75	9.12
4ppm	15.25±0.62	17.08±0.66	17.08±0.67	6.55
6ppm	9.75±0.62	14.5±0.79	14.5±0.79	18.43
F-cal	1532.165*	13255.92*	30120.66*	-

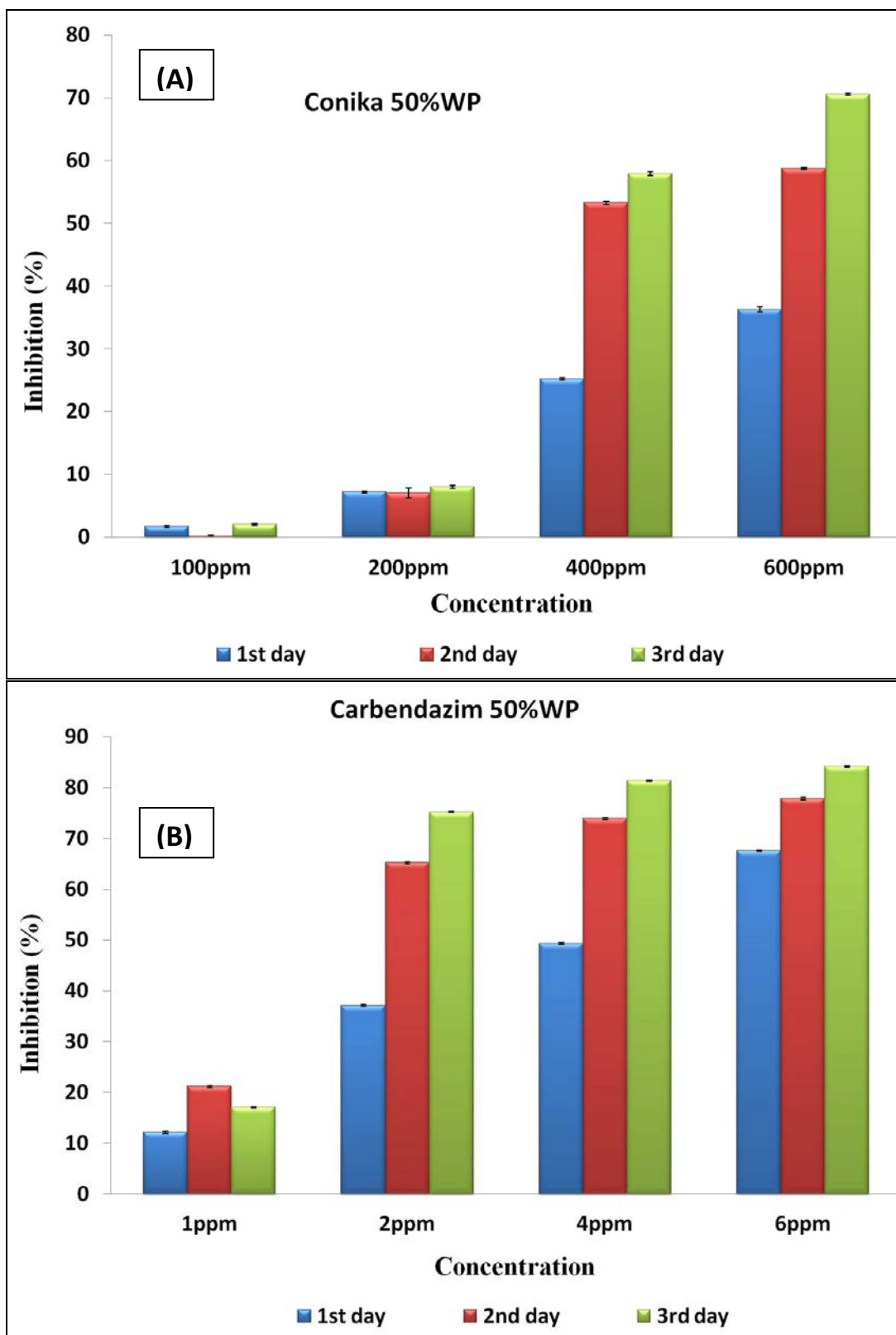
Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

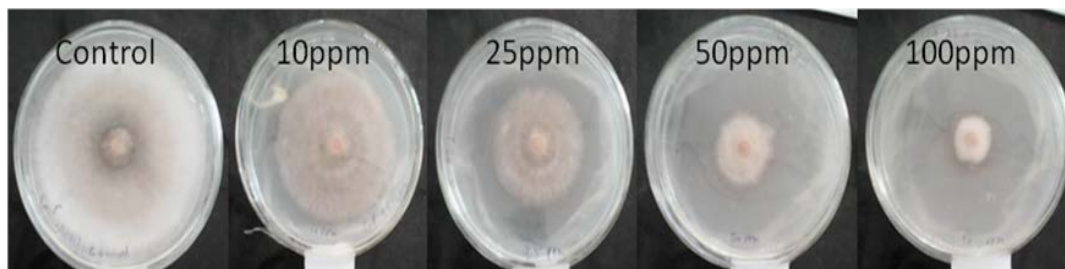
\*= Significant at p=0.05



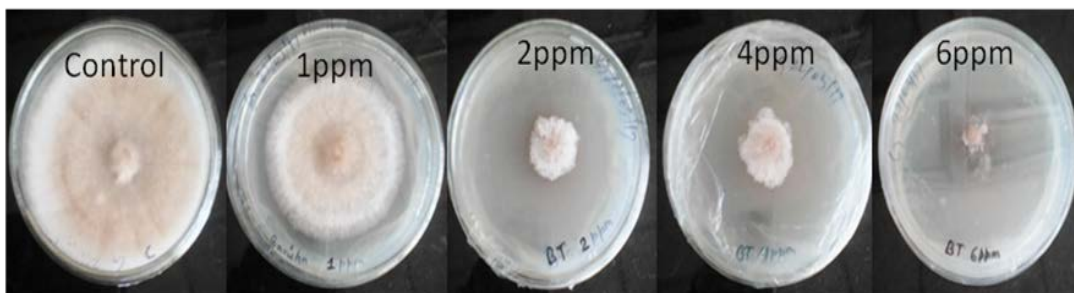
**Fig. 4.5:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with (A) Bioagent (B) Myclobutanil 10% WP under *in vitro* condition



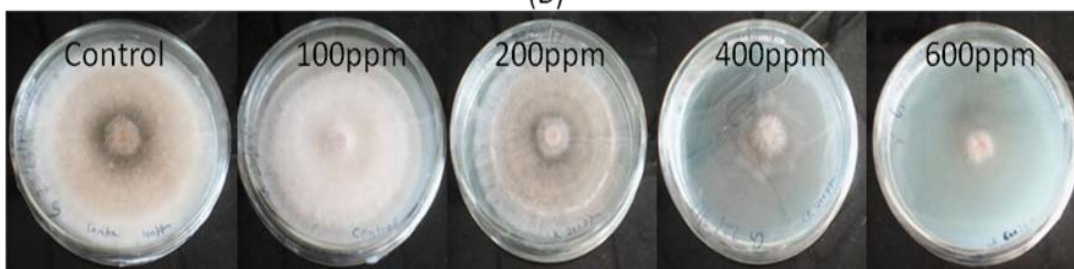
**Fig. 4.6:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with (A) Conika 50% WP (B) Carbendazim 50% WP under *in vitro* condition



(A)



(B)



(C)

**Fig. 4.7:** Antifungal activity of chemical fungicide (A) Myclobutanil 10% WP (B) Carbendazim 50% WP (C) Conika 50% WP at different concentration against *Sclerotium rolsii* using poisoned food technique on 3<sup>rd</sup> day incubation period

#### 4.7. Effect of plant extracts against *Sclerotium rolfsii*

The selected five plants viz. *Azadirachta indica* (leaf), *Ocimum tenuiflorum* (leaf), *Syzygium aromaticum* (oil), *Tinospora cordifolia* (stem), *Trachyspermum ammi* (seeds) were tested at different concentrations, to find out their antifungal activity against *S. rolfsii* and was found that they were significantly effective ( $P \leq 0.05$ ) (Appendix II–Table 13–27) against *S. rolfsii* under *in vitro* condition. *Azadirachta indica* inhibited the growth maximum (79.65%) at 75% v/v concentration followed by 18.59%, 32.10%, 71.05% at 10%, 25%, 50% v/v concentration on 3<sup>rd</sup> day against *S. rolfsii* (Table 4.9; Fig.4.8A, 4.11A). *Ocimum tenuiflorum* was tested at 10%, 25%, 50%, 75% concentration against *S. rolfsii* and 100% growth inhibition at 75% v/v concentration was recorded followed by 19.98%, 37.55%, 77.06% growth inhibition at 10%, 25%, 50% v/v concentration on 3<sup>rd</sup> day respectively (Table 4.10; Fig.4.8B, 4.11B). On another hand, *Syzygium aromaticum* oil inhibited 100% growth of *S. rolfsii* at 400 ppm v/v concentration while at 100 ppm, 200ppm v/v concentration it inhibited the growth by 13.23%, 79.65% of *S. rolfsii* on 3<sup>rd</sup> day respectively (Table 4.11; Fig.4.9A). *Tinospora cordifolia* showed antifungal activity against *S. rolfsii* and it inhibited the expansion by 100% at 75% v/v concentration followed by 39.56%, 61.34%, 73.59% at 10%, 25%, 50% v/v concentration respectively on 3<sup>rd</sup> day under *in vitro* condition (Table 4.12; Fig.4.9B, 4.11C). *Trachyspermum ammi* seeds powder inhibited the growth by 100% at 50% v/v concentration followed by 22.11%, 31.73% at 10%, 25% v/v concentration against *S. rolfsii* on 3<sup>rd</sup> day of incubation period under *in vitro* condition (Table 4.13; Fig.4.10). Among all tested medicinal plants, *Syzygium aromaticum* and *Trachyspermum ammi* inhibited the growth by 100% at 400 ppm (v/v) and 50% (v/v) concentration respectively while *Tinospora cordifolia* inhibited 100% at 75% (v/v) concentration on 3<sup>rd</sup> day. *Syzygium aromaticum* oil,

*Trachyspermum ammi* and *Tinospora cordifolia* was significantly effective compared to *Azadirachta indica* and *Ocimum tenuiflorum* at different concentration on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day under *in vitro* condition.

*Tinospora cordifolia* is a most effective ayurvedic medicine for both plants and animals. *Tinospora cordifolia* is a climbing shrub of family *Menispermaceae* contain anticancer, antiulcer, antidiabetic, antiinflammatory, anti AIDS, antiallergic, antioxidant, antitoxic and antimicrobial activities (Sarangi and Soni, 2013; Reddy and Reddy, 2015; Mittal et al., 2014). Mahato et al. (2018) studied about eight medicinal plant extracts viz. *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Andrographis paniculata*, *Curcuma longa*, *Catharanthus roseus*, *Ocimum sanctum* and *Zingiber officinale* against *S. rolfsii* at 5, 10 and 20% concentration under *in vitro* condition. They reported that *Allium sativum* showed maximum inhibition (84.89%) of *S. rolfsii* at 20% concentration followed by *Azadirachta indica* (80.86%), while *Ocimum sanctum* inhibited the growth by 53.47%. Gupta et al. (2012) evaluated the efficacy of some plant extracts like ashok (*Polyalthia longifolia*), bhang (*Cannabis sativa*), clerodendron (*Clerodendrum inerme*), eucalyptus (*Eucalyptus globulus*), garlic, ginger, lantana (*Lantana camara*), karanj (*Pongamia pinnata*), madar (*Calotropis gigantea*), marigold (*Tagetes erecta*), mehendi (*Lawsonia inermis*), neem (*Azadirachta indica*), onion, parthenium (*Parthenium hysterophorus*), sadabahar (*Catharanthus roseus*), tulsi (*Ocimum tenuiflorum*) against *S. rolfsii* causing collar rot of chickpea at 5 and 10% concentration under *in vitro* and found that garlic extract inhibited the expansion of *S. rolfsii* by 100% at 10% concentration on 42 hours compared to neem (97.0%), ginger (95.8%), marigold (95.6%), lantana (87.1%), madar (79.9%), ashok (77.4%), parthenium (70.8%), clerodendron (66.3%), eucalyptus (61.9%) and onion (55.9%). The plant extracts of *Azadirachta indica*, *Allium sativum* and *Catheranthus*

*roseus* at 10% concentration were effective against *S. rolfsii* under *in vitro* condition (HaralPatil and Raut 2008). The plants have thousand of constituent and biologically dynamic molecule having antimicrobial property. Scientists are investigating for plant product as antimicrobial properties. Thousand of phyto-chemical having antimicrobial activities against all types microorganisms under *in vitro* condition have been reported (Gurjar et al., 2012). Sumi and Ao, (2015) tested *Azadirachta indica* against *Sclerotium rolfsii* using poison food technique *in vitro*, and effectively inhibited mycelial growth of pathogen.

The medicinal plant parts and their essential oils are generally used for ayurvedic medicines and perfumes. The healthy plant materials are essential for maintaining products quality and quantity. Therefore, it is adversely affected by diseases (root rot, wilt, leaf spots) during plant development (Gahukar, 2018). The five botanical extracts *viz.* Clove, Thyme, Cinnamon, *Calotropis* and *Parthenium* at 5%, 10%, 15%, 25% and 25% concentrations which contain antifungal activity were tested against *S. rolfsii* under *in-vitro* condition and were found that the bud of clove possessed better antifungal activity as compared to others at low concentration (Singh et al., 2019).

**Table 4.9:** Effect of *Azadirachta indica* leaf extract at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±1.11	70.5±1.88	90.08±0.99	40.14
10%	28.25±0.96	56.91±1.44	73.33±1.07	35.81
25%	22±0.95	45.16±0.93	61.16±1.11	38.18
50%	14.58±0.90	22.33±1.30	26.08±0.90	23.61
75%	11±0.95	14.91±0.79	18.33±0.88	21.35
F-cal	851.571*	3676.523*	11369.65*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.10:** Effect of *Ocimum tenuiflorum* leaf extract at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±1.11	70.5±1.88	90.08±0.99	40.14
10%	26.58±0.99	58.25±1.21	72.08±1.37	36.99
25%	18.25±0.86	36.41±1.24	56.25±1.35	42.65
50%	12.16±0.83	17.75±0.96	20.66±1.07	21.88
75%	0±0.0	0±0.0	0±0.0	0
F-cal	2321.35*	6632.393*	13939.05*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.11:** Effect of *Syzygium aromaticum* oil at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±1.11	70.5±1.88	90.08±0.99	40.14
100ppm	20.25±3.51	49.58±2.53	78.16±1.74	48.89
200ppm	11.33±1.23	15.08±0.79	18.33±2.01	21.52
400ppm	0±0.0	0±0.0	0±0.0	0
600ppm	0±0.0	0±0.0	0±0.0	0
F-cal	667.725*	5652.262*	14047.61*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.12:** Effect of *Tinospora cordifolia* stem extract at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control

Treatment	1st day	2nd day	3rd day	CV%
Control	41.16±0.71	78.58±0.79	91.83±0.38	30.85
10%	22.33±0.77	37.16±2.72	55.5±0.52	36.13
25%	15.25±0.62	25.16±0.57	35.5±0.52	33.2
50%	0±0.0	11.25±0.45	24.25±0.52	85.3
75%	0±0.0	0±0.0	0±0.0	0
F-cal	11797.64*	6435.324*	73889.41*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

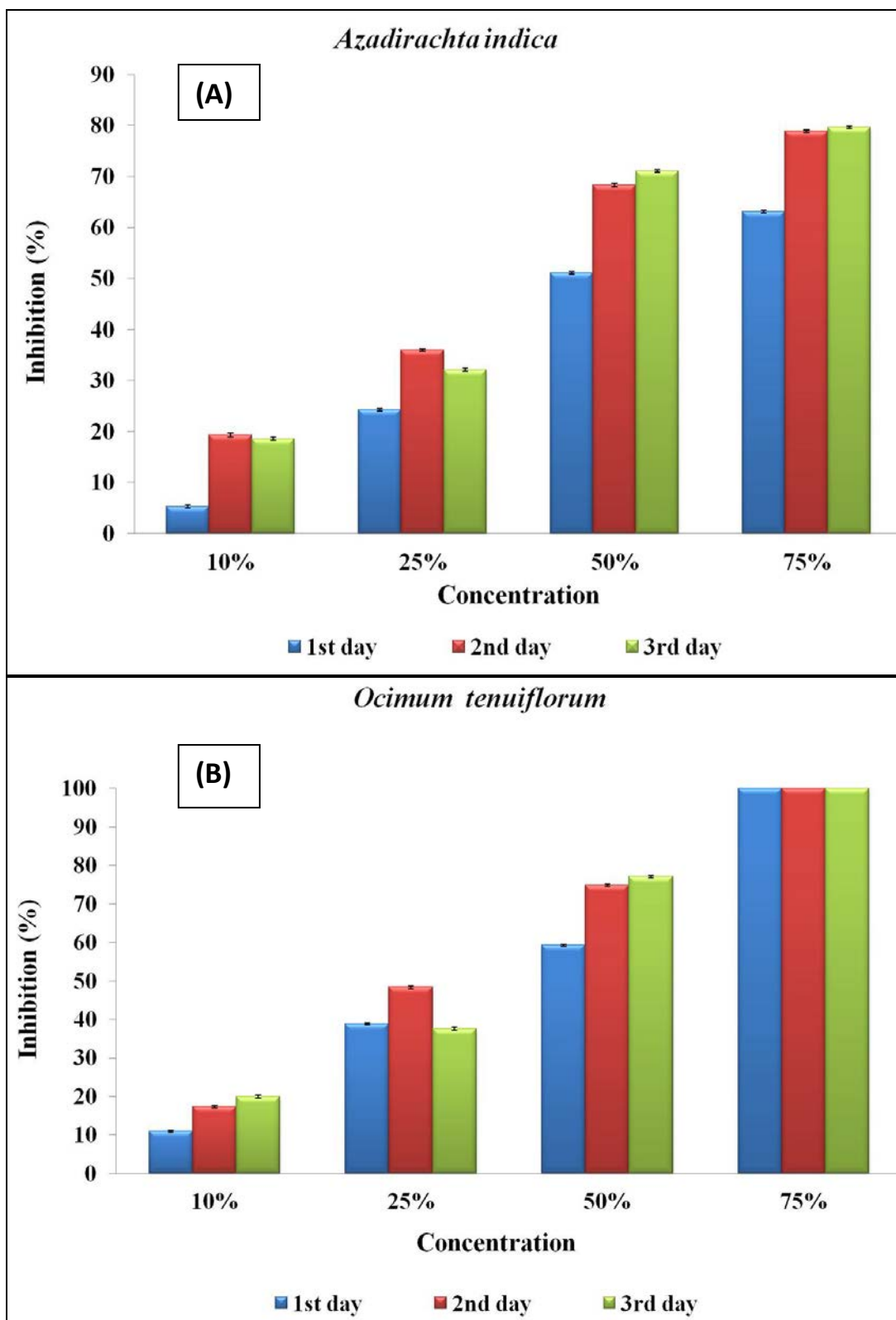
**Table 4.13:** Effect of *Trachyspermum ammi* seeds powder at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±1.11	70.5±1.88	90.08±0.99	40.14
10%	23.41±1.16	49.75±1.60	70.16±3.58	40.9
25%	20.5±1.44	41.33±3.14	61.5±1.67	41.62
50%	0±0.0	0±0.0	0±0.0	0
75%	0±0.0	0±0.0	0±0.0	0
F-cal	2465.581*	3687.923*	6278.842*	-

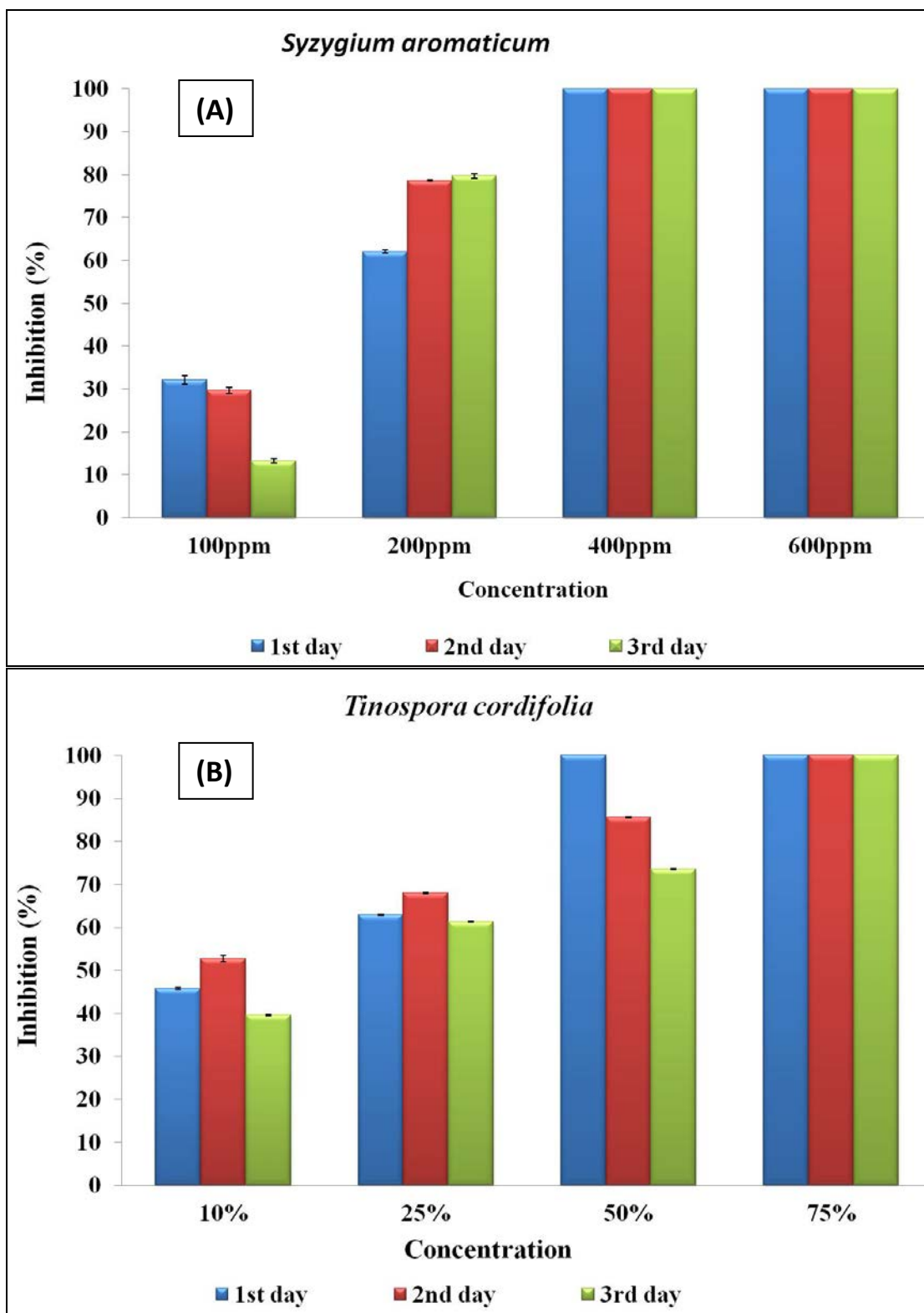
Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

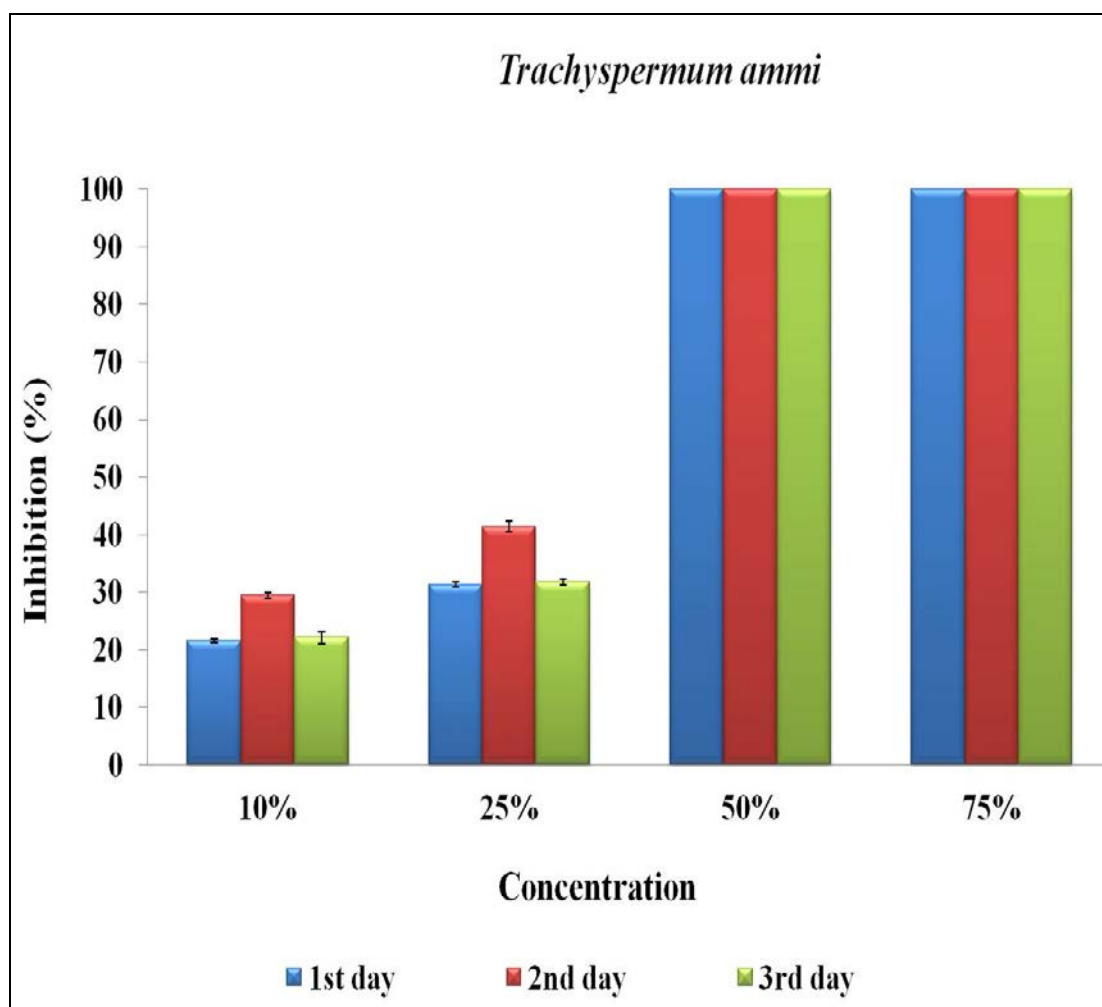
\*= Significant at p=0.05



**Fig. 4.8:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with (A) *Azadirachta indica* leaf extract (B) *Ocimum tenuiflorum* leaf extract under *in vitro* condition



**Fig. 4.9:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with (A) *Syzygium aromaticum* oil (B) *Tinospora cordifolia* under *in vitro* condition



**Fig. 4.10:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with *Trachyspermum ammi* seeds powder under *in vitro* condition



(A)

(B)



(C)

**Fig. 4.11:** Antifungal activity of (A) *Azadirachta indica* (B) *Ocimum tenuiflorum* (C) *Tinospora cordifolia* against *Sclerotium rolfsii* using poisoned food technique on 3<sup>rd</sup> day incubation period

#### 4.8. Effect of metabolites of mycoflora against *Sclerotium rolfii*

The selected six metabolite of fungal species viz. *Aspergillus flavus*, *A. niger*, *Trichoderma viride*, *Penicillium citrinum*, *P. italicum* and *Penicillium* sp. were tested against *Sclerotium rolfii* for their antifungal activity (Fig. 4.16, 4.17, 4.18). They were significantly ( $P \leq 0.05$ ) (Appendix II–Table 28–45) effective against *S. rolfii* under *in vitro* condition. *Aspergillus flavus* inhibited 68.32%, 58.97%, 58.20%, 70.04% mycelial growth of *S. rolfii*, while *A. niger* inhibited 69.18%, 70.62%, 76.81%, 76.52% at 10%, 25%, 50%, 75% v/v concentration respectively on 3<sup>rd</sup> day of incubation period (Table 4.14, 4.15; Fig. 4.12A, 4.12B). *Trichoderma viride* inhibited the growth by 76.63% at 75% v/v concentration followed by 61.06%, 74.91%, 67.75% at 10%, 25%, 50% v/v concentration respectively against *S. rolfii* on 3<sup>rd</sup> day of incubation period (Table 4.16; Fig.4.14B). Whereas *Penicillium citrinum* and *P. italicum* inhibited 100% mycelial growth of *S. rolfii* at 75% v/v concentration while 84.40%, 82.38%, 84.51% and 71.52%, 70.94%, 83.35% growth inhibition was recorded at 10%, 25%, 50% v/v concentration respectively on 3<sup>rd</sup> day (Table 4.17, 4.18; Fig.4.13A, 4.13B). *Penicillium* sp. inhibited the expansion of *S. rolfii* by 78.92%, 53.71%, 48.60%, 68.14% at 10%, 25%, 50%, 75% v/v concentration respectively on 3<sup>rd</sup> day of incubation period (Table 4.19; Fig.4.14A). It may be concluded that among the all tested fungi *Aspergillus flavus* (70.04%), *A. niger* (76.52%), *Trichoderma viride* (76.63%), *Penicillium citrinum* (100%), *P. italicum* (100%) and *Penicillium* sp (68.14%) were most effective against *S. rolfii* at 75% concentration on 3<sup>rd</sup> day. *Penicillium citrinum* and *P. italicum* were significantly effective compared to *Aspergillus flavus*, *A. niger*, *Trichoderma viride* and *Penicillium* sp. at 10%, 25%, 50%, 75% on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day respectively.

*Trichoderma* sp. viz. *T. harzianum*, *T. viride* and *T. hamanatum* produces specific volatile and non-volatile substances which contain antibiotic properties that suppressed the radial enlargement of *S. rolfsii* (Srinivasulu et al., 2005). They were reported that the bioagents for tomato at sowing were most proficient for controlling of fungal soil borne disease. The biocontrol agents are ecofriendly and protect the environment from pollution and maintenance of human health. (Zaghloul et al., 2008). Curtis et al. (2010) found that *Rhizoctonia solni* and *S. rolfsii* causing root-rot in tomato plants significantly checked by biocontrol agent as eco-compatible farming. Twenty isolates of *Trichoderma* spp which produces growth-promoting metabolites viz. IAA, phosphate and siderophore. They also showed antagonistic potentiality against *S. rolfsii* under *in vitro* condition. Out of these 20 isolates, *T. viride* produces maximum inorganic phosphate, IAA and siderophore showed high antagonistic and plant growth promoting capability (Kotasthane et al., 2015).

The metabolites of *Trichoderma harzianum* and *T. viride* inhibited collar rot disease incidence in sunflower caused by *S. rolfsii* and improved the crop yield (Maji and Nath, 2016). Jana and Malay (2017) reported that *Trichoderma* sp. has inhibitory activity against *S. rolfsii* under laboratory situation. Kushwaha et al. (2018) studied three bioagents viz. *Trichoderma viride*, *T. harzianum* and *T. virens* were tested against *Sclerotium rolfsii* under *in vitro* condition. They were reported that all three bioagents *T. viride* (50.85%), *T. harzianum* (63.60%) and *T. virens* (51.5 %) have effectively inhibited mycelial growth of *S. rolfsii* on 72 hours. They also reduced sclerotia formation by 91.31% (*T. viride*), 84.92% (*T. harzianum*) and 84.29% (*T. virens*) after 15 days of incubation. The culture filtrates of *Trichoderma harzianum* and *T. viride* effectively inhibited radial growth of *S. rolfsii* at 15% concentration by

57.46% and 49.62% respectively. They also reduced sclerotia formation by 98.20% (*T. harzianum*) and 99.83% (*T. viride*) of *Sclerotium rolfsii* (Kushwaha et al., 2018).

**Table 4.14:** Effect of metabolite of *Aspergillus flavus* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
Control	46.83±4.44	59.58±1.56	87.33±1.23	26.88
10%	20.16±0.71	25.25±0.75	27.66±0.88	13.38
25%	25.66±1.07	31±0.73	35.83±0.71	13.91
50%	24.33±0.65	30.66±0.88	36.5±0.52	16.65
75%	21.08±0.51	26.16±0.71	26.16±0.71	10.25
F-cal	326.534*	2501.583*	10719.78*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.15:** Efficacy of metabolite of *Aspergillus niger* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
Control	46.83±4.44	59.58±1.56	87.33±1.23	26.88
10%	20.5±0.52	26.5±0.52	26.91±2.06	13.03
25%	20.5±1.08	24.66±0.65	25.66±0.65	10.16
50%	16±0.73	20.16±0.57	20.25±0.45	11.11
75%	16.25±0.62	20.5±0.52	20.5±0.52	11
F-cal	453.175*	4409.644*	7436.492*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.16:** Toxicity of culture filtrate of *Trichoderma viride* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
<b>Control</b>	46.83±4.44	59.58±1.56	87.33±1.23	26.88
<b>10%</b>	26.08±1.37	31.91±1.37	34±0.85	11.74
<b>25%</b>	17.66±1.49	21.91±0.99	21.91±0.99	11.36
<b>50%</b>	19.25±1.35	23.66±1.55	28.16±1.02	16.5
<b>75%</b>	15.33±0.49	20.41±0.79	20.41±0.79	13.46
<b>F-cal</b>	379.479*	1902.96*	9484.648*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.17:** *In vitro* evaluation of toxicity of culture filtrate of *Penicillium citrinum* at different concentration and incubation period against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
<b>Control</b>	35.66±0.88	63.75±0.96	86.58±1.56	34.09
<b>10%</b>	13.5±1.44	13.5±1.44	13.5±1.44	10.37
<b>25%</b>	15.25±1.28	15.25±1.28	15.25±1.28	8.19
<b>50%</b>	13.41±1.31	13.41±1.31	13.41±1.31	9.47
<b>75%</b>	0±0.0	0±0.0	0±0.0	0
<b>F-cal</b>	1570.875*	5659.317*	9049.605*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.18:** Culture filtrate toxicity of *Penicillium italicum* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
<b>Control</b>	35.66±0.88	63.75±0.96	86.58±1.56	34.09
<b>10%</b>	18.66±1.23	24.66±1.37	24.66±1.37	13.85
<b>25%</b>	20.75±1.42	25.16±1.58	25.16±1.58	10.89
<b>50%</b>	14.41±1.37	14.41±1.37	14.41±1.37	9.22
<b>75%</b>	0±0.0	0±0.0	0±0.0	0
<b>F-cal</b>	1582.333*	4642.639*	7542.84*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

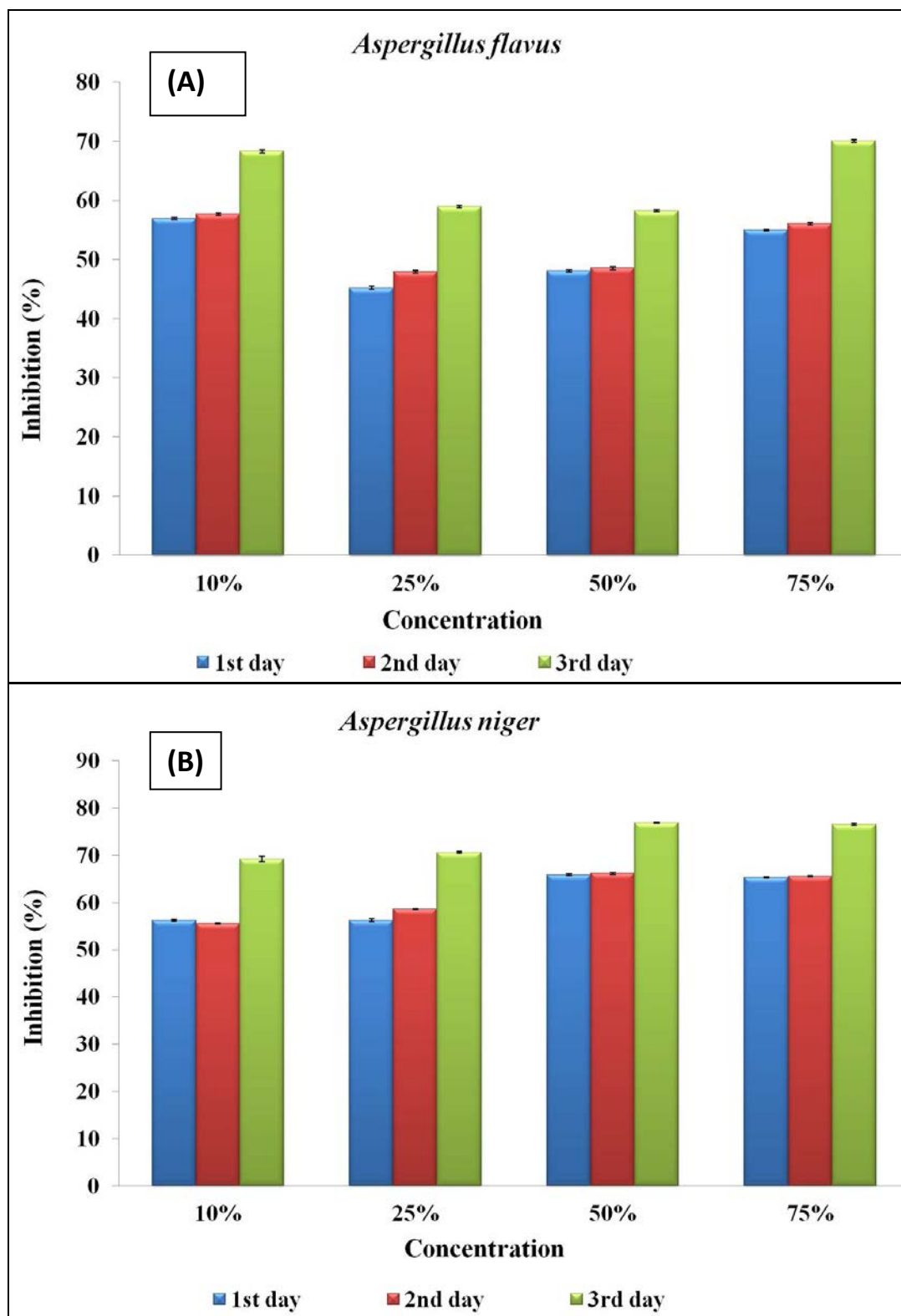
**Table 4.19:** Culture filtrate toxicity of *Penicillium* sp. at different concentration and incubation period against mycelial growth (in mm) of *Sclerotium rolfsii* under *in vitro* condition

Treatment	1st day	2nd day	3rd day	CV%
<b>Control</b>	35.66±0.88	63.75±0.95	86.58±1.56	34.09
<b>10%</b>	14.91±0.99	16.75±0.96	18.25±0.86	9.92
<b>25%</b>	20.33±1.07	30.16±1.11	40.08±0.66	27.26
<b>50%</b>	26.16±1.19	33.33±1.15	44.5±1.24	22.3
<b>75%</b>	18.91±1.72	22.5±1	27.58±1.16	16.65
<b>F-cal</b>	529.491*	3660.97*	6324.312*	-

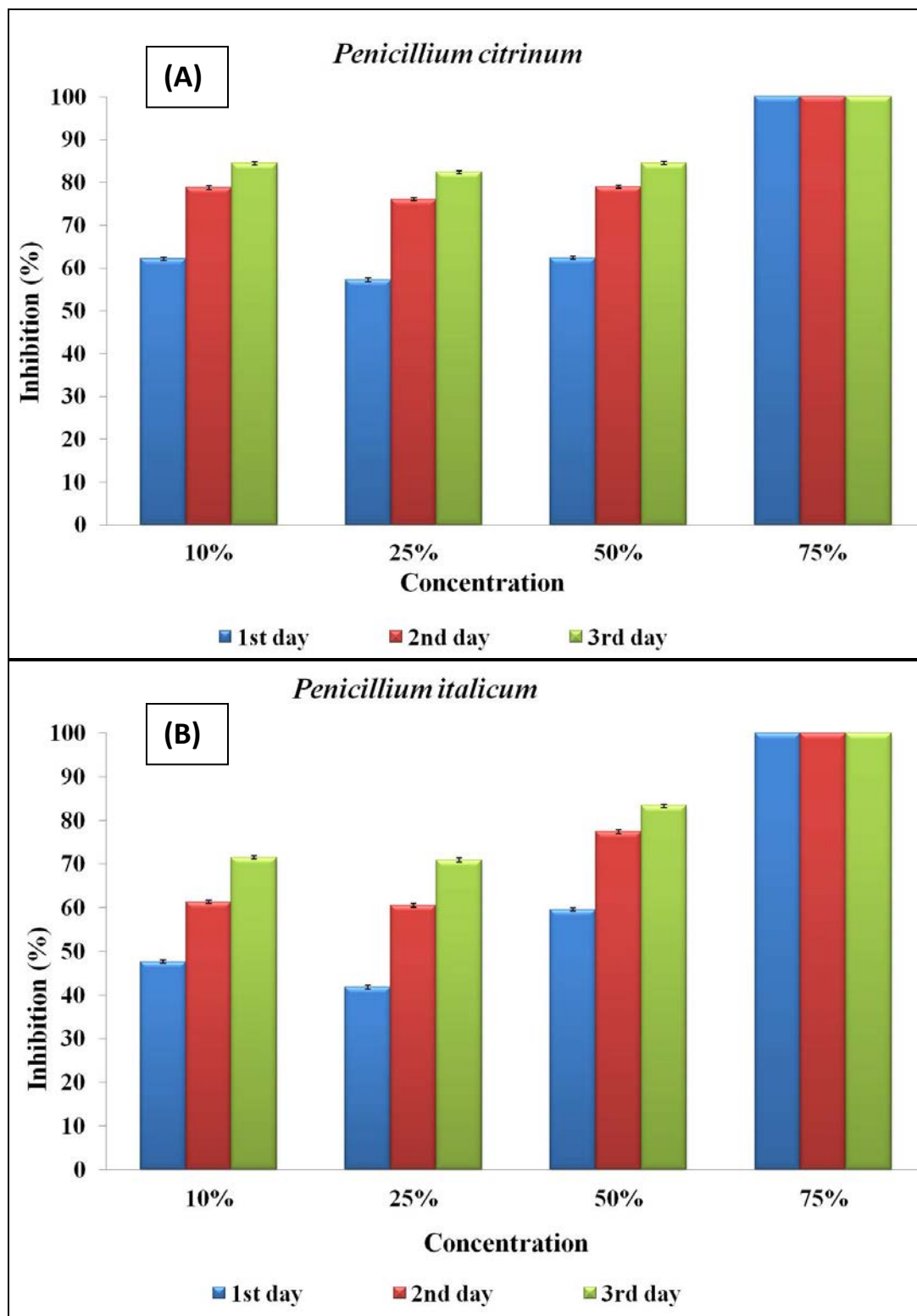
Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

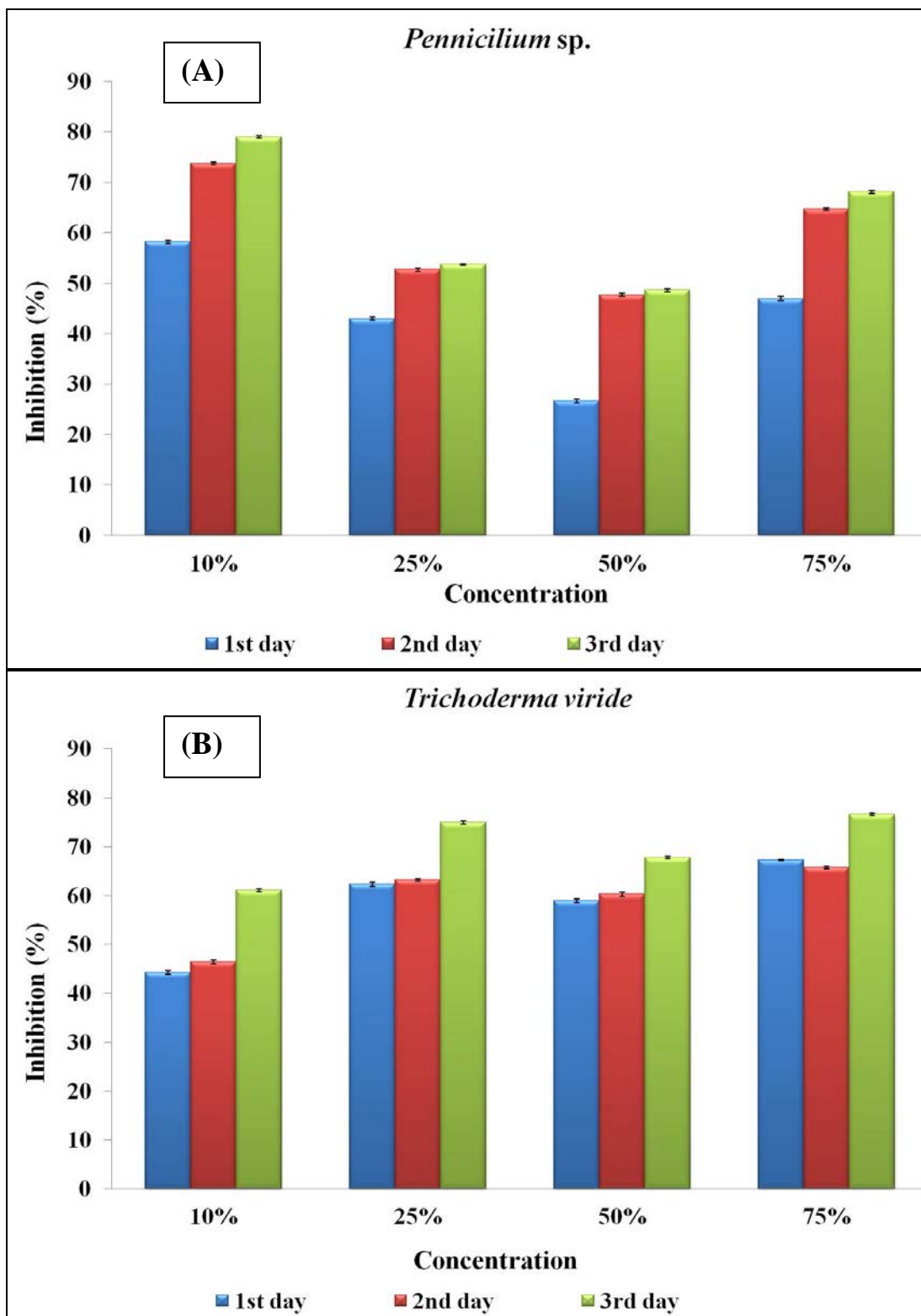
\*= Significant at p=0.05



**Fig. 4.12:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with culture filtrate of (A) *Aspergillus flavus* (B) *Aspergillus niger* under *in vitro* condition



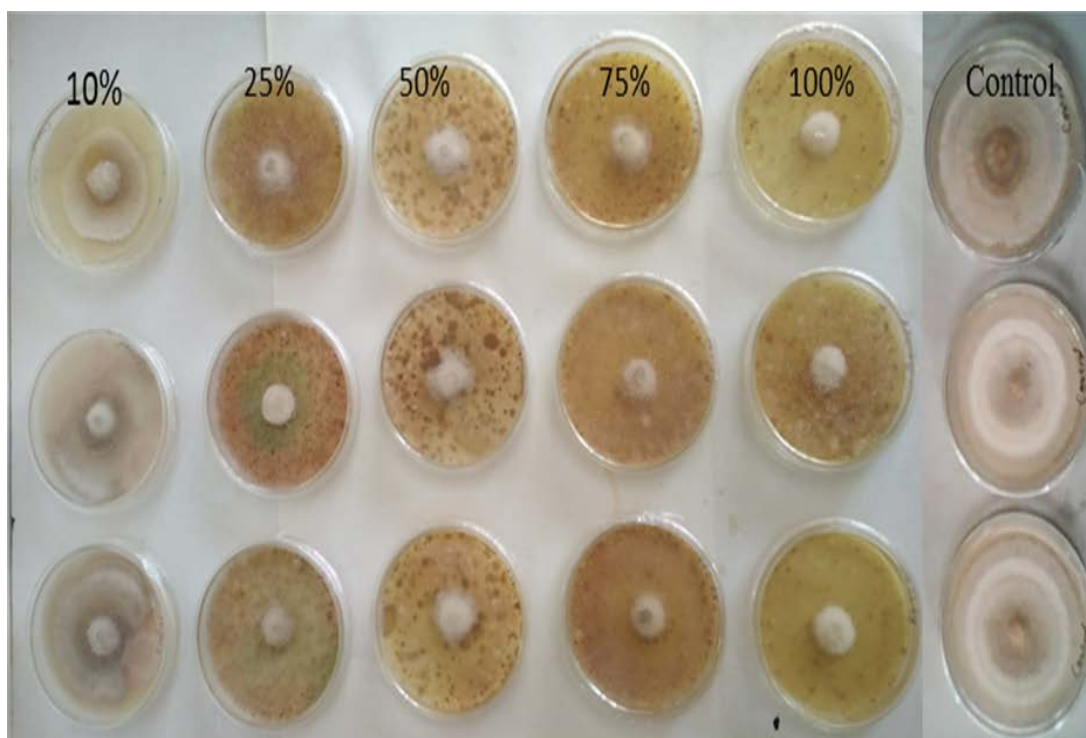
**Fig. 4.13:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with culture filtrate of (A) *Penicillium citrinum* (B) *Penicillium italicum* under *in vitro* condition



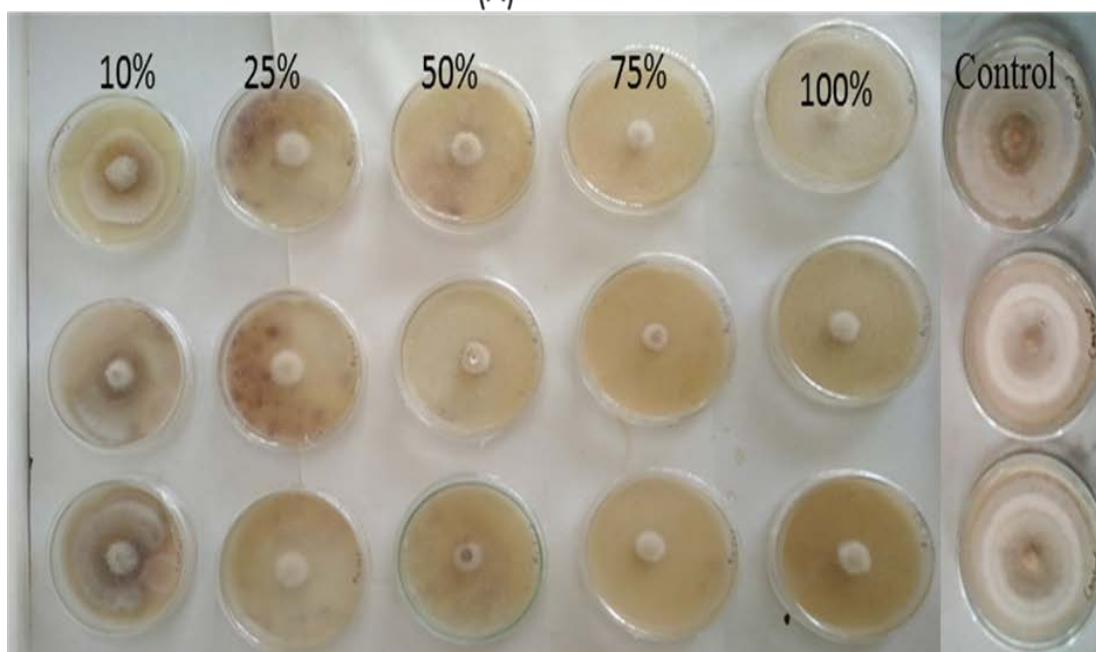
**Fig. 4.14:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with culture filtrate of (A) *Penicillium sp* (B) *Trichoderma viride*



**Fig. 4.15:** Preparation of metabolite of Bioagents using broth culture; Pc=*Penicillium citrinum*, Ps=*Penicillium* sp., Pi=*P. italicum*, Af= *Aspergillus flavus*, An= *A. niger*, Tv=*Trichoderma viride*

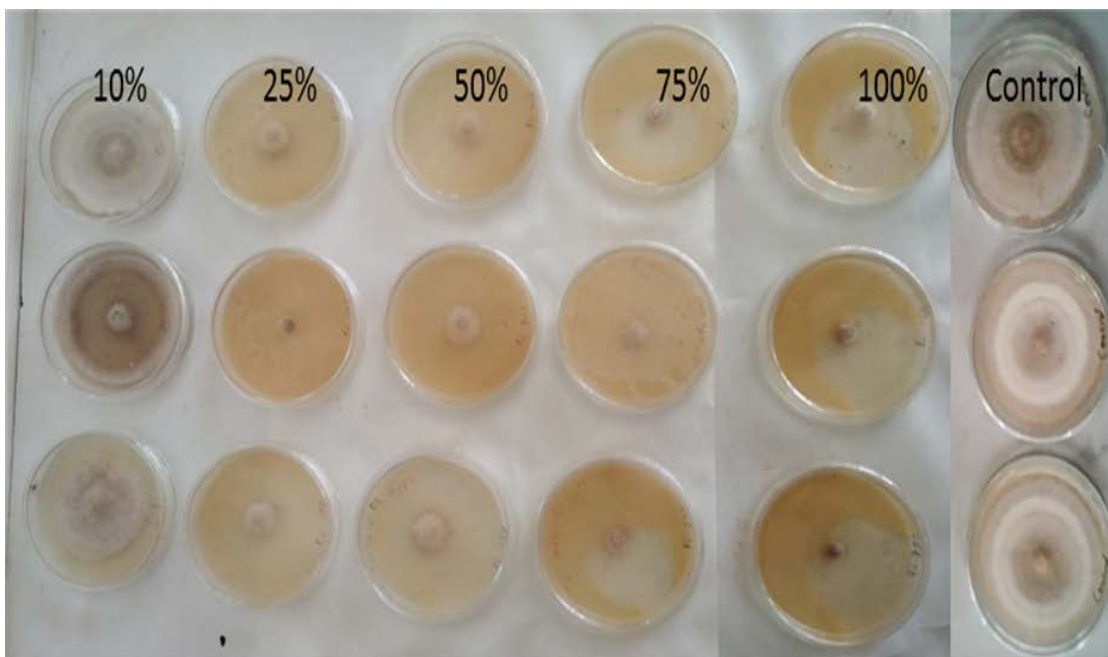


(A)

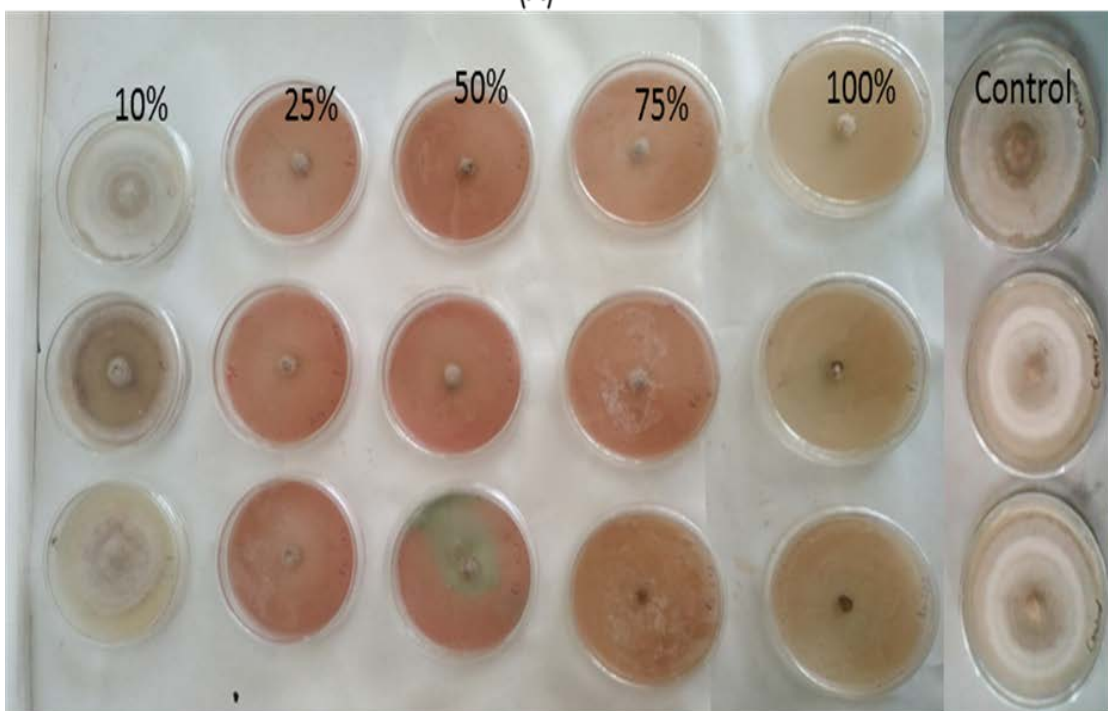


(B)

**Fig. 4.16:** Treatment of *Sclerotium rolfsii* with metabolite of (A) *Aspergillus flavus* (B) *Aspergillus niger* using food poisoned technique on 3<sup>rd</sup> day incubation period

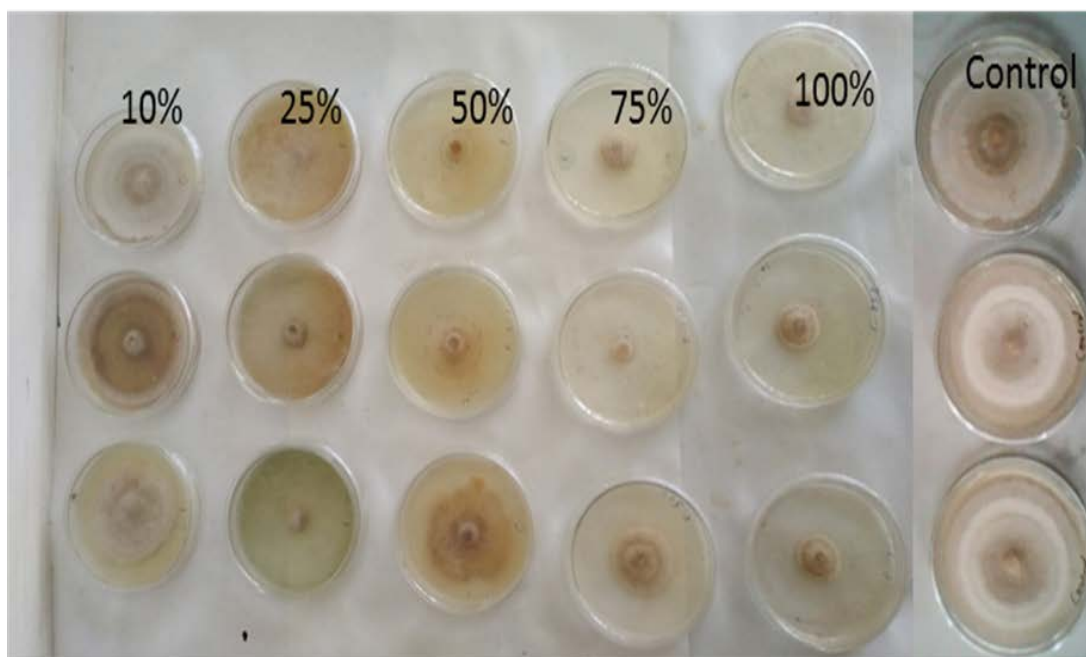


(A)

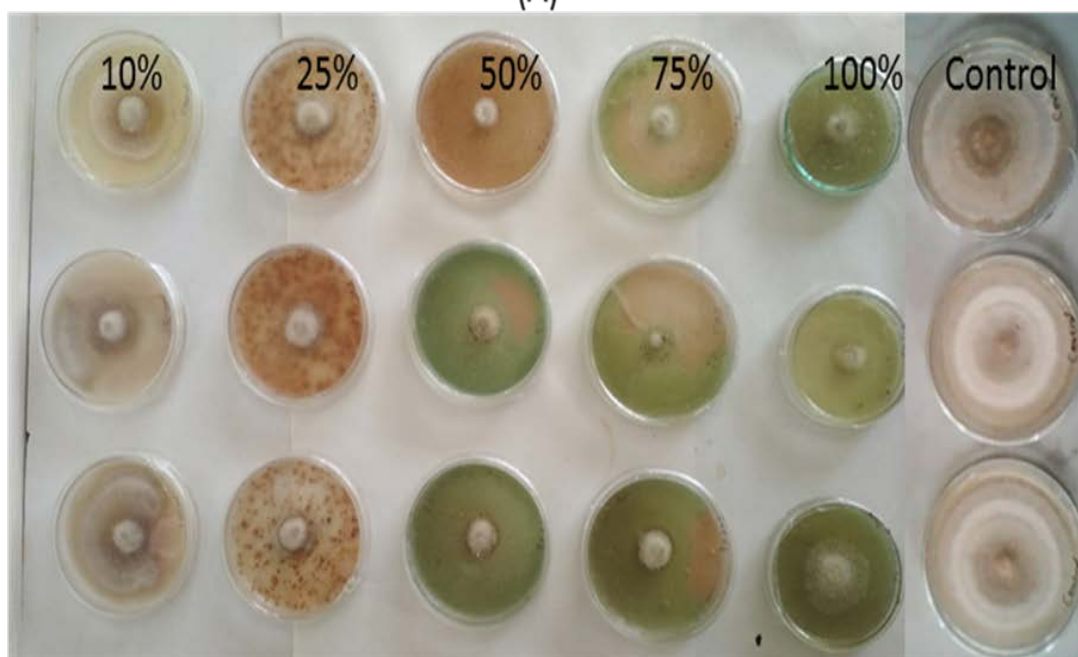


(B)

**Fig. 4.17:** Treatment of *Sclerotium rolfsii* with metabolite of (A) *Penicilium citrinum* (B) *Penicilium italicum* using food poisoned technique on 3<sup>rd</sup> day incubation period



(A)



(B)

**Fig. 4.18:** Treatment of *Sclerotium rolfsii* with metabolite of (A) *Penicilium* sp. (B) *Trichoderma viride* using food poisoned technique on 3<sup>rd</sup> day incubation period

#### 4.9. Evaluation of integrated management of *Sclerotium rolfsii* under *in vitro* condition

The composite *T. viride*+Myclobutanil 10%WP inhibited 100% mycelial growth of *S. rolfsii* at 75 %+100 ppm concentration followed by 32.66%, 52.37%, 74.27% at 10%+10 ppm, 25%+25ppm, 50%+50 ppm concentration on 3<sup>rd</sup> day respectively (Table 4.20; Fig.4.19A, 4.21A). Whereas composite *Ocimum tenuiflorum*+Myclobutanil 10%WP and *Ocimum tenuiflorum*+*T. viride* inhibited 100% mycelial expansion of *S. rolfsii* at 50%+50 ppm (v/v) and 50%+50% (v/v) concentration while at 10%+10 ppm, 25%+25ppm and 10%+10%, 25%+25% (v/v) concentration the growth was inhibited by 21.95%, 78.97% and 3.18%, 69.24% on 3<sup>rd</sup> day respectively (Table 4.21, 4.22; Fig.4.19B, 4.20A, 4.21B, 4.22A). On other hand the composite *T.viride*+Mycobutanil 10%WP +*Ocimum tenuiflorum* inhibited 100 % mycelial expansion of *S. rolfsii* at 75 %+100ppm+75% (v/v) concentration followed by 30.14%, 51.85%, 71.13% at 10%+10ppm+10%, 25%+25ppm+25%, 50%+50ppm+50% (v/v) concentration on 3<sup>rd</sup> day under *in vitro* condition using poisoned food technique (Table 4.23; Fig.4.20B, 4.22B). All composite *T. viride*+Myclobutanil 10%WP, *Ocimum tenuiflorum*+Myclobutanil 10%WP, *Ocimum tenuiflorum*+*T. viride*, *T.viride*+Mycobutanil 10%WP +*Ocimum tenuiflorum* have significantly ( $P \leq 0.05$ ) (Appendix II–Table 46–57) effective against mycelial growth of *S. rolfsii* under *in vitro* condition.

Composite *T. virens* + Metalaxyl + Mancozeb was most significantly efficient against collar rot disease in lentil caused by *S. rolfsii* and also killed the sclerotia. The combination of *T. viride* and Bavistin was also very effective to control of *S. rolfsii* which agrees the findings of Pandey (2013). Pawar et al. (2015) conducted an experiment with five fungicides Carbendazim, Captan, Thiram, Thiram +

Carbendazim, Chlorothalonil and two bioagents *T. viride* and *T. harzianum* against *S. rolfsii* and reduced the disease incidence and enhanced seed germination of soybean. Suryawanshi et al. (2015) evaluated some bioagents and plant extract under *in vitro* condition. Amongst them *T. viride* (87.85%) and plant extract *Ocimum tenuiflorum* (52.30%) inhibited the mycelial enlargement of *S. rolfsii* causing collar rot in brinjal. Bhat et al. (2015) evaluated chemical fungicides viz. mancozeb, captan, hexaconazole, carboxin, carboxin+thiram, tebuconazole, carbendazim and bioagents (*T. viride*, *T. harzianum*) against *S. rolfsii* during 2011-12 in both conditions and found similar result as in the present study. The management of root and foot rot diseases of betel vine caused by *Sclerotium rolfsii* was studied with 6 fungicides, 5 plants extracts, 2 bio-agents. They were reported that among all treatments, chemical fungicide Bavistin, plant extract of garlic and clove and bioagent *Trichoderma harzianum* had greatest efficiency to control the mycelial expansion of *Sclerotium rolfsii* under *in vitro* condition compared to untreated control (Parvin et al., 2016).

**Table 4.20:** Effect of composite *Trichoderma viride*+Myclobutanil 10%WP at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
Control	29±1.27	65±0.85	91.33±0.88	41.97
10%+10ppm	23.33±0.77	43.41±0.79	61.5±0.67	37
25%+25ppm	19.16±0.93	29±0.85	43.5±0.67	33.25
50%+50ppm	15.75±0.62	20.5±0.52	23.5±0.52	16.47
75%+100ppm	0±0.0	0±0.0	0±0.0	0
F-cal	2045.667*	15176.11*	37339.67*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.21:** Efficacy of composite *Ocimum tenuiflorum*+Myclobutanil 10% WP at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
Control	35.08±1.08	70.08±1.08	90.33±0.88	35.54
10%+10ppm	21±0.73	47.5±0.52	70.5±0.52	44.29
25%+25ppm	0±0.0	15.25±0.62	19±0.60	73.09
50%+50ppm	0±0.0	0±0.0	0±0.0	0
75%+100ppm	0±0.0	0±0.0	0±0.0	0
F-cal	9095.546*	31676.3*	73969.38*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

**Table 4.22:** Effect of composite *Ocimum tenuiflorum*+*Trichoderma viride* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
Control	41.16±0.71	78.58±0.79	91.83±0.38	30.85
10%+10%	40.16±0.71	69.83±1.26	88.91±0.90	30.7
25%+25%	14.75±0.75	19.66±0.49	28.25±0.62	27.25
50%+50%	0±0.0	0±0.0	0±0.0	0
75%+75%	0±0.0	0±0.0	0±0.0	0
F-cal	15757.58*	35049.09*	93453.74*	-

Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*= Significant at p=0.05

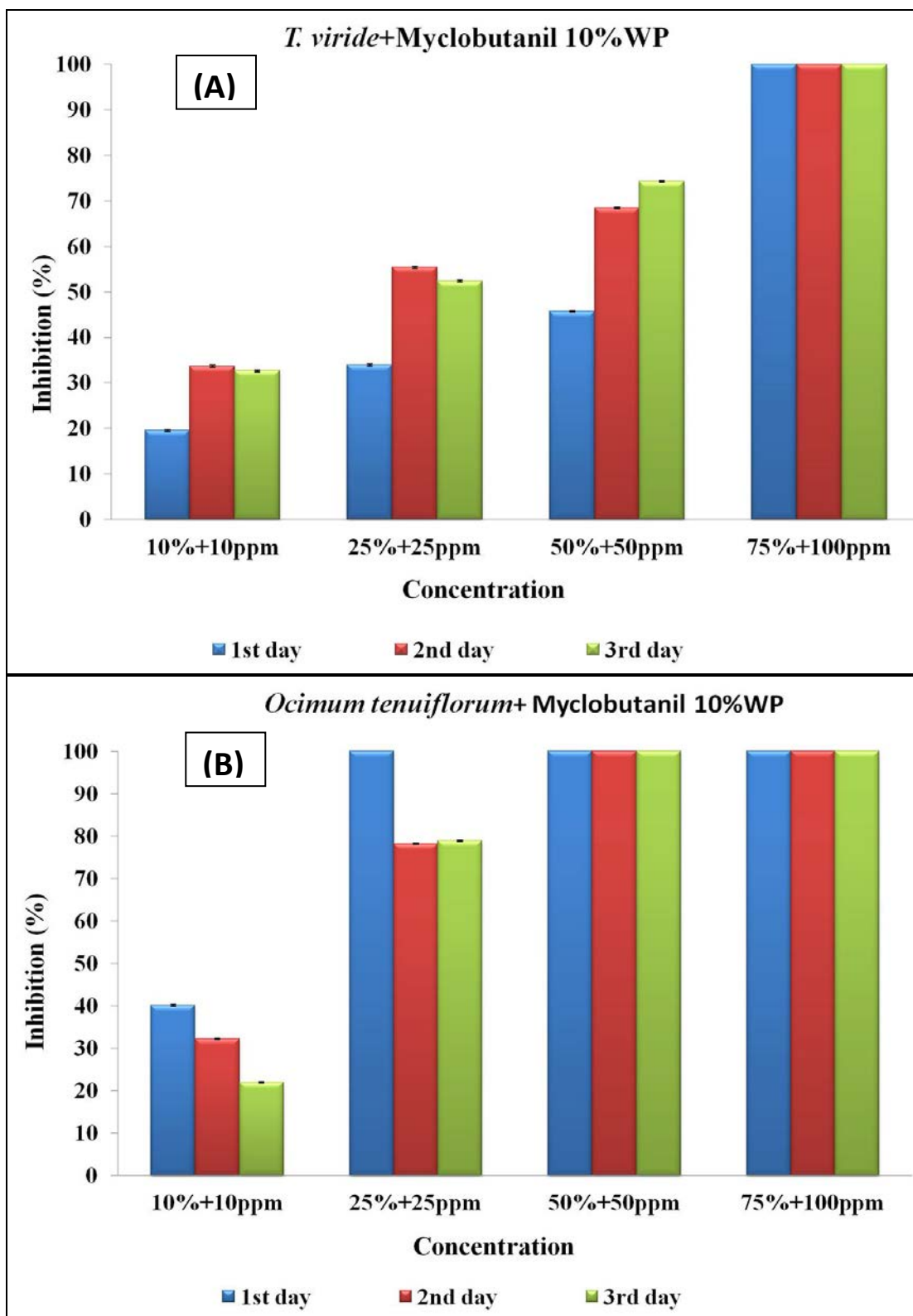
**Table 4.23:** Effect of composite *T.viride*+Mycobutanil 10%WP+ *Ocimum tenuiflorum* at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii*

Treatment	1st day	2nd day	3rd day	CV%
<b>Control</b>	31.91±1.44	63.83±0.71	86±1.90	37.22
<b>10%+10ppm+10%</b>	23±0.60	45.33±0.88	60.08±1.08	36.16
<b>25%+25ppm+25%</b>	20.58±0.66	35.33±0.65	41.41±0.90	27.43
<b>50%+50ppm+50%</b>	17.16±1.02	21.08±1.50	24.83±1.11	16.12
<b>75%+100ppm+75%</b>	0±0.0	0±0.0	0±0.0	0
<b>F-cal</b>	2080.97*	8788.603*	9443.141*	-

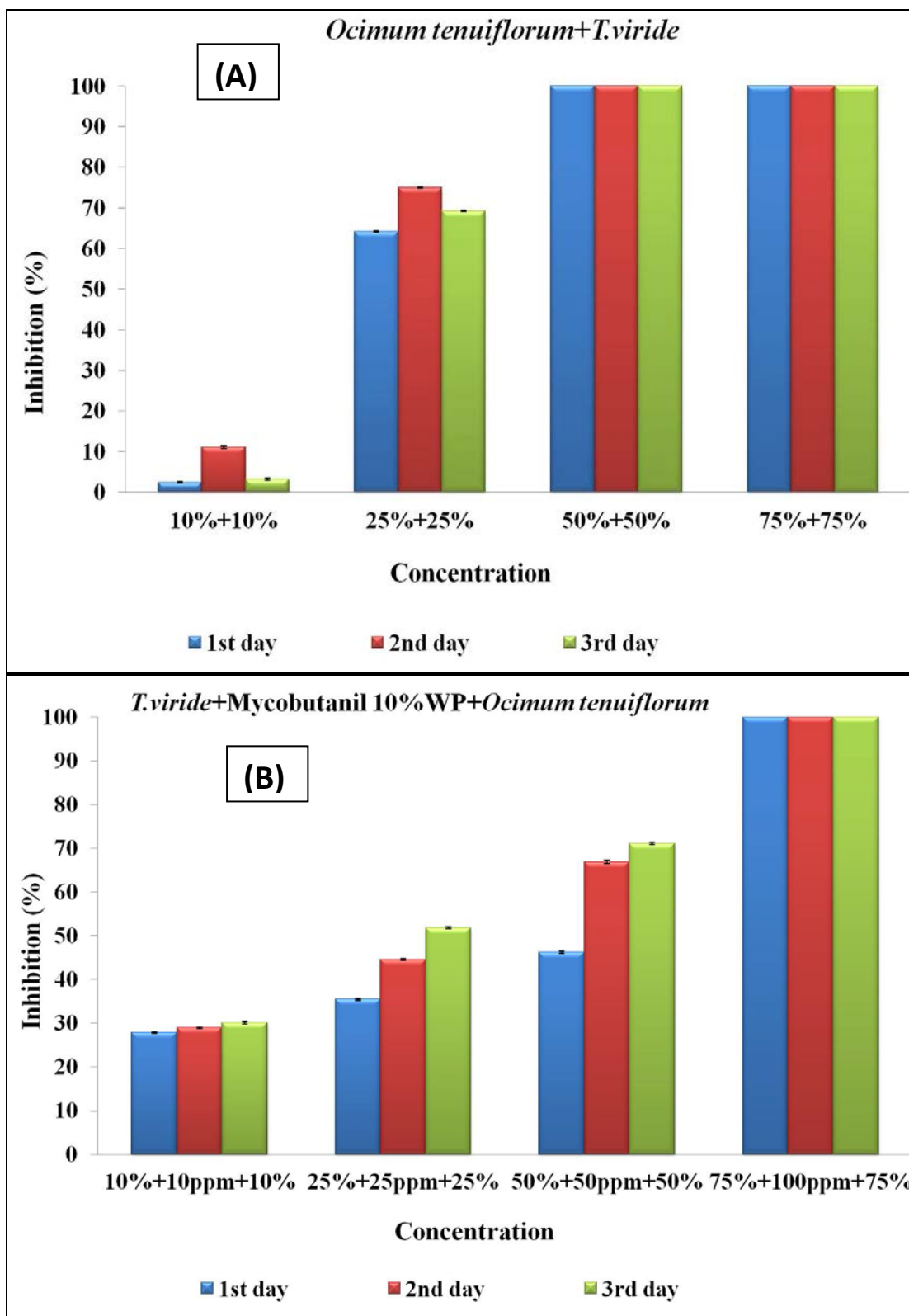
Mean±SD, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

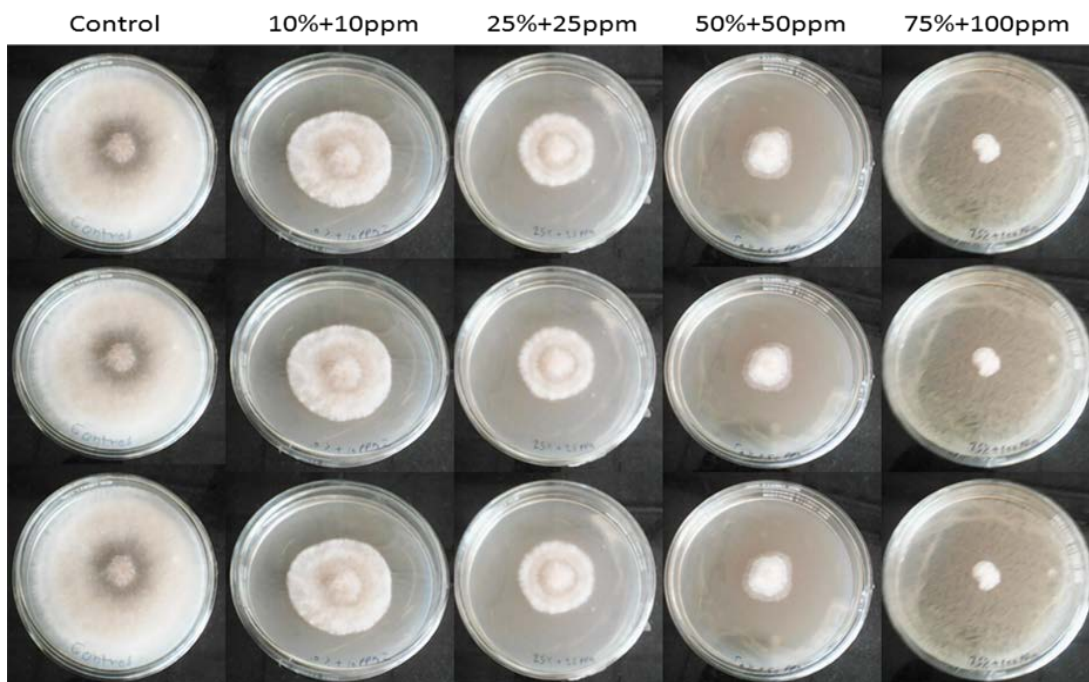
\*= Significant at p=0.05



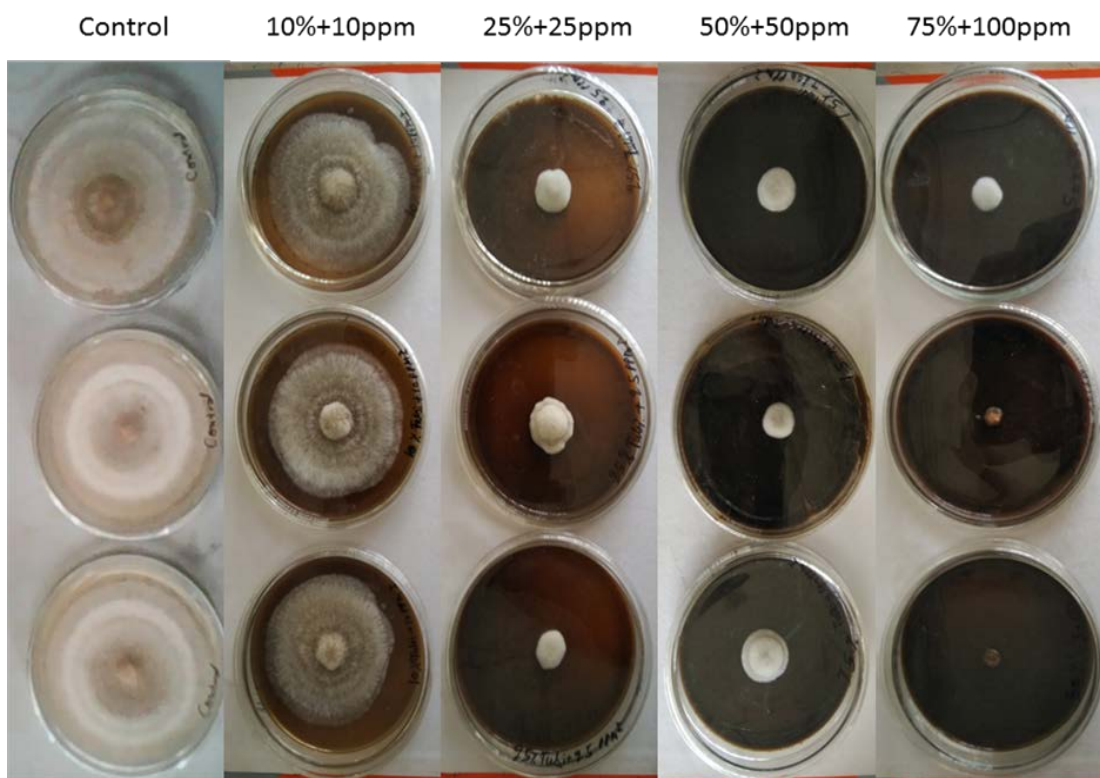
**Fig. 4.19:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with (A) *T. viride*+Myclobutanil 10%WP (B) *Ocimum tenuiflorum*+Myclobutanil 10%WP under *in vitro* condition



**Fig. 4.20:** Percent inhibition of mycelial growth of *Sclerotium rolfsii* treated with (A) *Ocimum tenuiflorum*+*T.viride* (B) *T.viride*+Mycobutanil 10% WP+*Ocimum tenuiflorum* under *in vitro* condition

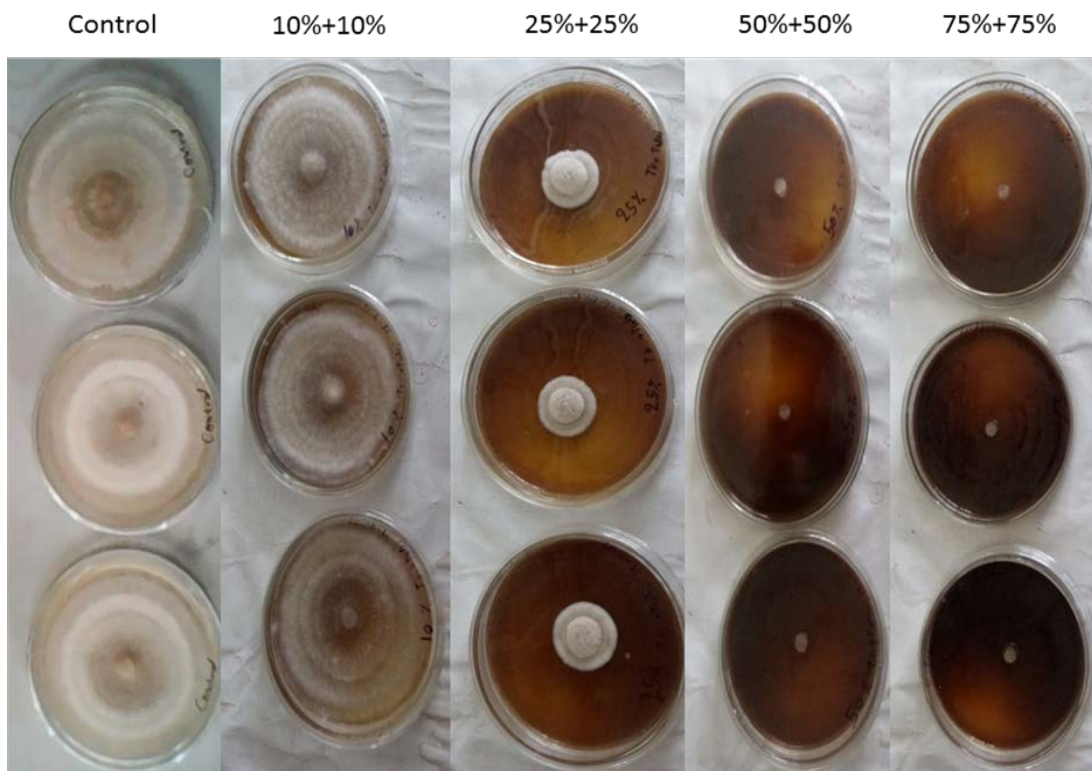


(A)

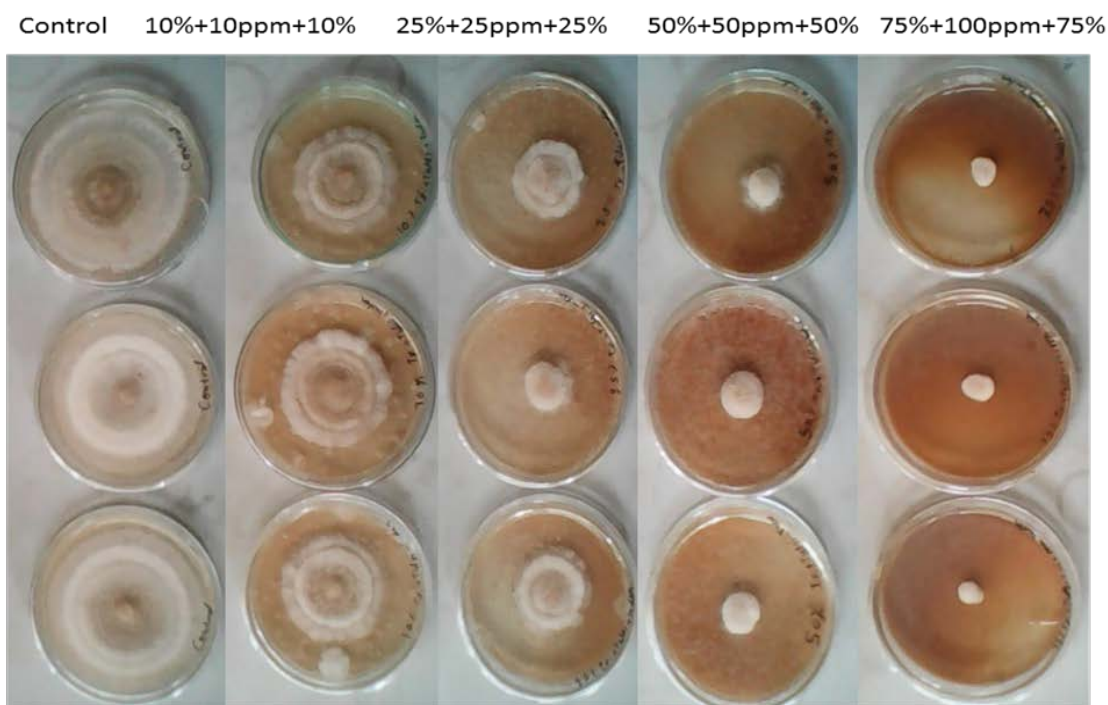


(B)

**Fig. 4.21:** *Sclerotium rolfsii* treated with composite (A) *Trichoderma viride* + Myclobutanil 10% WP (B) *Ocimum tenuiflorum* + Myclobutanil 10% WP using food poisoned technique under *in vitro* on 3<sup>rd</sup> day incubation period



(A)



(B)

**Fig. 4.22:** *Sclerotium rolfsii* treated with composite (A) *Trichoderma viride* + *Ocimum tenuiflorum* (B) *Trichoderma viride* + *Ocimum tenuiflorum* + Myclobutanil 10% WP using food poisoned technique under *in vitro* on 3<sup>rd</sup> day incubation period

#### 4.10. *In vivo* evaluation of selected bioagent, plant extract and chemical against root rot disease in *Zea mays*

The results obtained with selected bioagent, plant extract and chemical in pot experiment are presented in Table 4.24 and 4.25. All the treatments were effective to check the root rot disease incidence caused by *Sclerotium rolfsii*.

##### 4.10.1. Efficacy of Bioagent against *Sclerotium rolfsii*

In the present study, the disease incidence in *Zea mays* plant was checked by 100% with inocula of *T. viride* at 4% w/w concentration and 100% healthy plant of *Zea mays* on 50<sup>th</sup> day was recorded (Table 4.25). The maximum shoot length (61.67 cm/plant), root length (25.33 cm/plant), fresh weight (98 gm/plant) and dry weight (33.33 gm/plant) of treated plants was recorded at 4% w/w concentration (Tv3) compared to untreated plants after sowing seeds on 50<sup>th</sup> day (Table 4.24; Fig.4.23, 4.24).

Among all treatments, *T. viride* was most significantly ( $P \leq 0.05$ ) (Appendix II–Table 62–64) effective and promoted the shoot length, root length and fresh weight of plant compared to *O. tenuiflorum* and myclobutanil on 50<sup>th</sup> day. *Trichoderma viride* was most effective against stem rot disease of groundnut caused by *S. rolfsii* and suppressed the disease incidence by 91.88% on 60<sup>th</sup> day after sowing in pot (Karthikeyan et al., 2006). *Trichoderma viride* is an effective bioagent and checked the southern blight disease in tomato caused by *S. rolfsii* and also improved the shoot and root development of plant (Ekundayo et al., 2015; Patro and Madhuri, 2013). Sennoi et al. (2013) reported that *T. harzianum* and *Glomus clarum* were effective but *Glomus clarum* was significantly most effective against southern stem-rot caused by *S. rolfsii* in *Jerusalem artichoke* under greenhouse condition compared to *T. harzianum*. *Trichoderma viride* and *T. harzianum* secreted antibiotics and hydrolytic enzymes which suppressed root-rot disease caused by *S. rolfsii* (Basumatary et al.,

2015). The inocula of *T. viride* checked the growth of root rot disease and enhanced the plant height at 6gm/kg on 60<sup>th</sup> day under *in vivo* study (Rani and Hegde, 2016).

The inoculum of *T. harzianum* checked root rot disease in groundnut caused by *S. rolfsii* and also enhanced the shoot and root length (Rani, 2017). *Trichoderma* sp. suppressed collar rot disease and also enhanced the germination percentage, root and shoot growth (Islam et al., 2017). Mahato et al. (2017) studied that when inocula of *T. harzianum* @2.5kg/ha was added in collar rot disease infected field of tomato, the disease incidence was suppressed by 65.01% on 65<sup>th</sup> day and enhanced the productivity of tomato. Faruk (2019) reported that inocula of *Trichoderma* reduced the seedling mortality of barley and enhanced the shoot length, shoot weight and yield in field experiment during 2014-16 cropping years.

#### **4.10.2. Efficacy of plant extract against *Sclerotium rolfsii***

In the present study, the leaf extract of *Ocimum tenuiflorum* at 1%, 2% and 3% v/w concentration was treated against *Sclerotium rolfsii* causing root rot disease in *Zea mays*. On 50<sup>th</sup> day, under *in vivo* evaluation and 80% plants were healthy at 3% v/w concentration (Table 4.25). The healthy plants had maximum shoot length (52.33 cm/plant), root length (19.67 cm/plant), fresh weight (93.67 gm/plant) and dry weight (34 gm/plant) recorded at 3% v/w concentration (Ot3) compared to untreated plants on 50<sup>th</sup> day (Table 4.24; Fig.4.23, 4.24).

In pot experiment the neem cake and mahua cake effectively suppressed the stem rot disease incidence by 85% and 89.38% on 60<sup>th</sup> day after sowing and improved the yield of groundnut (Karthikeyan et al., 2006). They reported that neem (seeds) and ginger (rhizome) were effective to check the *S. rolfsii* causing damping-off disease in tomato under pot experiment (Okereke and Wokocha, 2006). The chickpea and sorghum plants may be defensive to *S. rolfsii* due to secretion of phenolic compound

(Maurya et al., 2005, 2007). Gupta et al.(2012) studied some selected plant extracts viz. ashok (*Polyalthia longifolia*), bhang (*Cannabis sativa*), clerodendron (*Clerodendrum inerme*), eucalyptus (*Eucalyptus globulus*), garlic, ginger, lantana (*Lantana camara*), karanj (*Pongamia pinnata*), madar (*Calotropis gigantea*), marigold (*Tagetes erecta*), mehendi (*Lawsonia inermis*), neem (*Azadirachta indica*), onion, parthenium (*Parthenium hysterophorus*), sadabahar (*Catharanthus roseus*), tulsi (*Ocimum tenuiflorum*) at 5 and 10% concentration against *S. rolfsii* causing collar rot of chickpea under *in vivo* condition and found that the collar rot disease was checked by garlic (76.7%) followed by neem (75.7%), ginger (74.4%), marigold (71.7%), lantana (68.3%), madar (67.9%), ashok (66.0%), parthenium (65.0%), clerodendron (65.0%) and tulsi (52.3%).

Islam and Faruq (2012) evaluated extract of some plants viz. allamonda leaf, bel leaf, clove, garlic, kalijira seed, ginger rhizome, katamehedi leaf, neem leaf, onion bulb and turmeric rhizome against damping-off disease in tomato and reported that neem (leaf extract) was most effective compared to other plant extracts. *Alternaria* leaf blight, Black rot, *Cercospora* leaf spot, Southern blight, Watery soft rot disease in *Daucus carota* caused by *S. rolfsii* was evaluated with *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Occimum sanctum* at 50, 75, 100 ppm concentration and they reported that *A. indica*, *A. sativum* were most effective and checked the disease incidence compared to *A. cepa* and *O. sanctum* at different levels (Singh et al., 2014). Junsopa et al. (2016) reported that sorghum seeds controlled the stem rot disease incidence in *Jerusalem artichoke* caused by *S. rolfsii*.

The leaf extract of *Azadirachta indica* and *Ocimum sanctum* was effective in collar rot disease in sunflower caused by *S. rolfsii* with promising yield (Maji and Nath, 2016). Mahato et al. (2017) evaluated neem seeds cake @150 kg/ha and Garlic

extract @20% checked collar rot disease by 60.01% and 55% in tomato caused by *S. rolfisii* on 65<sup>th</sup> day. Mahato et al. (2018) studied collar rot disease incidence and reported that *Azadirachta indica* checked the growth by 67.69% followed by *Allium sativum* (62.65%) and *Ocimum sanctum* (25.30%) at 20% concentration. When 100% concentrated leaf extract of *Ocimum basilicum* was added to pots (100 ml/pot), damping-off disease incidence in tomato was controlled by 60% after 10 days of inoculation under *in vivo* condition (Nugroho et al, 2019).

#### 4.10.3. Efficacy of chemical fungicide against *Sclerotium rolfisii*

The fungicide myclobutanil 10% WP checked 70% disease incidence at 1000 ppm v/w concentration on 50<sup>th</sup> day (Table 4.25). The results of healthy plants are presented in Table 4.24 with maximum shoot length (46.33 cm/plant), root length (17.67 cm/plant), fresh weight (80 gm/plant) and dry weight (30 gm/plant) at 1000 ppm concentration (Bn3) compared to untreated plants on 50<sup>th</sup> day under *in vivo* condition (Table 4.24; Fig.4.23, 4.24).

In a study reported that nine systemic fungicides were drenched with soil at 1000ppm, 2000ppm, 3000ppm concentration against *S. rolfisii* in pot experiment (Madhavi and Bhattiprolu, 2011) and they found that root-rot disease in chilli was inhibited by 100% at 3000 ppm concentration. Hoquel et al. (2014) reported that Secure 600 WG, bavistin 70 WP and captan 50 WP suppressed the root and foot rot disease incidence and enhanced the yield production of lentil in field condition. Among all the tested fungicides, the Secure 600 WG controlled highest disease incidence in lentil caused by *S. rolfisii*. Siddique et al. (2016) reported that Bavistin 50 WP reduced 89.06 % root and foot rot disease incidence and increased the yield productivity of eggplant under *in vivo* condition. Carbendazim was most effective fungicide to check root rot disease incidence; plant height and number of leaf per

plant was recorded maximum at 0.1% concentration on 60<sup>th</sup> day under *in vivo* study (Rani and Hegde, 2016). The chemical fungicide Carbendazim checked 80.24% collar rot disease in chickpea at 0.1% concentration agrees with the result of the present study (Wavare et al., 2017).

Table 4.24: Effect of *Ocimum tenuiflorum* leaf extract, *Trichoderma viride*, Myclobutanil 10% WP at different concentration against root rot of *Zea mays* and recorded shoot length, root length, fresh weight, dry weight on 50<sup>th</sup> day under *in vivo* condition

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)	F-cal
<b>Control</b>	42±2	14±1	78±1	27.67±1.52	1092.2*
<b>Ot1</b>	0±0	0±0	0±0	0±0	65535*
<b>Ot2</b>	45±1	16.33±1.52	82±2	30±1	1154.24*
<b>Ot3</b>	52.33±2.51	19.67±1.52	93.67±3.21	34±1	617.66*
<b>Tv1</b>	0±0	0±0	0±0	0±0	65535*
<b>Tv2</b>	45.67±1.52	17.67±2.08	83.33±2.08	29.33±1.52	737.53*
<b>Tv3</b>	61.67±1.52	25.33±4.72	98±1	33.33±2.08	432.3*
<b>Bn1</b>	0±0	0±0	0±0	0±0	65535*
<b>Bn2</b>	38±2.64	14±1	76.67±1.52	25.67±1.52	701.64*
<b>Bn3</b>	46.33±2.08	17.67±2.08	80±1	30±1	821.2*

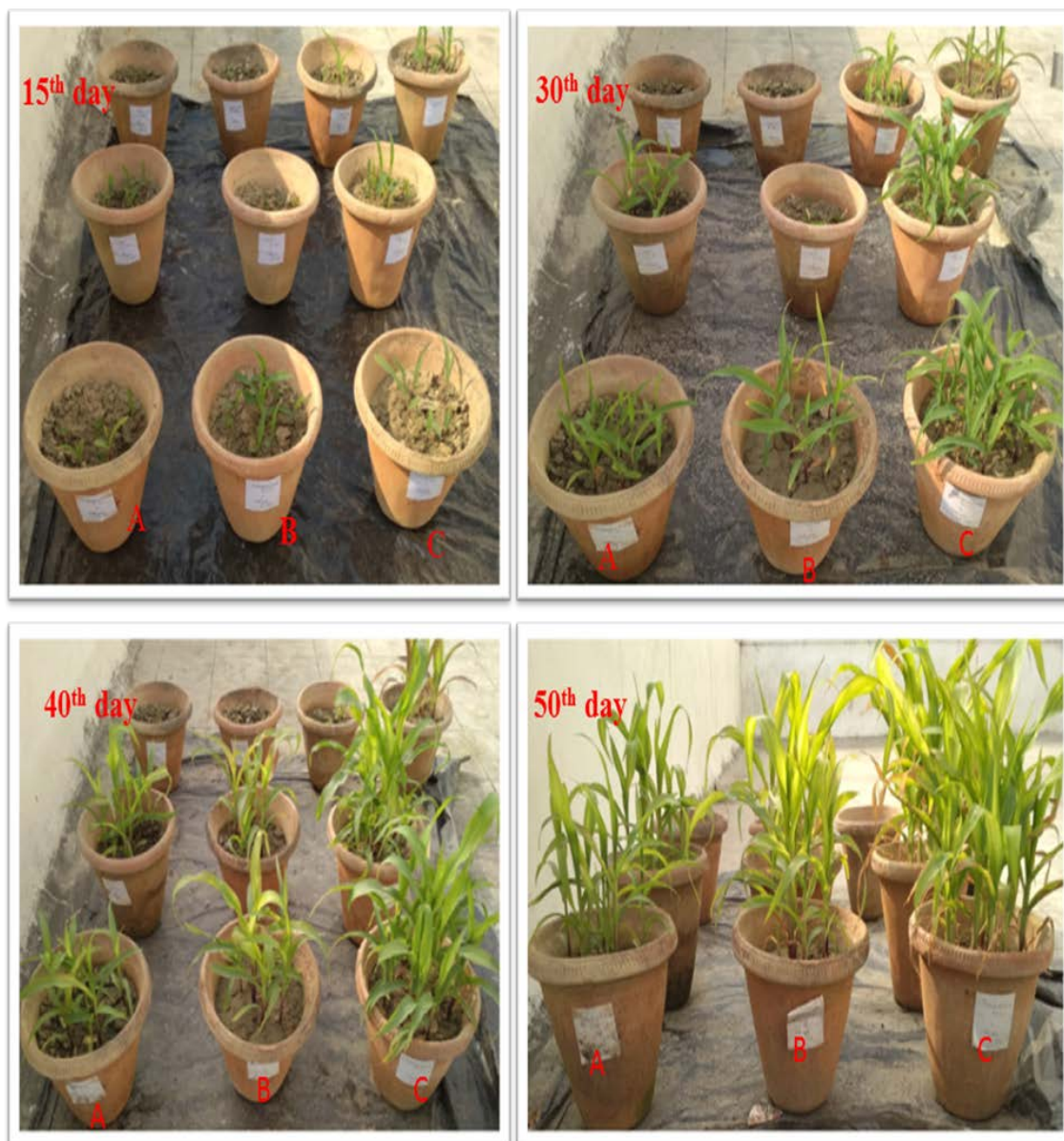
Mean±SD, F-cal= F calculated value at 5% level, \*= Significant at p=0.05 (Appendix II–Table 58–67)

Ot1= *Ocimum tenuiflorum* 1% v/w, Ot2= *Ocimum tenuiflorum* 2%v/w, Ot3= *Ocimum tenuiflorum* 3%v/w, Tv1= *Trichoderma viride* 1%w/w, Tv2= *Trichoderma viride* 2%w/w, Tv3= *Trichoderma viride* 4%w/w, Bn1= Myclobutanil 200 ppm v/w, Bn2= Myclobutanil 600 ppm v/w, Bn3= Myclobutanil 1000ppm v/w.

**Table 4.25:** Treatment of *Sclerotium rolfsii* with *Ocimum tenuiflorum* leaf extract, *Trichoderma viride*, Myclobutanil 10% WP at different concentration under *in vivo* condition and percent disease incidence and percentage of healthy plants on 30<sup>th</sup> and 50<sup>th</sup> day under *in vivo* condition

Day	Treatment																	
	Myclobutanil 10% WP						<i>Ocimum tenuiflorum</i>						<i>Trichoderma viride</i>					
	200 ppm v/w		600 ppm v/w		1000 ppm v/w		1% v/w		2% v/w		3% v/w		1%w/w		2% w/w		4% w/w	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
30 <sup>th</sup>	25	75	00	100	00	100	40	60	00	100	00	100	30	70	00	100	00	100
50 <sup>th</sup>	100	00	50	50	30	70	100	00	60	40	20	80	100	00	30	70	00	100

A= Infected plant (%), B=Healthy plant (%)



**Fig. 4.23:** Treatment of root rot disease in *Zea mays* with (A) Myclobutanil 10% WP (B) *Ocimum tenuiflorum* (C) *Trichoderma viride*  
As the incubation period was increased the plants became healthier from 15<sup>th</sup> day to 50<sup>th</sup> day



**Fig. 4.24:** Treatments of root rot disease in *Zea mays* with Myclobutanil 10% WP, *Ocimum tenuiflorum* and *Trichoderma viride* on 50th day; A2= Myclobutanil 600 ppm v/w, A3= Myclobutanil 1000ppm v/w, B2= *Ocimum tenuiflorum* 2%v/w, B3= *Ocimum tenuiflorum* 3%v/w, C2= *Trichoderma viride* 2%w/w, C3= *Trichoderma viride* 4%w/w on root length of the plant

#### 4.11. Evaluation of integrated management of root rot disease in *Zea mays* under *in vivo* condition

Under *in vivo* evaluation, the composite *Trichoderma viride*+Myclobutanil 10% WP, *Ocimum tenuiflorum*+Myclobutanil 10% WP, *Trichoderma viride*+*Ocimum tenuiflorum* were tested against root rot disease and suppressed 100% disease incidence in *Zea mays* plants at 4% w/w+1000ppm v/w (Tb3), 3% v/w+1000ppm v/w (Ob3), 4% w/w+3% v/w (To3) concentration respectively on 50<sup>th</sup> day (Table 4.27, 4.28). The composite *T. viride*+*Ocimum tenuiflorum*+Myclobutanil 10% WP checked the disease incidence by 100% in *Zea mays* plants at 4% w/w+3% v/w+ 1000 ppm v/w (Tob3) concentration on 50<sup>th</sup> day (Table 4.28). All integrated treatments had healthy plants with shoot length, root length, fresh weight and dry weight per plant (Table 4.26). All the composite treatments were significantly ( $P \leq 0.05$ ) (Appendix II–Table 68–80) effective and checked 100% root-rot disease in *Zea mays* crop under pot experiment. The composite *Trichoderma viride* +*O. tenuiflorum* checked 100% root-rot disease incidence in *Zea mays* and also promote the shoot length, root length and fresh weight of plant compared to other treatments (Fig.4.25, 4.26).

*Moringa* leaf extract+*Trichoderma* sp. checked effectively disease incidence by 94% and 70% in cowpea under greenhouse and field condition respectively (Adandonon et al., 2006). In pot experiment, the efficacy of the composite *T. viride*+neem cake checked significantly stem rot disease incidence by 95% compared to untreated groundnut plant on 60<sup>th</sup> day after sowing and enhanced the shoot and root length (Karthikeyan et al., 2006). They suggested to farmers for using Bavistin with garlic extract and *T. harzianum* as the ecofriendly measures to control foot and root rot in betel vine. Madhavi and Bhattiprolu (2011) reported that the composite carbendazim+ mancozeb and carbendazim+diathane M-45 suppressed the root-rot

disease in chilli caused by *S. rolfsii* in pot experiment. The crude extract of seven plant species (*Bauhinia purpurea*, *Cassia senna*, *Cassia fistula*, *Chrysanthemum frutescens*, *Caesalpinia gilliesii*, *Euonymus japonicus* and *Thespesia populnea* var. *acutiloba*) and some natural pesticides checked the damping-off disease in sugarbeet under laboratory and in greenhouse condition (Derbalah et al., 2012). These plant extracts contain hexadecanoic acid, linalool-1,8, pentadecanoic acid, phytol, tetradecanoic acid, and 9, 12, 15-octadecanoic acid that suppressed *Sclerotium rolfsii* causing damping-off disease in sugarbeet. They reported that the highest (61%) growth inhibition of pathogen with *T. viride* followed by *T. harzianum* (55%). subsequently, *Trichoderma* sp. are extremely valuable as biocontrol agents. They were also recorded that *Trichoderma* sp. reduced significantly expansion of soil borne pathogen and therefore, they can be incorporated for integrated management of soil borne plant pathogen (Heena et al., 2015).

The composite treatment of Carbendazim 25%+mancozeb 50% were most effective chemical fungicide against root rot disease and maximum plant height was recorded compared to untreated plant at 0.1% concentration on 60<sup>th</sup> day under *in vivo* study (Rani and Hegde, 2016). Foot and root rot disease reduces the yield and quality in betel vine caused by *S. rolfsii*, when treated with Bavistin, Topgan, garlic and clove extract, and *T. harzianum* significantly suppressed the disease incidence and increased the yield quantity (Parvin et al., 2016). Rani (2017) studied integrated management of stem rot disease of groundnut caused by *S. rolfsii* and reported that inocula of *T. harzianum*+carbendazim reduced stem rot disease by 60% and enhanced the yield production. The integrated management of collar rot disease in tomato was controlled by composite neem seed cake+*T. harzianum* and Garlic extract+*T. harzianum* and checked the disease incidence by 94.99% and 75.02% respectively with development

of shoot length, root length and yield compared to untreated plant (Mahato et al., 2017). The combination of soil treatment with maize grain based culture of *T. harzianum* (10 gm per pot) with Vitavax seed treatment @ 2 gm/kg seed demonstrated highest disease control (79.95%) and significantly reduced the seedling mortality of chickpea (Ahsan et al., 2018). Shyam and Tiwari (2018) reported that *Trichoderma harzianum* with fungicides as integrated management of collar rot disease in chickpea caused by *Sclerotium rolfsii* was most effective for seed germination, mortality and grain yield. Management of disease using fungicides, microbial biocontrol agents and plant extracts were found very effective in agriculture. Another method is also useful for pathogen control such as crop rotation, use of disease resistant plants, soil health management etc. They concluded that use of biological agents, botanical extracts, antagonists, microbials and organic pesticides for efficient management of diseases (Tulipa De and De, 2019). The soil borne pathogen *Sclerotium rolfsii* causing seedling disease of barley which is most common and prevalent disease that affected seed germination and seedling survival in the field. The composite bioagent (*Trichoderma harzianum*) and chemical fungicide (Provax 200 WP) were most effective and significantly reduced seedling mortality of barley and accelerate the plant growth with increasing yield of barley (Faruk, 2019).

**Table 4.26:** Effect of composite *Trichoderma viride*+Myclobutanil 10% WP, *Ocimum tenuiflorum*+ Myclobutanil 10% WP, *Trichoderma viride*+ *Ocimum tenuiflorum*, *T. viride*+*Ocimum tenuiflorum*+ Myclobutanil 10% WP at different concentration under *in vivo* condition on shoot length, root length, fresh weight, dry weight of *Zea mays* plant on 50<sup>th</sup> day

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)	F-cal
Control	61.67±1.52	32.33±2.51	92.33±2.08	31.33±1.52	654.97*
Tb1	57±1	34±1	90.66±1.52	32.67±2.08	1019.01*
Tb2	69±1	40.33±1.52	125±1	43.33±1.52	2769.51*
Tb3	83.33±208	52±2	145.33±2.08	65±1	1499.66*
Ob1	55±2	30±2	88.33±1.52	30.33±1.52	719.32*
Ob2	67.67±2.51	39.67±1.52	120.33±1.52	42.33±2.51	969.1*
Ob3	82.67±2.51	48.33±1.52	147±3	62.33±2.51	951.27*
To1	58.33±1.52	32.67±1.52	90±1	31.67±1.52	1132.94*
To2	68.33±1.52	41.33±1.52	119.67±1.52	39±1	2109.15*
To3	86.67±1.52	51.67±1.52	142±2.64	63.33±2.08	1207.24*
Tob1	63.67±3.21	34.67±1.52	93.33±2.08	35.67±1.52	479.65*
Tob2	72.67±2.08	42.33±1.52	124±1	40.33±1.52	1827.42*
Tob3	90±2	52.33±1.52	147.33±2.51	65.67±1.52	1411.34*

Mean±SD, F-cal= F calculated value at 5% level, \*= Significant at p=0.05(Appendix II–Table 68–80)

Tb1= *Trichoderma viride* 1%w/w+Myclobutanil 200 ppm v/w, Tb2= *Trichoderma viride* 2% w/w+Myclobutanil 600ppmv/w, Tb3= *Trichoderma viride* 4%w/w+Myclobutanil 1000 ppm v/w, Ob1= *Ocimum tenuiflorum* 1%v/w+ Myclobutanil 200 ppm v/w, Ob2= *Ocimum tenuiflorum* 2%v/w+ Myclobutanil 600 ppm v/w, Ob3= *Ocimum tenuiflorum* 3%v/w+ Myclobutanil 1000 v/w, To1= *Trichoderma viride* 1%w/w+ *Ocimum tenuiflorum* 1% V/W, To2= *Trichoderma viride* 2%w/w+ *Ocimum tenuiflorum* 2%v/w, To3= *Trichoderma viride* 4%w/w+ *Ocimum tenuiflorum* 3%v/w, Tob1= *T. viride* 1%w/w+*Ocimum tenuiflorum* 1%v/w+ Myclobutanil 200 ppm v/w, Tob2= *T. viride* 2%w/w+*Ocimum tenuiflorum* 2%v/w+ Myclobutanil 600 ppm v/w, Tob3= *T. viride* 4%w/w+*Ocimum tenuiflorum* 3%v/w+ Myclobutanil 1000 ppm v/w.

**Table 4.27:** Effect of composite *Trichoderma viride*+Myclobutanil 10% WP and *Ocimum tenuiflorum*+ Myclobutanil 10% WP on *Sclerotium rolfsii* at different concentration under *in vivo* condition and percent disease incidence in *Zea mays* plants on 30<sup>th</sup> and 50<sup>th</sup> day

Day	Treatment											
	<i>Trichoderma viride</i> +Myclobutanil 10% WP						<i>Ocimum tenuiflorum</i> + Myclobutanil 10% WP					
	1%w/w+200ppm v/w		2%w/w+600 ppm v/w		4%w/w+1000ppm v/w		1%v/w+200ppmv/w		2% v/w+600ppmv/w		3%v/w+1000 ppm v/w	
	A	B	A	B	A	B	A	B	A	B	A	B
30 <sup>th</sup>	20	80	00	100	00	100	20	80	00	100	00	100
50 <sup>th</sup>	60	40	20	80	00	100	60	40	20	80	00	100

**Table 4.28:** Effectiveness of different treatments on root-rot disease incidence in *Zea mays* due to *Sclerotium rolfsii* at different concentration under *in vivo* condition on 30<sup>th</sup> and 50<sup>th</sup> day

Day	Treatment											
	<i>Trichoderma viride</i> + <i>Ocimum tenuiflorum</i>						<i>T. viride</i> + <i>Ocimum tenuiflorum</i> + Myclobutanil 10% WP					
	1%w/w+1%v/w		2%w/w+2%v/w		4%w/w+3%v/w		1%w/w+1%v/w+200ppmv/w		2%w/w+2%v/w+ 600ppmv/w		4%w/w+3%v/w+1000ppm v/w	
	A	B	A	B	A	B	A	B	A	B	A	B
30 <sup>th</sup>	20	80	00	100	00	100	00	100	00	100	00	100
50 <sup>th</sup>	40	60	20	80	00	100	00	100	00	100	00	100

A= Infected plant (%), B=Healthy plant (%)



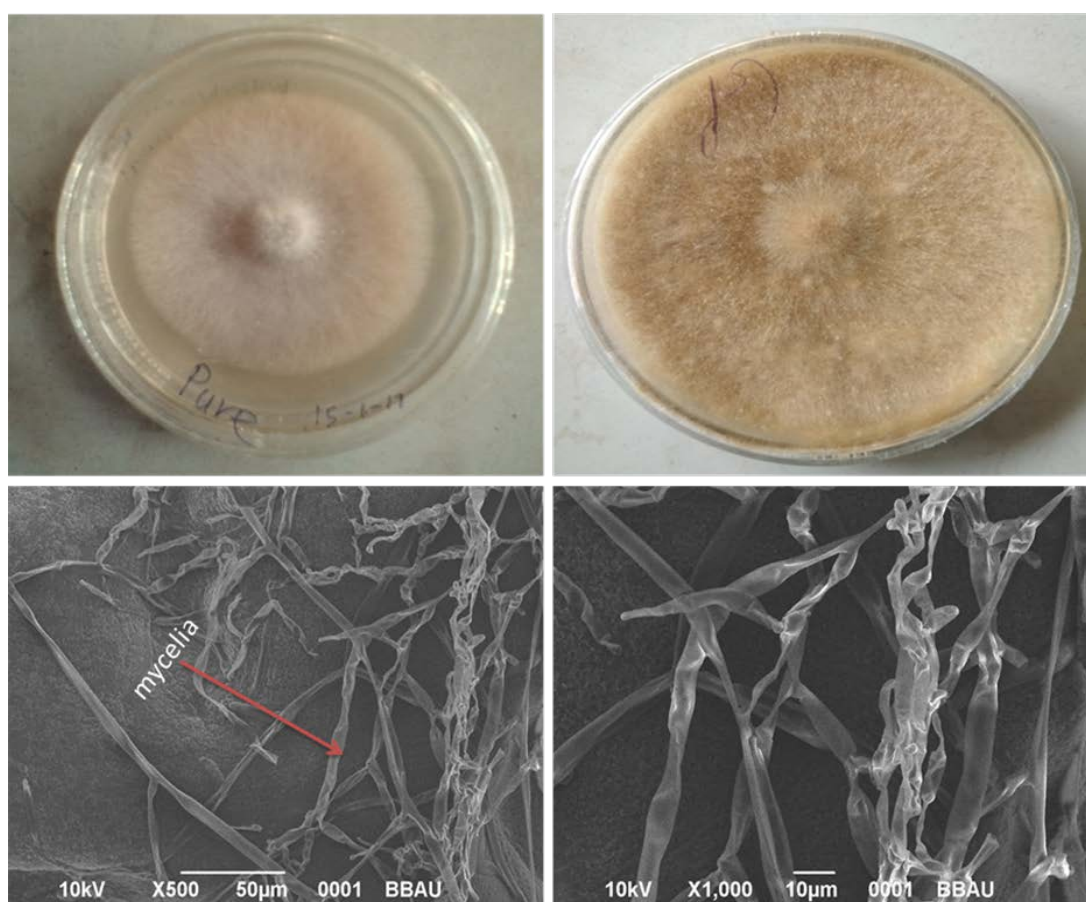
**Fig. 4.25:** Treatment of root rot disease in *Zea mays* as integrated management with (A) *Trichoderma viride*+Myclobutanil 10% WP (B) *Ocimum tenuiflorum*+ Myclobutanil 10% WP (C) *Trichoderma viride*+ *Ocimum tenuiflorum*  
As the incubation period was increased the plants became healthier from 15<sup>th</sup> day to 50<sup>th</sup> day



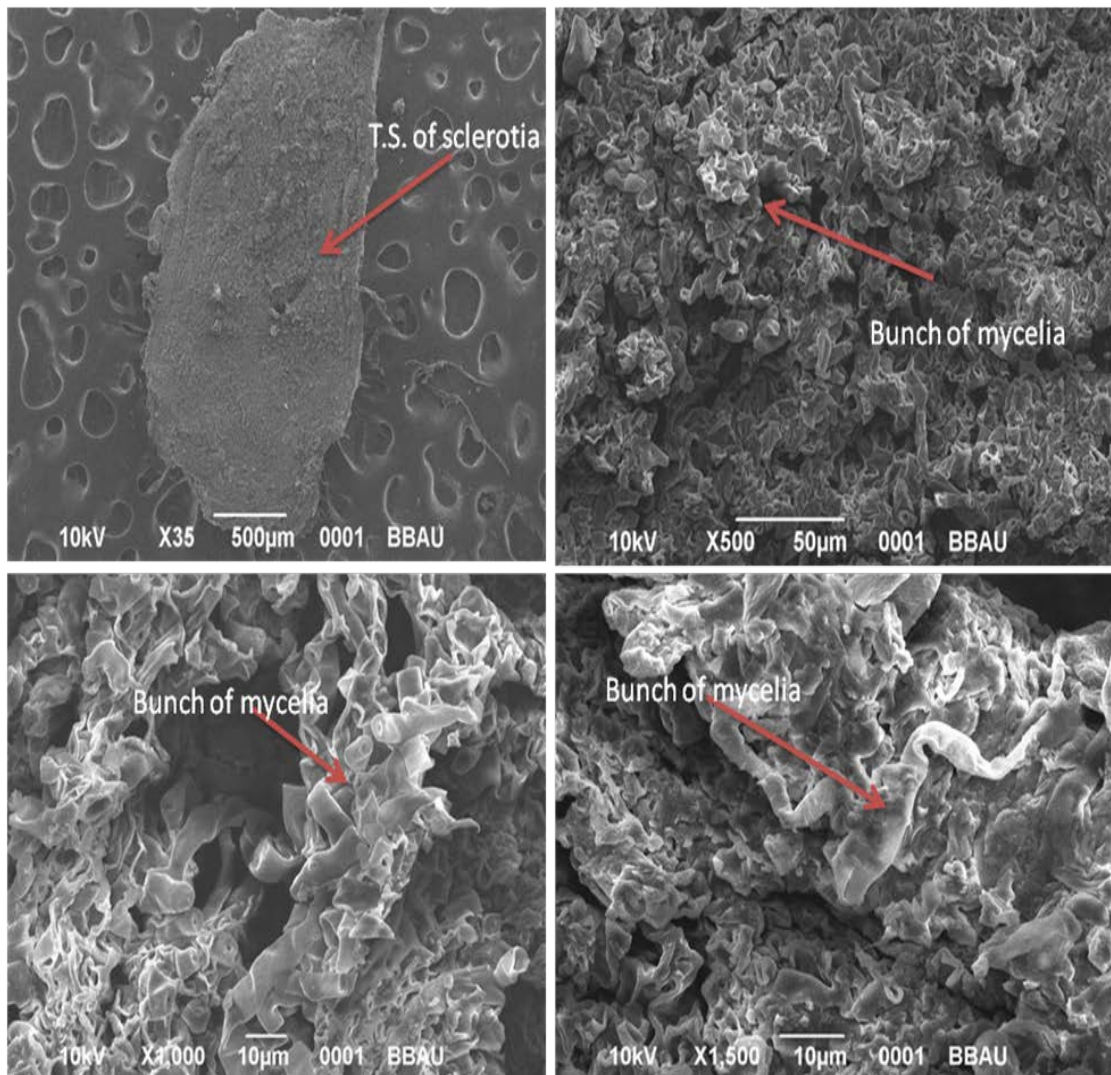
**Fig. 4.26:** Treatment of root rot disease in *Zea mays* as integrated management with *Trichoderma viride*+Myclobutanil 10% WP, *Ocimum tenuiflorum*+ Myclobutanil 10% WP and *Trichoderma viride*+ *Ocimum tenuiflorum*; A1= *Trichoderma viride* 1% w/w+Myclobutanil 200 ppm v/w, A2= *Trichoderma viride* 2% w/w+Myclobutanil 600ppmv/w, A3= *Trichoderma viride* 4%w/w+Myclobutanil 1000 ppm v/w, B1= *Ocimum tenuiflorum* 1%v/w+ Myclobutanil 200 ppm v/w, B2= *Ocimum tenuiflorum* 2%v/w+ Myclobutanil 600 ppm v/w, B3= *Ocimum tenuiflorum* 3%v/w+ Myclobutanil 1000 v/w, C1= *Trichoderma viride* 1%w/w+ *Ocimum tenuiflorum* 1% V/W, C2= *Trichoderma viride* 2%w/w+ *Ocimum tenuiflorum* 2%v/w, C3= *Trichoderma viride* 4%w/w+ *Ocimum tenuiflorum* 3%v/w on root length of the plant

#### 4.12. Histopathological study

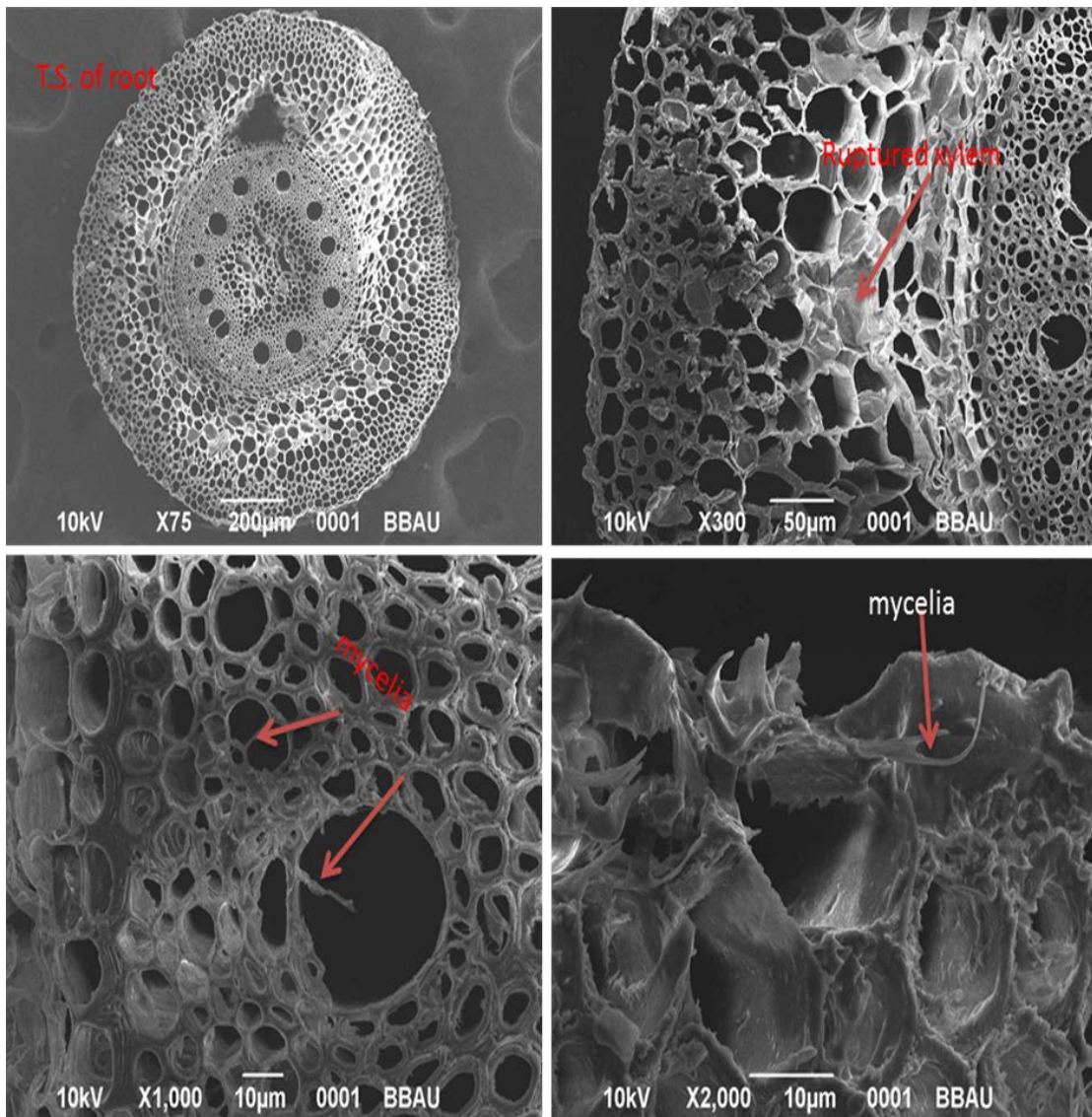
The pure culture of *Sclerotium rolfsii* observed under SEM images at different magnification showed that the mycelia are rod shaped structure (Fig. 4.27). T.S. of sclerotia showed bunch shaped structure in SEM images at different magnification (Fig.4.28). The histopathological study of root and stem of chemically and biologically controlled *Zea mays* plants in SEM showed that xylem vessels were not ruptured while in root rot disease infected plants the xylem vessels of plants were ruptured (Fig.4.29, 4.30). The SEM observation showed the straight penetration of fungal hyphae throughout the cuticle within 24 HAI of inoculation and no mycelial growth occurred in treatment case (Rajasekhar et al., 2019).



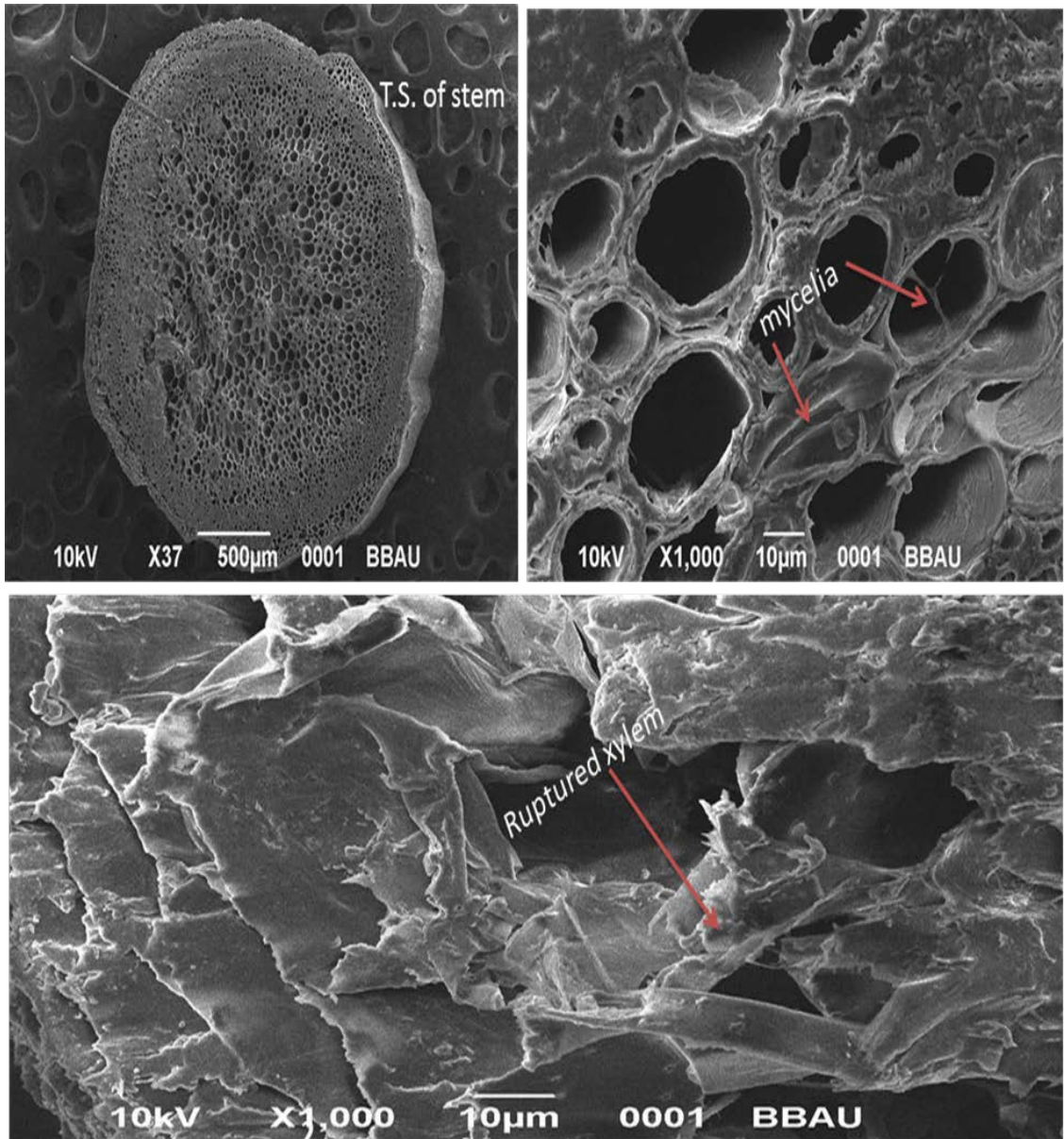
**Fig. 4.27:** Pure culture of *Sclerotium rolfsii* and scanning electron microscope (SEM) images at x500 and x1000 magnification



**Fig. 4.28:** T.S. of sclerotia of *Sclerotium rolfsii* in scanning electron microscope (SEM) images at different magnifications



**Fig. 4.30:** T.S. of root of *Zea mays* infected from *Sclerotium rolfsii* in scanning electron microscope (SEM) images at different magnifications



**Fig. 4.30:** T.S. of stem of *Zea mays* infected from *Sclerotium rolfsii* in scanning electron microscope (SEM) images at different magnifications

# CHAPTER - 5

## SUMMARY



## SUMMARY

---

*Zea mays* seeds are nutritive and edible cereal enriched with carbohydrates, protein, minerals and vitamins (A, B, C, K). It is used as food sources for people and animals. It is short day with large grain and C<sub>4</sub> plant. The diversity of *Zea mays* plants were recorded in temperate zone in sandy soil during 2017-18. Plant density and cultivar was affected on morphological trait of *Zea mays* such as stem diameter and leaf length of the plant but plant height and number of leaf per plant was not affected. Soil borne fungus (*Sclerotium rolfsii*) affected the growth and yield production in *Zea mays*. The root-rot disease is caused by *S. rolfsii* in root of *Zea mays*. Apart from root-rot disease, *S. rolfsii* causes different types of diseases viz. collar-rot, *Sclerotium* wilt, seedling blight, damping-off of seedlings, stem canker, crown blight, crown, bulb rot, tuber rot, fruit rot, crown rot, systemic wilt or blight of whole plant, stem-rot, charcoal rot, foot-rot and stem blight. It also affected to other plants including tomato, chilli, maize, groundnut, sunflower, cucumber, brinjal, soybean, bean, watermelon.

The soil samples were collected from rhizospheric and non-rhizospheric region of healthy *Zea mays* plant from Jaunpur, Barabanki and Lucknow. The soil texture, temperature, pH, organic carbon and organic matter were analyzed from the soil samples. The highest sand particle (33.33%) was found in sample no. 4 compared to sample no. 1, 2 and 3. The maximum silt particle (38.09%) was recorded in sample no. 3 followed by sample no. 1, 2 and 4. The highest clay particle (41.67%) was in sample no. 2&4 and minimum clay particle (33.33%) was recorded in sample no. 3. The soil pH (9.34) was recorded in sample no. 2 followed by sample no. 1, 3 and 4. The maximum soil temperature (27.15<sup>0</sup> C) was recorded in sample no. 2 and

minimum temperature (24.23<sup>0</sup> C) in sample no. 4. These factors are also responsible for growth and development of soil mycoflora. The organic carbon and organic matter is an important part of soil, the maximum organic carbon (2.00%) and organic matter (5.79%) was recorded in sample no. 1 compared to sample no. 2, 3, 4.

Twelve soil mycoflora have been isolated from collected soil samples using serial dilution technique [1/1000 (10<sup>-3</sup>), 1/10000 (10<sup>-4</sup>), 1/100000 (10<sup>-5</sup>) dilution]. The isolated soil mycoflora are *Aspergillus flavus*, *A. fumigatus*, *A. luchuensis*, *A. niger*, *A. sydowii*, *Alternaria solani*, *Fusarium solani*, *Penicillium citrinum*, *Penicillium italicum*, *Penicillium* species, *Trichoderma viride* and white sterile mycelium, isolated from four soil samples. The rich fungal diversity was recorded in soil sample no. 1. The percent frequency and percent occurrence of *Aspergillus flavus* and *Aspergillus luchuensis* were highest compared to other soil mycoflora. *Sclerotium rolfisii* was isolated on potato dextrose agar (PDA) Petriplates from infected root of *Zea mays*. The pathogen produced cottony white mycelia on PDA Petriplate after one day, on the 5<sup>th</sup> day cottony white mycelia changed into light brown and after seven days light brown mycelia changed into dark brown and become hard mustard seed like “sclerotia” (2-4 mm diameter) are formed which is a characteristic feature of *S. rolfisii*.

The pathogen grows around the *Zea mays* and enters into the root of the *Zea mays*. After infection, the crop growth was inhibited resulting in drying of the plant. Histopathological study of the infected root of the plants with Scanning Electron Microscope (SEM) showed ruptured xylem vessels. The bioagents viz. *Aspergillus flavus*, *A. niger*, *Trichoderma viride*, *Penicillium citrinum*, *P. italicum* and *Penicillium* sp. had antagonistic activity against mycelial growth of *S. rolfisii* on 3<sup>rd</sup> day under *in vitro* condition. Among the selected test fungi, *T. viride* and *Penicillium* sp. were most

effective ( $p \leq 0.05$ ) bioagents against *S. rolfsii* and inhibits the growth of *S. rolfsii* at different levels.

Three chemical fungicides viz. Myclobutanil 10% WP, Carbendazim 50% WP and Conika 50% WP, procured from local market of Lucknow, were tested against *S. rolfsii* using poisoned food technique. The fungicides were significantly ( $P \leq 0.05$ ) effective against mycelial growth of *S. rolfsii* under *in vitro* condition on 3<sup>rd</sup> day. Myclobutanil 10% WP inhibited the growth by 77.16% at 100 ppm concentration while Conika 50% WP inhibited 70.57% mycelial growth of *S. rolfsii* at 600 ppm concentration on 3<sup>rd</sup> day. Carbendazim 50% WP inhibited 84.19% mycelial growth of *S. rolfsii* at 6 ppm concentration on 3<sup>rd</sup> day. We observed that Carbendazim 50% WP was most effective fungicide compared to Myclobutanil 10% WP and Conika 50% WP against *S. rolfsii* at different concentration on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day under *in vitro* condition.

The plants viz. *Azadirachta indica* (leaf), *Ocimum tenuiflorum* (leaf), *Syzygium aromaticum* (oil), *Tinospora cordifolia* (stem), *Trachyspermum ammi* (seeds) contained antifungal activity against *S. rolfsii*. The leaves extract of *A. indica* inhibited 79.65% mycelial growth of *S. rolfsii* at 75% concentration on 3<sup>rd</sup> day. At 75% concentration, the leaves extract of *O. tenuiflorum* inhibited 100% mycelial expansion of *S. rolfsii* on 3<sup>rd</sup> day. *Syzygium aromaticum* oil inhibited 100% growth of *S. rolfsii* at 400 ppm concentration on 3<sup>rd</sup> day. The stem extract of *T. cordifolia* showed antifungal activity against *S. rolfsii* and inhibited the growth by 100% at 75% concentration on 3<sup>rd</sup> day. *Trachyspermum ammi* seeds powder inhibited 100% growth of test fungus at 50% concentration on 3<sup>rd</sup> day. Among all the tested extract of medicinal plants, *S. aromaticum* and *T. ammi* inhibited the growth (100%) at 400 ppm

and 50% concentration respectively while *T. cordifolia* inhibited 100% growth at 75% concentration on 3<sup>rd</sup> day after inoculation. The extract of *S. aromaticum*, *T. ammi* and *T. cordifolia* were most significantly effective compared to *A. indica* and *O. tenuiflorum* at different concentration on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day.

The metabolite of fungal species viz. *Aspergillus flavus*, *A. niger*, *Trichoderma viride*, *Penicillium citrinum*, *P. italicum* and *Penicillium* sp. were significantly effective against *S. rolfisii* on 3<sup>rd</sup> day under *in vitro* condition. Amongst all metabolites of test fungi, *A. flavus* inhibited the growth of test fungus by 70.04%, followed by *A. niger* (76.52%), *T. viride* (76.63%), *P. citrinum* (100%), *P. italicum* (100%) *Penicillium* sp (68.14%) at 75% concentration on 3<sup>rd</sup> day. The metabolite of *P. citrinum* and *P. italicum* were significantly effective to inhibit the growth compared to *A. flavus*, *A. niger*, *T. viride* and *Penicillium* sp. at 10%, 25%, 50%, 75% on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day.

The composite *O. tenuiflorum*+Myclobutanil 10%WP and *O. tenuiflorum*+*T. viride* inhibited 100% mycelial growth of *S. rolfisii* at 50%+50 ppm and 50%+50% concentration respectively on 3<sup>rd</sup> day. At 75 %+100 ppm and 75 %+100ppm+75 % concentration the composite *T. viride*+Myclobutanil 10%WP and *T.viride* +Mycobutanil 10%WP+*O. tenuiflorum* inhibited 100 % mycelial growth of *S. rolfisii* on 3<sup>rd</sup> day after inoculation under *in vitro* condition.

*Sclerotium rolfisii* was treated with bioagent, plant extract and chemical fungicide at different concentration against *S. rolfisii* in pot experiments. The bioagent and plant extract were most significantly and ecofriendly effective compared to chemical fungicide under *in vivo* condition. The treatment with myclobutanil 10% WP showed 100% healthy plant at 1000 ppm v/w on 30<sup>th</sup> day while on 50<sup>th</sup> day, 30%

plants were infected from disease and 70% plants were healthy with shoot length (46.33 cm), root length (17.67 cm), fresh weight (80 gm), dry weight (30 gm) at 1000 ppm concentration compared to 200 ppm, 600 ppm under *in vivo* condition. In treatment with leaf extract of *O. tenuiflorum* at 3% v/w concentration, 100% healthy plants were recorded on 30<sup>th</sup> day while on the 50<sup>th</sup> day, 20% plants were unhealthy and 80% plants were healthy under *in vivo* study. The healthy plants recorded shoot length (52.33 cm), root length (19.67 cm), fresh weight (93.67 gm), dry weight (34 gm) at 3% v/w concentration, while in case of *T. viride* at 4% w/w concentration, 100% healthy plants were recorded on both 30<sup>th</sup> and 50<sup>th</sup> day after inoculation and their shoot length (61.67 cm), root length (25.33 cm), fresh weight (98 gm), dry weight (33.33 gm) were more developed under *in vivo* condition.

The treatment with composite *T. viride*+Myclobutanil 10% WP, *O. tenuiflorum*+Myclobutanil 10% WP *T. viride*+*O. tenuiflorum* showed 100% healthy plants at 4% w/w+1000ppm v/w, 3% v/w+1000ppm v/w, 4% w/w+3% v/w concentration on 30<sup>th</sup> and 50<sup>th</sup> day respectively with healthy shoot length, root length, fresh weight, dry weight. At 4% w/w+3% v/w+ 1000ppm v/w concentration, composite *T. viride*+*O. tenuiflorum*+Myclobutanil 10% WP showed 100% healthy plants with shoot length (90cm), root length (52.33cm), fresh weight (147.33gm), dry weight (65.67gm) on 30<sup>th</sup> and 50<sup>th</sup> day under in pot experiment.

In histopathological study, the T. S. of root & stem of infected *Zea mays* plants images was taken with SEM and showed that the xylem vessels of root and stem were ruptured from *S. rolfisii*.

# CHAPTER - 6

# CONCLUSION



**CONCLUSION**

A large, red, distressed-style stamp of the word "CONCLUSION" is centered on the page. The text is slanted upwards from left to right and is enclosed within a thick red circular border. The stamp has a grainy, ink-like texture.

## CONCLUSION

---

*Zea mays* seeds are nutritive, edible cereal and have great potential as food sources for people. *Zea mays* plants (growth, development and yield) are affected by soil borne fungus (*Sclerotium rolfsii*) and causes root-rot disease.

The soil texture (sand, silt and clay), soil temperature, pH, organic carbon and organic matter were analyzed in four soil samples. Out of all four soil samples, the highest sand particles (33.33%), silt particles (38.09%), clay particles (41.67%), soil pH (9.34) and soil temperature (27.15<sup>0</sup> C) was recorded in sample no. 4, 3, 2&4, 2 and 2 respectively. These above factors are responsible for growth and development of soil mycoflora. The organic carbon and organic matter is an important part of soil, the maximum organic carbon (2.00%) and organic matter (5.79%) was recorded in sample no. 1.

In the present study twelve soil mycoflora viz. *Aspergillus flavus*, *A. fumigatus*, *A. luchuensis*, *A. niger*, *A. sydowii*, *Alternaria solani*, *Fusarium solani*, *Penicillium citrinum*, *Penicillium italicum*, *Penicillium* species, *Trichoderma viride*, white sterile mycelium were isolated from four soil samples using serial dilution technique on 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> dilution. Amongst four soil samples the rich fungal diversity was recorded in soil sample no. 1. The bioagents viz. *Aspergillus flavus*, *A. niger*, *Trichoderma viride*, *Penicillium citrinum*, *P. italicum* and *Penicillium* sp. effectively inhibited mycelial growth of *S. rolfsii* on 3<sup>rd</sup> day under *in vitro* condition. Amongst the antagonists, *T. viride* and *Penicillium* sp. were most effective compared to other antagonist against *S. rolfsii*.

The chemical fungicides viz. Index (Myclobutanil 10% WP), Bavistin (Carbendazim 50% WP) and Conika 50% WP (Kasugamycin 5% + Copper oxychloride 45 % WP) were effective against *S. rolfsii*. Carbendazim 50% WP (84.19%) was most effective chemical fungicide compared to Myclobutanil 10% WP (77.16%) and Conika 50% WP (70.57%) at 6ppm, 100 ppm and 600 ppm concentration respectively.

In the present study the extract of plants viz. *Azadirachta indica*, *Ocimum tenuiflorum*, *Syzygium aromaticum*, *Tinospora cordifolia*, *Trachyspermum ammi* with antifungal activity checked the mycelial growth of *S. rolfsii* under *in vitro* condition. We observed that among all the tested extract of medicinal plants, *Syzygium aromaticum* and *Trachyspermum ammi* inhibited the growth by 100% at 400 ppm and 50% concentration respectively while *Ocimum tenuiflorum* and *Tinospora cordifolia* inhibited 100% growth at 75% concentration on 3<sup>rd</sup> day after inoculation. The extract of *O. tenuiflorum*, *S. aromaticum*, *T. ammi* and *T. cordifolia* were most significantly effective compared to *A. indica* on 3<sup>rd</sup> day.

The metabolite of fungal species viz. *Aspergillus flavus*, *A. niger*, *Trichoderma viride*, *Penicillium citrinum*, *P. italicum* and *Penicillium* sp. were effective against *S. rolfsii* under *in vitro* condition. We observed that all test fungi viz. *A. flavus* (70.04%), *A. niger* (76.52%), *T. viride* (76.63%), *P. citrinum* (100%), *P. italicum* (100%) and *Penicillium* sp (68.14%) inhibited the growth of *S. rolfsii* at 75% concentration on 3<sup>rd</sup> day. The culture filtrate of *P. citrinum* and *P. italicum* were most effective compared to *A. flavus*, *A. niger*, *T. viride* and *Penicillium* sp. at 10%, 25%, 50%, 75% on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day.

The composite *O. tenuiflorum*+Myclobutanil 10%WP and *O. tenuiflorum*+*T. viride* inhibited 100% mycelial growth of *S. rolfisii* at 50%+50 ppm and 50%+50% concentration on 3<sup>rd</sup> day respectively whereas composite *T. viride*+Myclobutanil 10%WP and *T. viride* +Myclobutanil 10%WP+*O. tenuiflorum* inhibited the mycelial growth of *S. rolfisii* completely at 75 %+100 ppm and 75 %+100ppm+75 % concentration on 3<sup>rd</sup> day.

In pot experiment, the treatment with *T. viride*, *O. tenuiflorum* and Myclobutanil 10% WP at different concentration were significantly effective compared to Myclobutanil 10% WP under *in vivo* condition. Under *in vivo* study, Myclobutanil 10% WP checked root rot disease incidence by 70% with healthy shoot length (46.33 cm), root length (17.67 cm), fresh weight (80 gm), dry weight (30 gm) at 1000 ppm concentration on 50<sup>th</sup> day. On 50<sup>th</sup> day, 20% plants showed disease incidence and 80% plants were healthy with shoot length (52.33 cm), root length (19.67 cm), fresh weight (93.67 gm), dry weight (34 gm) when treated with leaf extract of *O. tenuiflorum* at 3% v/w concentration. After treatment of *T. viride* at 4% w/w concentration, 100% healthy plants were recorded on 50<sup>th</sup> day having shoot length (61.67 cm), root length (25.33 cm), fresh weight (98 gm), dry weight (33.33 gm) under *in vivo* condition.

The results obtained with integrated management of root rot disease when treated with composite *T. viride*+Myclobutanil 10% WP, *O. tenuiflorum*+Myclobutanil 10% WP, *T. viride*+*O. tenuiflorum*, 100% healthy plants were recorded at 4%w/w+1000ppm v/w, 3%v/w+1000ppm v/w, 4%w/w+3%v/w concentration on 50<sup>th</sup> day respectively and plants having healthy shoot length, root length, fresh weight, dry weight. The composite *T. viride*+*O. tenuiflorum*+Myclobutanil 10% WP checked the disease incidence by 100% in *Zea*

*mays* at 4% w/w+3% v/w+ 1000ppm v/w concentration with shoot length (90cm), root length (52.33cm), fresh weight (147.33gm), dry weight (65.67gm) on 50<sup>th</sup> day in pot experiment. The histopathological study on T. S. of root & stem of infected *Zea mays* plant was analyzed with SEM, showed that the xylem vessels of root and stem were ruptured by *S. rolfsii*.

## RECOMMENDATION

---

The result obtained during the present research work has developed new idea for management of root rot disease in *Zea mays* caused by *Sclerotium rolfsii* using chemical fungicides, bioagents and plants extract means. On the basis of present study following recommendations are suggested.

The chemical fungicide Bavistin (Carbendazim 50% WP), extract of plants *O. tenuiflorum*, *S. aromaticum*, *T. ammi*, *T. cordifolia*, culture filtrate of *T. viride*, *P. citrinum*, *P. italicum* may be exploit to check the growth of *S. rolfsii*. The composite *O. tenuiflorum*+Myclobutanil 10%WP, *O. tenuiflorum*+*T. viride*, *T. viride*+Myclobutanil 10%WP, *T. viride* +Mycobutanil 10%WP+*O. tenuiflorum* were very effective to control the growth of *S. rolfsii*. On the basis of pot experiment, *T. viride*, *O. tenuiflorum* and myclobutanil 10% WP as well as composite *T. viride*+Myclobutanil 10% WP, *O. tenuiflorum*+Myclobutanil 10% WP, *T. viride*+*O. tenuiflorum* may be used to check the root rot disease incidence in *Zea mays* caused by *S. rolfsii*. The above treatments may be effectively useful to control *S. rolfsii* in field condition.



## REFERENCES

---

- Aanuoluwa, E.E., Kehinde, A., Esthe, B.B., Julie, A.B. & Clement, A.F. (2015). Optimizing culture conditions for the antagonistic activities of *Trichoderma viride* against *Sclerotium rolfsii* causative agent of southern blight disease of tomato. *Malaysian Journal of Microbiology*, 11(3):240-245.
- Abd-Allah, E.F. (2005). Effect of a *Bacillus subtilis* isolates a Southern blight (*Sclerotium rolfsii*) and lipid composition of peanut seeds. *Phytopathology*, 33(5):460-466.
- Adandonon, A., Aveling, T.A.S., Labuschagne, N. & Tamo, M. (2006). Biocontrol agents in combination with *Moringa oleifera* extract for integrated control of *Sclerotium*-caused cowpea damping-off and stem rot. *European Journal of Plant Pathology*, 115: 409–418.
- Adhilakshmia, M., Latha, P., Paranidharana, V., Balachandarb, Ganesamurthy, K. & Velazhahan, R. (2014). Biological control of stem rot of groundnut (*Arachis hypogaea* L.) caused by *Sclerotium rolfsii* Sacc. with actinomycetes. *Archives of Phytopathology and Plant Protection*, 47(3): 298–311.
- Ahsan, M.S., Kumar, M. & Upadhya, J.P. (2018). Integrated approach for the management of collar rot of Chickpea. *International Journal of Current Microbiology and Applied Sciences*, 7(5): 3560-3569.
- Ahsan, S., Kumar, M., Upadhyay, J.P., Hussain, A., Gupta, P.K. & Singh, A. (2018). Effect of different doses of *Trichoderma harzianum* and Fungicides for the management of collar rot of Chickpea caused by *Sclerotium rolfsii*. *International Journal of Pure & Applied Bioscience*, 6(1): 1656-1660.

- Akgull, D.S., Ozgonen, H. & Erkilic, A. (2011). The effects of seed treatments with fungicides on stem rot caused by *Sclerotium rolfsii* sacc., in Peanut. *Pakistan Journal of Botany*, 43(6): 2991-2996.
- Akram, A., Shaheen, I., Akhund, S., Nayyar, B.G. & Seerat, W. (2016). *In vitro* antifungal activity of *Pongamia pinnata* against collar rot pathogen (*Sclerotium rolfsii*) of Chickpea. *Pure and Applied Biology*, 5 (3): 520-528.
- Amin, R., Sarker, B.C., Adhikary, S.K., Sultana, S. & Zubair, T. (2013). Effect of some botanical extracts and cow's urine on *Sclerotium rolfsii* causal agent of foot and root rot of betel vine. *The International Journal of Engineering and Science*. 2(9): 77-82.
- Anahosur, K.H. (2001). Integrated management of potato *Sclerotium* wilt caused by *Sclerotium rolfsii*. *Indian Phytopathology*, 54 (2): 158-166.
- Anand, S. & Reddy, J. (2009). Biocontrol potential of *Trichoderma sp.* against plant pathogens. *International Journal of Agriculture Science*, 1(2):30-39.
- Anees, M., Tronsmo, A., Edel-Hermann, H., Raud, L.G. & Steinberg, C. (2010). Characterization of field isolates of *Trichoderma* antagonistic against *Rhizoctonia solani*. *Fungal Biology*, 114 (9): 691–701.
- Arunasri, P., Chalam, T. V., Reddy, N. E., Reddy, S. T., & Reddy, R. (2011). Investigations on fungicidal sensitivity of *Trichoderma* spp and *Sclerotium rolfsii* (collar rot pathogen) in Crossandra. *International Journal of Applied Biology and Pharmaceutical Technology*, 2(2):290-293.
- Aycock R.(1966). Stem rot and other diseases caused by *Sclerotium rolfsii*: or the status of Rolfs' fungus after 70 years. *North Carolina state university, Technical Buletin*, 174:202.

- Ayed, F., Jabnoun-Khiareddine, H., Abdallah, R.A.B. & Daami-Remadi, M. (2018). Effect of temperatures and culture media on *Sclerotium rolfii* mycelial growth, sclerotial formation and germination. *Journal of Plant Pathology & Microbiology*, 9(8):1-9.
- Babu, G.P., Paramageetham, ch. (2013). Biocontrol of *Sclerotium rolfii* – a polyphagous plant pathogen by *Pseudomonas aeruginosa* isolated from forest litter. *International Journal of research in plant science*, 3(1):1-4.
- Bagwan, N.B. (2011). Evaluation of biocontrol potential of *Trichoderma* species against *Sclerotium rolfii*, *Aspergillus niger* and *Aspergillus flavus*. *International Journal of Plant Protection*, 4(1): 107-111.
- Bankoliya, M.K., Kumar, S. & Ashish, S. (2015). Effect of intercropping on the fungicides of foot and collar rot in soybean. *International Journal of Plant Protection*, 8(2): 375-378.
- Banyal, D.K., Mankotia, V. & Sugha, S.K. (2008). Integrated management of Tomato collar rot caused by *Sclerotium rolfii*. *Journal of Mycology and Plant Pathology*, 38(2): 164-167.
- Barnett, H. L., & Bary, H. (1998). Illustrated genera of imperfect fungi . The American Phytopathological Society. *US Department of Agriculture, Agricultural Research Service, Washington State University, Pullman. APS Press. USA. St. Paul, Minnesota USA.* pp. 218.
- Bartholomew, N.A., Emylia, J.T. & Chibuzor, N. (2017). Preventive and curative control of *sclerotium* rot disease of cocoyam cormel (*Colocasia esculenta* [L., Scott]) using plant extracts and *Trichoderma koningii*. *Journal of Applied Biology & Biotechnology*, 5(6): 40-44.

- Basumatary, M., Dutta, B.K., Singha, D.M. & Das, N. (2015). Some *in vitro* observations on the biological control of *Sclerotium rolfsii*, a serious pathogen of various agricultural crop plants. *IOSR Journal of Agriculture and veterinary science*, 8(2)II: 87-94.
- Beales, N. (2004) Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Comprehensive Reviews in Food Science and Food Safety*, 3:1-20.
- Bhat, M.N., Sardana, H.R., Singh, D., Srivastava, Ch. & Ahmad, M. (2015). Evaluation of chemicals and bioagents against *Sclerotium rolfsii* causing southern blight of bell pepper (*Capsicum annuum*). *Indian Phytopathology*, 68 (1) : 97-100.
- Bhuiyan, M. A. H. B., Rahman, M. T., & Bhuiyan, K. A. (2012). *In vitro* screening of fungicides and antagonists against *Sclerotium rolfsii*. *African Journal of Biotechnology*, 11(82): 14822-14827.
- Biswas, K.K. & Sen, C. (2000). Management of stem rot of groundnut caused by *Sclerotium rolfsii* through *Trichoderma harzianum*. *Indian phytopathology*, 53 (3): 290-295.
- Bosah, O., Igalek, C.A. & Omorusi, V.I. (2010). *In vitro* microbial control of pathogenic *Sclerotium rolfsii*. *International Journal of Agriculture & Biology*, 12(3): 474-476.
- Bot, A. & Benites, J. (2005). *The importance of soil organic matter: Key to drought-resistant soil and sustained food production*. Food & Agriculture Organization of the United Nations, Bulletin No. 80.

- Boukaew, S., Chuenchit, S. & Petcharat, V. (2011). Evaluation of *Streptomyces* sp for biological control of *Sclerotium* root and stem & *Ralstonia* wilt of chili pepper. *International Organisation for Biological Control*, 56:365-374.
- Butt, B.Z., Sana, N. & Butt, S. (2016). Management of *Sclerotium rolfsii* through methanolic leaf extract of *Alstonia scholaris* and *Azadirachta indica*. *Journal of Bioresource Management*, 3(2): 1-8.
- Campbell, C. A., McConkey, B. G., Zentner, R., Selles, F. & Curtin, D. (1996). Long-term effects of tillage and crop rotations on soil organic C and total N in a clay soil in southwestern Saskatchewan. *Canadian Journal of Soil Science*, 76(3): 395-401.
- Chandra, S. (2014). Biological control of southern stem blight of Soybean (*Glycine max Merrill*) by the selected *Trichoderma* species under Glasshouse. *An International Quarterly Journal of Life Sciences*, 1(1):18-21.
- Chaurasia, A.K., Chaurasia<sup>1</sup>, S., Chaurasia, S. & Chaurasia, S. (2014). *In vitro* efficacy of fungicides against the growth of foot-rot pathogen (*Sclerotium rolfsii* Sacc.) of Brinjal. *International Journal of Current Microbiology and Applied Science*, 3(12): 477-485.
- Chaurasia, S., Chaurasia, A.K., Chaurasia, S. & Chaurasia, S. (2014). Pathological studies of *Sclerotium rolfsii* causing foot rot disease of Brinjal (*Solanum melongena*). *International Journal of Pharmaceutical and Life Sciences*, 5(1):3257-3264.
- Chet, I. (1975). Ultrastructural basis of *Sclerotium rolfsii* survival in soil. *Microbial Ecology*, 2: 194-200.
- Conway, K.E. & Tomasino, S.F. (1996). *Sclerotium rolfsii* a problem to apple nursery stock in olahoma. *Phytopathology*, 75-449.

- Cook, A. A. (1975). Diseases of tropical and subtropical fruits and nuts. *Hafner Press, a division of Macmillan publishing co., New York, USA*, pp. 207-212.
- Curl, E. A. (1961). Influence of sprinkler irrigation and 4 forage crops on populations of soil microorganisms including those antagonistic to *Sclerotium rolfsii* Sacc. *Plant Disease Reporter*, 45(7): 517-519.
- Curtis, F.D., Lima, G., Vitullo, D., & Cicco, V.D. (2010). Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering antagonistic bacteria through a drip irrigation system. *Crop Protection*, 29: 663–670.
- Dania, V.O., Fadina, O.O., Ayodele, M. & Kumar, P.L. (2014). Efficacy of *Oryza sativa* husk and *Quercus phillyraeoides* extract for the *in vitro* and *in vivo* control of fungal rot disease of white yam (*Dioscorea rotundata* Poir). *Springer plus*, 3:711.
- Darma, R., Purnamasari, I. M., Agustina, D., Pramudito, T. E., Sugiharti, M., & Suwanto, A. (2016). A strong antifungal—Producing bacteria from bamboo powder for biocontrol of *Sclerotium rolfsii* in melon (*Cucumis melo* var. *amanta*). *Journal of Plant Pathology & Microbiology*, 7(2): 334.
- Darvin, G. & Kumari, V.P. (2013). Effect of bio-control agents on radial growth of *Sclerotium rolfsii* *in vitro*. *International Journal of Applied Biology and Pharmaceutical Technology*, 4(4):61-64.
- Darvin, G., Venkatesh, I. & Reddy, G.N. (2013). Evaluation of *Trichoderma* spp. against *Sclerotium rolfsii* *in vitro*. *International journal of applied biology and pharmaceutical technology*, 4(4):268-272.
- Derbalah, A.S., Dewir, Y.H. & El-Sayed, A.E.-N.B. (2012). Antifungal activity of some plant extracts against sugar beet damping-off caused by *Sclerotium rolfsii*. *Annals of Microbiology*, 62:1021–1029.

- Doley, K. & Jite, P.K. (2013). Management of stem rot of Groundnut (*Arachis hypogaea*) cultivar in the field. *Notulae Scientia Biologicae*, 5(3):316-324.
- Dwivedi, R.S. (1970). Evaluation of methods for measuring productivity in wheat (*Triticum aestivum*) and maruel grass (*Dicanthium annulatum*). *Indian Journal of Agriculture Sciences*, 40:81-88.
- Dwivedi, S.K. & Prasad, G. (2016). Integrated management of *Sclerotium rolfsii*: an overview. *European Journal of Biomedical and Pharmaceutical Sciences*, 3(11): 137-146.
- Dwivedi, S.K. & Sangeeta (2015). Efficacy of some medicinal plant extract against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *Asian Journal of Crop Science*, 7 (2): 138-146.
- Eid, K. E. (2014). Biological control of bean damping-off caused by *Sclerotium rolfsii*. *Egypt Journal of Phytopathology*, 42(1), 1-11.
- Ekundayo, E.A., Boboye, B.E. & Adetuyi, F.C. (2015). *In vitro* antifungal efficacies of maize associated microorganisms. *Advances in Microbiology*, 5: 258-268.
- Ekundayo, E.A., Ekundayo, F.O. & Osinowo, I.A. (2015). Antifungal activities of *Trichoderma viride* and two fungicides in controlling diseases caused by *Sclerotium rolfsii* on tomato plants. *Advances in Applied Science Research*, 6(3): 12-19.
- Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection*, 19:709-714.
- Elanchezhian, K., Keerthana, U., Nagendran, K., Prabhukarthikeyan, S. R., Prabakar, K., Raguchander, T. & Karthikeyan, G. (2018). Multifaceted benefits of *Bacillus amyloliquefaciens* strain FBZ24 in the management of wilt disease in

- tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. *Physiological and molecular plant pathology*, 103: 92-101.
- El-Mohamedy, R. S., Jabnoun-Khiareddine, H. & Daami-Remadi, M. (2014). Control of root rot diseases of tomato plants caused by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* using different chemical plant resistance inducers. *Tunisian Journal of Plant Protection*, 9(1): 45-55.
- El-Mougy, N. S., Abdel-Kader, M. M., Aly, M., & Lashin, S. M. (2012). Application of fungicides alternatives as seed treatment for controlling root rot of some vegetables in pot experiments. *Advances in Life Sciences*, 2(3): 57-64.
- Elshahawya, I.E., Haggard, K.H.E. & El-Khaira, H.A. (2016). Compatibility of *Trichoderma* sp. with seven chemical fungicides used in the control of soil-borne plant pathogens. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 7(1):1772.
- Epps, W. M., Patterson, J. C., & Freeman, I. E. (1951). Physiology and parasitism of *Sclerotium rolfsii*. *Phytopathology*, 41(3): 245-256.
- Eziashi, E. I., Omamor, I. B., & Odigie, E. E. (2007). Antagonism of *Trichoderma viride* and effects of extracted water soluble compounds from *Trichoderma* species and benlate solution on *Ceratocystis paradoxa*. *African Journal of Biotechnology*, 6(4):388–392.
- Faruk, M.I. (2019). Management of barley seedling disease caused by *Sclerotium rolfsii* through soil amendment with tricho-compost. *European Journal of Biophysics*, 7(1): 1-7.
- Flados, N. D. (1958). Ecological factors affecting the growth of *Sclerotium-rolfsii*. *In Phytopathology*, 48(6): 342-342.

- Gahukar, R.T. (2018). Management of pests and diseases of important tropical/subtropical medicinal and aromatic plants: A review. *Journal of applied research on medicinal and aromatic plants*, 9: 1-18.
- Ganesan, S., Kuppusamy, R.G., & Sekar, R. (2007). Integrated management of stem rot disease (*Sclerotium rolfsii*) of groundnut (*Arachis hypogaea* L.) using *Rhizobium* and *Trichoderma harzianum* (ITCC-4572). *Turkish Journal of Agriculture and Forestry*, 31(2): 103-108.
- Ghildiyal, A., & Pandey, A. (2008). Isolation of cold tolerant antifungal strains of *Trichoderma* sp. from glacial sites of Indian Himalayan region. *Research Journal of Microbiology*, 3(8): 559-564.
- Gilman, J. C. (2001). A manual of soil fungi, 2<sup>nd</sup> Indian edition. *Biotech Books. New Delhi*.
- Gopalakrishnan, S., Beale, M.H., Ward, J.L. & Strange, R.N.(2005). Chickpea wilt: identification and toxicity of methyl fusarubin from *Fusarium acutatum*. *Phytochemistry*. 66:1536-1539.
- Grantina, L., Seile, E., Kenigvalde, K., Kasparinskis, R., Tabors, G., Nikolajeva, V., Jungerius, P. & Muiznieks, I. (2011). The influence of the land use on abundance and diversity of soil fungi: comparison of conventional and molecular methods of analysis. *Environmental and Experimental Biology*, 9: 9–21.
- Grover, R.K. & Moore, J.D. (1962) Toximetric studies of fungicides against the brown rot organisms, *Sclerotinia fructicola* and *S. laxa*. *Phytopathology*, 52: 876-879.
- Guerra, Y.L., Oliveira, T.A.S., Laranjeira, D., Lima, L.M., Filho, P.A.M. & Santos, R.C. (2015). Control of *Sclerotium rolfsii* in peanut by using *Cymbopogon*

- martinii* essential oil. *African Journal of Microbiology Research*, 9(27): 1684-1691.
- Gupta, C.P., Dubey, R.C., Kang, S.C. & Maheshwari, D.K. (2001). Antibiosis mediated necrotrophic effect of *Pseudomonas* GRC2 against two fungal plant pathogens. *Current Science*, 81:91–94.
- Gupta, R. P., Singh, S. K., Mishra, R. S. & Gupta, S. (2012). Efficacy of botanicals against *Sclerotium rolfsii* causing collar rot of Chick pea. *Annals of Plant Protection Sciences*, 20(2): 486-487.
- Gurjar, M. S., Ali, S., Akhtar, M., & Singh, K. S. (2012). Efficacy of plant extracts in plant disease management. *Agricultural Sciences*, 3(3): 425-433.
- Hagvar, S. (1998). The relevance of the Rio-Convention on biodiversity to conserving the biodiversity of soil. *Applied Soil Ecology*, 9:1-7.
- Han, K.S., Lee, S.C., Lee, J.S., Soh, J.W. & Kim, S. (2012). First report of *Sclerotium* rot on *Cymbidium* orchids caused by *Sclerotium rolfsii* in Korea. *Mycobiology*. 40(4): 263-264.
- HaralPatil, S. K., & Raut, S. P. (2008). Efficacy of fungicides, bio-agents and botanicals against collar rot of betelvine. *Journal of Plant Disease Sciences*, 3(1): 93-96.
- Haran, S., Schikler, H. & Chet, I. (1996a). Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. *Microbiology*, 142:2321-2331.
- Hareesh, V. (Head of Commodity Research) Maize special report June 2016. *Geofin Research Desk, Kochi*.

- Hari, B. V. S. C., Chiranjeevi, V., Sitaramaiah, K. A. & Subrhamaniyam, K. (1988). *In vitro* screening of fungicides against groundnut isolate of *Sclerotium rolfsii* Sacc. by soil vial technique. *Pesticides*, 10: 47-49.
- Hawksworth, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research*, 105(12): 1422-1432.
- Heena, J. P., Bodar, N. P., Bhadja, N. V., Patel, S. V., Kandoliya, U. K., & Golakiya, B. A. (2015). *In vitro* antagonism between biocontrol agent *Trichoderma* and pathogen *Sclerotium rolfsii* causing stem rot in *arachis hypogaea* L. *Journal of Cell and Tissue Research*, 15(1): 4921-4928.
- Hermosa, M.R., Grondona, I., Iturriaga, E.A., Diz-Minguez, J.M., Castro, C., Monte, E. & Garcia-Acha, I. (2000). Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. *Applied Environmental Microbiology*, 66:1890-1898.
- Higgins, B. B. (1927). Physiology and parasitism of *Sclerotium rolfsii* Sacc. *Phytopathology*, 17: 417-448.
- Hirpara, D.G., Gajera, H.P., Hirpara, H.Z. & Golakiya, B.A. (2017). Molecular diversity and fingerprints of *Trichoderma* associated with antagonistic potentials against *Sclerotium rolfsii* Sacc. *Journal of Plant Diseases and Protection*, 124(1):31-40.
- Hirpara, D.G., Gajera, H.P., Patel, A.K., Katakpara, Z.A. & Golakiya, B.A. (2019) Molecular insights into development of *Trichoderma interfusants* for multistress tolerance enhancing antagonism against *Sclerotium rolfsii* Sacc. *Journal of Cell Physiology*, 234:7368–7383.
- Hooda, K.S., Bhatt, J.C., Joshi, D. Sushil, S.N. & Gupta, H.S. (2008). Biocontrol agents vis-à-vis fungicides in managing various diseases of tomato

- (*Licopersicon ulescentum* mill.) in hills of Uttarakhand. *Indian Phytopathology*, 61:331.
- Hoque<sup>1</sup>, M. A., Hamim, I., Haque, M. R., Ali, M.A. & Ashrafuzzaman, M. (2014). Effect of some fungicides on foot and root rot of Lentil. *Universal Journal of Plant Science*, 2(2): 52-56.
- Hosen, S., Shamsi, S. & Bashar, M. (2016). *In vitro* biological control of *Colletotrichum gloeosporioides* (penz.) sacc. and *Sclerotium rolfsii* Sacc., causal agent of anthracnose and soft rot of *Corchorus capsularis* L. *Bangladesh Journal of Botany*, 45(1): 175-180.
- Huang, H.C. & Hoes, J.A. (1976). Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minutans*. *Canadian journal of Botany*, 54: 406-410.
- Islam, M. M., Hossain, D. M., Nonaka, M., & Harada, N. (2017). Biological control of tomato collar rot induced by *Sclerotium rolfsii* using *Trichoderma* species isolated in Bangladesh. *Archives of Phytopathology and Plant Protection*, 50(3-4): 109-116.
- Islam, M.T. & Faruq, A.N. (2012). Effect of some medicinal plant extracts on damping-off disease of winter vegetable. *World Applied Sciences Journal*, 17(11): 1498-1503.
- Jacob, S., Sajjalaguddam, R. R., Kumar, K. V. K., Varshney, R., & Sudini, H. K. (2016). Assessing the prospects of *Streptomyces* sp. RP1A-12 in managing groundnut stem rot disease caused by *Sclerotium rolfsii* Sacc. *Journal of General Plant Pathology*, 82(2): 96-104.

- Jana, S. C., & Malay, M. (2017). Antagonistic effect of *Trichoderma* isolates on *Sclerotium rolfsii*. *Journal of Experimental Biology and Agricultural Sciences*, 5(4): 506-514.
- Jantasorn, A., Moungrimuangdee, B., & Dethoup, T. (2016). *In vitro* antifungal activity evaluation of five plant extracts against five plant pathogenic fungi causing rice and economic crop diseases. *Journal of Biopesticides*, 9(1): 1-7.
- Jegathambigai, V., Wijeratnam, R. W., & Wijesundera, R. L. C. (2010). Effect of *Trichoderma* sp. on *Sclerotium rolfsii*, the causative agent of collar rot on *Zamioculcas zamiifolia* and an on farm method to mass produce *Trichoderma* species. *Plant Pathology Journal*, 9(2): 47-55.
- John, D., Gupta, P., & Gupta, V. (2006). Etiology and management of guava wilt disease in Allahabad and Kaushambi districts of Uttar Pradesh, India. *Journal of Mycology and Plant Pathology*, 36(2): 365-372.
- John, N.S., Anjanadevi, I.P., Nath, V.S., Sankar, S.A., Jeeva, M.L., John, K.S. & Misra, R.S. (2015). Characterization of *Trichoderma* isolates against *Sclerotium rolfsii*, the collar rot pathogen of *Amorphophallus* – A polyphasic approach. *Biological Control* 90: 164–172.
- Johnson, L. F., & Curl, E. A. (1972). Methods for research on the ecology of soil-borne plant pathogens. *Minneapolis, Burgess Publishing Co. University of Michigan*. pp. 247.
- Jothi, L.N. & Reddy, E.C.S. (2015). Biocontrol potential of indigenous fluorescent *Pseudomonas* species against *Sclerotium rolfsii* is causing stem rot of Groundnut. *2th International Conference on Exhibition Biotechnology*, 2015.
- Junsopa, Ch., Jogloy, S., Saksirirat, W., Songsri, P., Kesmala, Th., Shew, B.B., & atanothai, A. (2016). Inoculation with *Sclerotium rolfsii*, cause of stem rot in

- Jerusalem artichoke*, under field conditions. *European Journal of Plant Pathology*, 146:47–58.
- Kapadiya, H.J. & Moradiya, A.M. (2017). Management of groundnut disease through different organic practices and compared with chemical fungicide. *International Journal of Chemical Studies*, 5(6): 690-693.
- Karthikeyan, V., Sankaralingam, A. & Nakkeeran, S. (2006) Biological control of groundnut stem rot caused by *Sclerotium rolfsii* (Sacc.). *Archives of Phytopathology and Plant Protection*, 39(3): 239-246.
- Kator, L., Hosea, Z.Y. & Oche, O.D. (2015). *Sclerotium rolfsii*; a causative organism of southern blight, stem rot, white mold and Sclerotia rot disease. *Annals of biological research*, 6(11):78-89.
- Keyser, H. A., & Ferreira, J. H. S. (1988). Chemical and biological control of *Sclerotium rolfsii* in grapevine nurseries. *South African Journal of Enology and Viticulture*, 9(1): 43-44.
- Khalequzzaman, K.M. (2015). Seed treatment with *Rhizobium* biofertilizer for controlling foot and root rot of Chickpea. *International Journal of Scientific Research in Agricultural Sciences*, 2(6): 144-150.
- Khalequzzaman, K.M. (2016). Control of foot and root rot of lentil by using different management tools. *ABC Journal of Advanced Research*, 5(1): 35-42.
- Khan, I.H. & Javaid, A. (2015). Chemical control of collar rot disease of Chickpea. *Pakistan Journal of Phytopathology*, 27 (01):61-68.
- Khati, D. V. S., Bhargava, S. N., & Shukla, D. N. (1983). Some studies on the saprophytic survival of *Sclerotium rolfsii* in soil. *Journal of Plant Diseases and Protection*, pp. 382-387.

- Koch, R. (1880). Investigations into the etiology of traumatic infective diseases. *New Sydenham Society*, Vol. 88.
- Kotasthane, A., Agrawal, T., Kushwah, R., & Rahatkar, O. V. (2015). *In-vitro* antagonism of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and their response towards growth of cucumber, bottle gourd and bitter gourd. *European Journal of Plant Pathology*, 141(3): 523-543.
- Kumar, B., Chikkappa, G., Karjagi, S. L., Jat, C.M., Yathish, K.R.P., Singh, V., Hooda K. S., Dass, AK, Mukri, G., Sekhar, J.C. Kumar, R. & Kumar, R.S. (2012). Maize biology: An introduction, directorate of Maize research. *Indian Council of Agricultural Research (ICAR), Technical Bulletin. 2: 32.*
- Kumar, D. & Jhariya, A.N. (2013). Nutritional, medicinal and economical importance of Corn: A mini review. *Research Journal of Pharmaceutical Sciences. 2(7): 7-8.*
- Kushwaha, S. K., Kumar, S., & Chaudhary, B. (2018). Efficacy of *Trichoderma* against *Sclerotium rolfsii* causing collar rot disease of lentil under *in vitro* conditions. *Journal of Applied and Natural Science*, 10(1): 307-312.
- Kwon, J.H. & Park, C.S. (2002). Stem rot of tomato caused by *Sclerotium rolfsii* in Korea. *Microbiology*, 30(4):244-246.
- Lal, M., Singh, A. P., Tomar, S., Hussain, T., Sharma, S., Kaushik, S. K., & Singh, B. P. (2013). Antagonistic effect of bioagents against three potato fungal diseases and their fungicidal sensitivity. *Vegetos*, 26: 362-367.
- Latha, P. & Rajeswari, E. (2019). Evaluation of biocontrol agents, fungicides and organic amendments against *Sclerotium* wilt (*Sclerotium rolfsii* Sacc) of

- jasmine (*Jasminum sambac* (L.) Aiton). *Journal of Pharmacognosy and Phytochemistry*, SP2: 897-902.
- Lewis, J.A. & Papavizas, G.C. (1991). Biocontrol of plant diseases: the approach for tomorrow. *Crop Protection*, 10:95-105.
- Lingaraju, S. (1977). Studies on *Sclerotium rolfsii* Sacc. with respect to the survival in soil. *M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore*, pp.87.
- Linskens, H.F. & Haage, P. (1963). Cutinase-Nachweis in phytopathogenen Pilzen. *Journal of phytopathology*, 48 (3): 306-311.
- Madhavi, G.B. & Bhattiprolu, S.L. (2011). Integrated disease management of dry root rot of chilli incited by *Sclerotium rolfsii* (Sacc.). *International Journal of Plant, Animal and Environmental Sciences*. 1(2):31-37.
- Mahadevakumar, S., Yadav, V., Tejaswini, G. S & Janardhana, G. R. (2016). Morphological and molecular characterization of *Sclerotium rolfsii* associated with fruit rot of *Cucurbita maxima*. *European Journal of Plant Pathology*. 145:215–219.
- Mahato, A. & Mondal, B. (2014). *Sclerotium rolfsii*: its isolates variability, pathogenicity and an eco-friendly management option. *Journal of Chemical, Biological and Physical Sciences Sec. B*, 4(4): 3334-3344.
- Mahato, A., Biswas, M.K. & Patra, S. (2017). Eco-friendly management of collar rot disease of tomato caused by *Sclerotium rolfsii* (Sacc.). *International Journal of Pure & Applied Bioscience*, 5(1): 513-520.
- Mahato, A., Biswas, M.K. & Patra, S. (2018). Efficacy of medicinal plant extracts against collar rot of tomato caused by *Sclerotium rolfsii* (Sacc.). *International Journal of Microbiology Research*, 10(5):1224-1227.

- Mahato, A., Mondal, B., Dhakre, D.H. & Khatua, D.C. (2014). *In vitro* sensitivity of *Sclerotium rolfsii* towards some fungicides and botanicals. *Scholars Academic Journal of Biosciences*, 2(7):467-471.
- Majdah, M.Y., Al-Tuwajjri & Aboellil, A.H. (2015). Associated change in metabolic activity of host pathogen system in some infected plants by bio-inducer. *International Journal of Advanced Research in Biological Sciences*, 2(3):86-100.
- Maji, A. & Nath, R. (2016). Management through eco-friendly approach of collar rot disease of sunflower caused by *Sclerotium rolfsii* sacc in sundarbans of West Bengal. *International Journal of Agricultural Science and Research*, 6(4):9-12.
- Mandal, M. & Jana, S.C. (2015). Antagonistic effects of Bacteriocin and Bacteriocinogenic *Lactobacillus* on stem rot causing *Sclerotium rolfsii* in Betel. *International Journal of Bioassays*, 4(11): 4454-4457.
- Manu, T.G., Nagaraja, A., Chetan, Janaward, S. & Hosamani, V. (2012). Efficacy of fungicides and biocontrol agents against *Sclerotium rolfsii* causing foot rot disease of finger millet, under *in vitro* conditions. *Global Journal of Biology, Agriculture & Health Sciences*, 1(2):46-50.
- Maurya, S., Singh, R., Singh, D. P., Singh, H. B., Srivastava, J. S. & Singh, U.P. (2007). Phenolic compounds of *Sorghum vulgare* in response to *Sclerotium rolfsii* infection. *Journal of Plant Interactions*, 2(1): 25–29.
- Maurya, S., Singh, U. P., Singh, D. P., Singh, K. P. & Srivastava, J. S. (2005). Secondary metabolites of Chickpea (*Cicer aritenum*) and their role in pathogenesis after infection by *Sclerotium rolfsii*. *Journal of Plant Diseases and Protection*. 122(2): 118–123.

- Mittal, J., Sharma, M.M. & Batra, A. (2014). *Tinospora cordifolia*: a multipurpose medicinal plant- A review. *Journal of Medicinal Plants Studies*, 2(2):32-47.
- Mohiddin, F. A., & Khan, M. R. (2013). Tolerance of fungal and bacterial biocontrol agents to six pesticides commonly used in the control of soil borne plant pathogens. *African Journal of Agricultural Research*, 8:5272.
- Mukadam, D. S. (1997). The illustrated kingdom of fungi (some selected genera). *Published by Akshar Ganga prakashan, Aurangabad, India*.
- Muthukumar, A. & Venkatesh, A. (2014). Biological inductions of systemic resistance to collar rot of peppermint caused by *Sclerotium rolfsii*. *Acta Physiologiae Plantarum*, 36:1421-1431.
- Muthukumar, A., Nakkeeran, S., Eswaran, A., & Sangeetha, G. (2010). *In vitro* efficacy of bacterial endophytes against the chilli damping-off pathogen *Pythium aphanidermatum*. *Phytopathologia Mediterranea*, 49(2):179–186.
- Nagamma, G. & Nagaraja, A. (2015). Efficacy of biocontrol agents against *Sclerotium rolfsii* causing collar rot disease of Chickpea, under *in vitro* conditions. *International Journal of Plant Protection*, 8(2):222-227.
- Najera, J.F.D., Castellanos, J.S., Hernandez, M.V., Serna, S.A., Gomez, O.G.A., Verduzco, C.V. & Ramos, M.A. (2018). Diagnosis and integrated management of fruit rot in *Cucurbita argyrosperma*, caused by *Sclerotium rolfsii*. *The Plant Pathology Journal*, 34(3): 171-181.
- Nalisa, I., Muskhazli, M. & Farizan, T. (2006). Production of the bioactive compound by *Bacillus subtilis* against *Sclerotium rolfsii*. *Malaysian Journal of Microbiology*, 2(2):19-23.
- Nevarez, L., Vasseur, V., Le Madec, A., Le Bras, M.A., Coroller, L., Leguerinel, I. & Barbier, G. (2009). Physiological traits of *Penicillium glabrum* strain LCP

- 08.5568, a filamentous fungus isolated from bottled aromatized mineral water. *International Journal of Food Microbiology*, 130(3):166-171.
- Nielson, M.N., Sorensen, J., Fels, J. & Pedersen, H.C. (1998). Secondary metabolite and endochitinase dependent antagonism towards plant pathogenic microfungi of *Pseudomonas fluorescens* isolates from sugar beet rhizosphere. *Applied Environmental Microbiology*, 64:563–3569.
- Nugroho, C., Mirnia, E., & Cumagun, C. J. R. (2019). Antifungal activities of sweet basil (*Ocimum basilicum* L.) aqueous extract against *Sclerotium rolfsii*, causal agent of damping-off on tomato seedling. *AGRIVITA Journal of Agricultural Science*, 41(1): 149-157.
- O’Sullivan, D.J. & O’Gara, F. (1992). Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiological Reviews*, 56:662-676.
- Okereke, V.C. & Wokocho, R..C. (2006). Effects of some tropical plant extracts, *Trichoderma harzianum* and captan on the damping-off disease of tomato induced by *Sclerotium rolfsii*. *Agricultural Journal*, 1(2):52-54.
- Okigbo, R.N., Opara, P.U. & Anuagasi, C. L. (2015). Efficacy of extracts of water yam (*Dioscorea alata*) and aerial yam (*Dioscorea bulbifera*) peels in the control of white yam (*Dioscorea rotundata*) rot. *Journal of Agricultural Technology*, 11(8): 1823-1842.
- Oladejo, J.A. & Adetunji, M.O. (2012). Economic analysis of maize (*Zea mays* L.) production in Oyo state of Nigeria. *Agricultural Science Research Journal*, 2(2): 77-83.

- Olsen, S.R. & Dean, L.A. (1965). Methods of soil analysis, Agronomy serial No. 9, part 2. *American Society of Agronomy, Madison. Phosphorus*, in C.A. Black (Ed.), pp. 1035-1049.
- Palakshappa, M. G., Kulkarn, S. & Hegde, R. K. (1986). Studies on foot rot of betel vine caused by *Sclerotium rolfsii* Sacc. *Karnataka. M. Sc.(Agri.) Thesis, University of Agricultural Sciences, Bangalore.*
- Pandey, R.K. (2013). Integrated management of collar rot disease caused by *Sclerotium rolfsii* on lentil crops through biological and chemical treatments. *Journal of Natural Resource and Development*, 8(1): 1-7.
- Paramageetham, Ch. & Babu, G.P. (2012). Antagonistic activity of fluorescent *Pseudomonas* against a polyphagous soil-born plant pathogen – *Sclerotium rolfsii*. *Open Access Scientific Reports*, 1:436.
- Parvin, R., Aminuzzaman, F. M., Islam, M. S., Hasan, N., Begum, K., & Roni, M. Z. K. (2016). *In vitro* efficacy of some fungicides, plant extracts and bio-agents against *Sclerotium rolfsii* and control of foot and root rot disease of betel vine. *International Journal of Business, Social and Scientific Research*, 4(3): 211-220.
- Parvin, R., Islam, M.R., Islam, M.S., Hasan, N., Begum, K. & Roni, M.Z.K. (2016). Comparative efficacy of different fungicides against foot and root rot disease of betel vine caused by *Sclerotium rolfsii*. *Bangladesh Research Publication Journal*, 12(1): 09-15.
- Pastor, N. A., Reynoso, M. M., Tonelli, M. L., Masciarelli, O., Rosas, S. B., & Rovera, M. (2010). Potential biological control *Pseudomonas* sp. PCI2 against damping-off of tomato caused by *Sclerotium rolfsii*. *Journal of Plant Pathology*, 92:737–745.

- Patil, N. N., Waghmode, M. S., Gaikwad, P. S., Gajbhiye, M. H., Gunjal, A. B., Nawani, N. N., & Kapadnis, B. P. (2014). Potential of *Microbispora* sp.V2 as biocontrol agent against *Sclerotium rolfsii*, the causative agent of southern blight of *Zea may* (Baby corn) *in vitro* studies. *Indian Journal of Experimental Biology*, 52:1147-1151.
- Patro, T.S.S.K. & Madhuri, J. (2013). Evaluation of biocontrol agents against foot rot of *Finger millet* caused by *Sclerotium rolfsii*, under *in vitro* conditions. *International Journal of Food, Agriculture and Veterinary Sciences*, 3(3): 30-32.
- Pattanapitpaisal, P. & Kamlandham, R. (2012). Screening of chitinolytic *Actinomycetes* for biological control of *Sclerotium rolfsii* stem rot disease of Chilli. *Songklanakarinn Journal of Science and Technology*, 34(4):387-393.
- Pawar, K. I. R. T. I., Mishra, S. P., & Singh, R. K. (2015). Efficacy of bioagents and fungicides against seed borne fungi of soybean. *Annals of Plant and Soil Research*, 17(1): 77-81.
- Pinheiro, V.D.R., Seixas, C.D.S. Godoy, C.V., Soares, R.M., Oliveira, M.C.N. & Almeida, A.M.R. (2010). Development of *Sclerotium rolfsii*, sclerotia on soybean, corn and wheat straw, under different soil temperature and moisture contents. *Pesquisa Agropecuária Brasileira*, 45(3):332-334.
- Podmaja, M., Narendra, K., Swati, J., Sowjanya, K.M., Babu, P.J. & Satya, A.K. (2013). *In vitro* antagonism of native isolates of *Trichoderma* sp. against *Sclerotium rolfsii*. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 4(3):886-891.
- Poul, C.N., Hwang, E.J., Nam, S.S., Lee, H.U., Lee, J.S., Dan-Yu, G., Kang, Y.G., Lee, K.B., Go, S. & Yang, J.W. (2017). Phylogenetic placement and

- morphological characterization of *Sclerotium rolfsii* (Teleomorph: *Athelia rolfsii*) associated with blight disease of *Ipomoea batatas* in Korea. *Mycobiology*, 45(3): 129–138.
- Prasad, G. & Dwivedi, S. K. (2017). Efficacy of some bioagents against *Sclerotium rolfsii* sacc. causing root-rot in *zea mays* L. crop. *European Journal of Biomedical and Pharmaceutical Sciences*, 4(7): 478-481.
- Priyadharcini, M., Akila, R., Mini, M.L., Rajinimala, N. & Kannan, R. (2018). Exploration of *Trichoderma* spp. as an effective bio control agents against the Sclerotial wilt caused by *Sclerotium rolfsii* Sacc. *International Journal of Current Microbiology and Applied Science*, 7(8): 1672-1682.
- Punja, Z.K. (1985). The biology, ecology and control of *Sclerotium rolfsii*. *Annual review of Phytopathology*, 23:97-127.
- Punja, Z.K., Grogan, R.G. & Unruh, T. (1982). Chemical control of *Sclerotium rolfsii* on golf greens in northern California. *Plant Disease*, 66:108-111.
- Puri, S. (2016). Eco-friendly management strategies for soil borne plant pathogens. *Internal Journal of Advanced Research in Biological Sciences*, 3(1):69-75.
- Pushpker, R. and Saler, R.S. 2011. Use of eco-friendly components in integrated fungal disease management of groundnut C. V. SB11 (*Archis hypogea* L.). *Bionano Frontier*, 4 (2) July - Dec. 2011.
- Quadri, U. & Fatima, S. (2016). Eco-friendly management of *Sclerotium rolfsii* causing root rot disease in chilli. *International Journal of Advanced Research*, 4(5): 672-678.
- Quadri, U. & Fatima, S. (2016). Screening of medicinal plants against *Sclerotium rolfsii* isolated from root rot of Chilli. *International Journal of Innovative Research in Science, Engineering and Technology*, 5(5):8082-8088.

- Radwan, M., Fadel, A.L.B., Mahareeq, I. & Mohammad, I.A.L. (2006). Biological control of *Sclerotium rolfsii* by using indigenous *Trichoderma* spp. isolates from Palestine. *Hebron University Research Journal*, 2(2):27-47.
- Rahman, M., Ali, M.E., Alam, F., Banu, M.B., Faruk, M.I. & Bhuiyan, M.A.H. (2017). Biocontrol of foot and root rot disease of Grasspea (*Lathyrus sativus*) by dual inoculation with *Rhizobium* and Arbuscular Mycorrhiza. *Bangladesh Journal of Microbiology*, 34(2):109-117.
- Rajasekhar, S., Amaravathi, Y., Vijayalakshmi, R.P., Vasanthi, R.P. & Reddy, N.P.E. (2019). Study of plant pathogen interaction in Groundnut challenged with *Sclerotium rolfsii* by Scanning Electron Microscopy. *International Journal of Current Microbiology and Applied Sciences*, 8(5): 1031-1038.
- Rajendraprasad, M., Sagar, B.V., Devi, G.U. & Rao, S.R.K. (2017). Biological control of tomato damping off caused by *Sclerotium rolfsii*. *Journal of Entomology and Zoology Studies*, 5(5): 113-119.
- Rakh, R.R., Rout, L.S., Dalvi, S.M. & Manwar, A.V. (2011). Biological control of *Sclerotium rolfsii*, causing stem rot of Groundnut by *Pseudomonas cf. monteilii*. *9. Recent research in Science and Technology*, 3(3):26-34.
- Ramarao, P., & Raja, U. (1980). Effect of soil moisture on development of foot and root rot of wheat and on other soil microflora. *Indian Journal of Mycology and Plant Pathology*, 10(1): 17-22.
- Ramzan, N., Noreen, N., Perveen, Z., & Shahzad, S. (2016). Effect of seed pelleting with biocontrol agents on growth and colonisation of roots of mungbean by root-infecting fungi. *Journal of the Science of Food and Agriculture*, 96(11): 3694-3700.

- Rangarani, A., Rajan, C.P.D., Harathi, P.N., Bhaskar, B. & Sandhya, Y. (2017). Evaluation of fungicides and herbicides on *Sclerotium rolfsii*, incitant of stem rot diseases in Groundnut (*Arachis hypogea* L.). *International Journal of Pure and Applied Bioscience*, 5(3): 92-97.
- Rangaswami, G. & Bagyaraj, D.J. (1998). Agricultural microbiology II edition published by *prentice hall of India Pvt. Ltd New Delhi*.
- Rangeshwaran, R. & Prasad, R.D. (2000). Biological control of *Sclerotium*-rot of Sunflower. *Indian Phytopathology*, 53 (4): 444-449.
- Rani, A.R. (2017). Stem rot of Groundnut incited by *Sclerotium rolfsii* Sacc. and its management - A review. *International Journal of Agricultural Science and Research*, 7(3):327-338.
- Rani, N. & Hegde, Y.R. (2016). *In vitro* and *In vivo* management of root rot/wilt of fenugreek through biological and chemical methods. *Journal of Pure and Applied Microbiology*, 10(4): 2849-2856.
- Rao, S. N. & Kulkarni, S. (2003). Effect of *Trichoderma* spp. on the growth of *Sclerotium rolfsii* Sacc. *Journal of Biological Control*, 17(2): 181-184.
- Rasu, T., Sevugapperumal, N., Thiruvengadam, R. & Ramasamy, S. (2013). Biological control of sugarbeet root rot caused by *Sclerotium rolfsii*. *International Journal of Biological, Ecological and Environmental Sciences*, 2(1): 7-10.
- Reddy, N.M. & Reddy, R.N. (2015). *Tinospora cordifolia* chemical constituents and medicinal properties: A Review. *Scholars Academic Journal of Pharmacy*, 4(8): 364-369.
- Rekha, D., Patil, M.B., Shetty, P.S., Swamy, K.M. & Gamanagatti, R.B., (2012). *In vitro* screening of native *Trichoderma* isolates against *Sclerotium rolfsii* causing

- collar rot of ground nut. *International Journal of Science and Nature*, 3(1): 117-120.
- Rolfs, P.H. (1892). Tomato blight: some hints. *Bulletin Fla. Agric. Experimentation Station*, p. 18.
- Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R. & Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The International Society for Microbial Ecology Journal*, 4(10):1340-1351.
- Sab, J., Nagaraja, A. & Nagamma, G. (2014). Efficacy of bio-pesticides against *Sclerotium rolfsii* sacc. causing collar rot of Chickpea (*Cicer arietinum* ). *The Bioscan.*, 9(1): 335-339.
- Sacchi, S., Maurya, S. & Choudhary, A.K. (2016). Antifungal efficacy of Garlic and Ginger against *Sclerotium rolfsii*. *International Journal of Agricultural Science and Research*, 6(6):419-424.
- Sahani, S., Sharma, B.K. & Singh, K.P. (2008). Management of *Sclerotium rolfsii* with integration of non-conventional chemical, vermicompost and *Pseudomonas syringae*. *World Journal of microbiological and biotechnology*, 24:517-522.
- Samsuzzaman, M., Islam, S.A.T.M., Hossain, S.K.M.M., Amin, M.H.A. & Kaisher, M.S. (2012). Biological control of collar rot of tomato caused by *Sclerotium rolfsii*. *Bangladesh research publication Journal*, 6(3): 240-247.
- Sarangi, M.K. & soni, S. (2013). A review on Giloy: The magic herb. *Inventi Rapid: Planta Activa*, 2:1-4.
- Sennoi, R., Singkham, N., Jogloy, S., Boonlue, S., Saksirirat, W., Kesmala, T., & Patanothai, A. (2013). Biological control of southern stem rot caused by

- Sclerotium rolfii* using *Trichoderma harzianum* and arbuscular mycorrhizal fungi on *Jerusalem artichoke* (*Helianthus tuberosus* L.). *Crop Protection*, 54: 148-153.
- Shaigan, S., Seraji, A. & Moghaddam, S.A.M. (2008). Identification and investigation on antagonistic effect of *Trichoderma* sp. on Tea seedlings white foot and root rot (*Sclerotium rolfii* Sacc.) *in vitro* condition. *Pakistan Journal of Biological Sciences*, 11: 2346-2350.
- Sharma, R.K. & Dhruj, I.U. (2018). *In vitro* evaluation of some fungicides against Indian isolates of *Sclerotium rolfii* Sacc. [Teleomorph: *Athelia rolfii* (Curzi) Tu & Kimbrough]. *International Journal of Current Microbiology and Applied Sciences*, 7(11): 1561-1586.
- Sharma, V.V., Hyde, K.D. & Vittal, B.P.R. (2001). Frequency of occurrence of mangrove fungi from the east coast of India. *Hydrobiologia*, 455:41-53.
- Shwetha, G. S., & Hegde, Y. R. (2012). Management of *Sclerotium* wilt of *Stevia rebaudiana* through biorationals. *International Journal of Plant Protection*, 5(2), 248-251.
- Shyam, S., & Tiwari, R. K. S. (2018). Efficacy of *Trichoderma harzianum* alone and its integration with fungicides against collar rot caused by *Sclerotium rolfii* in Chickpea. *International Journal of Agriculture Sciences*, 10(9):5945-5947.
- Siddique, M. N. A., Ahmmed, A. N. F., Jahan, N., Mazumder, M. G. H., & Islam, M. R. (2018). Management of foot and root rot disease of Eggplant (*Solanum melongena* L.) caused by *Sclerotium rolfii* under *in vivo* condition. *The Agriculturists*, 16(1): 78-86.
- Siddique, M.N.A., Ahmmed, A.N.F., Mazumder1, M.G.H., Khaiyam, M.O. & Islam, M.R. (2016). Evaluation of some fungicides and bio-agents against *Sclerotium*

- rolfsii* and foot and root rot disease of Eggplant (*Solanum melongena* L.). *The Agriculturists*, 14(1): 92-97.
- Siddiqui, I., Bokhari, N. A., & Perveen, K. (2016). Anti-fungal ability of *Nerium oleander* against *Fusarium oxysporum*, *Sclerotium rolfisii* and *Macrophomina phaseolina*. *The Journal of Animal & Plant Sciences*, 26(1):269-274.
- Sindhu, S.S. & Dadarwal, K.R. (2001). Chitinolytic and cellulolytic *Pseudomonas* species antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* spp. in Chickpea. *Microbiological Research*, 56:662-676.
- Singh, J., Bhatnagar, S. K., & Tomar, A. (2019). Study on fungicidal effect of plant extracts on plant pathogenic fungi and the economy of extract preparation and efficacy in comparison to synthetic/chemical fungicides. *Journal of Applied and Natural Science*, 11(2): 333-337.
- Singh, P.K., Pandey, V, Singh, H. & Shukla, D.N. (2014). Effect of plant leaf extract on fungal diseases of carrot in spore germination. *European Journal of Experimental Biology*, 4(5): 138-142.
- Singh, R. Maurya, S. & Upadhyay, R.S. (2016). The improvement of competitive saprophytic capabilities of *Trichoderma* species through the use of chemical mutagens. *Brazilian Journal of Microbiology*, 47: 10–17.
- Singh, R., Singh, P.P. & Singh, V. (2006). Integrated management of collar rot of *amorphophallus paeoniifolius* blume caused by *Sclerotium rolfisii* Saccardo. *Vegetable Science*, 33(1):45-49.
- Srinivasulu, B., Kumar, K. V. K., Aruna, K., Prasadji, J. K., & Rao, D. V. R. (2005). *In vitro* antagonism of three *Trichoderma* spp. against *Sclerotium rolfisii* Sacc., a collar-rot pathogen in elephant foot yam. *Journal of Biological Control*, 19(2): 167-171.

- Suheri, H., Isnaini, M. & Rohyadi, A. (2014). Application of organic amendment and *Trichoderma* sp. to control basal *Sclerotium rolfsii* on peanut grown on partially degraded land. *Journal of Degraded and Mining Lands Management*, 1(3):131-136.
- Sumi, K., & Ao, N. T. (2015). Integrated management of stem rot of Sunflower (*Helianthus annuus* L.) incited by *Sclerotium rolfsii* Sacc. *International Journal of Bio-Resource & Stress Management*, 6(6).
- Suryawanshi, A.P., Borgaonkar, A.S., Kuldhar, D.P., & Dey, U. (2015). Integrated management of collar rot (*Sclerotium rolfsii*) of brinjal (*Solanum melongena*). *Indian Phytopathology*, 68 (2): 189-195.
- Swathi, B., Patibanda, A.K. & Prasuna, R.P. (2015). Antagonistic efficacy of *Trichoderma* species on *Sclerotium rolfsii* in vitro. *Journal of Agriculture and Veterinary Science*, 8(7):19-22.
- Taj, A. Kumar, N.K. & Kumar, V.B.S. (2014). Fungicidal evaluation and physiological studies of *Sclerotium rolfsii* causing collar root of Capsicum. *Environment & Ecology*, 32 (2A) : 758—761.
- Tang, W., Kuang, J. & Qiang, S. (2015). The pathogenicity of *Sclerotium rolfsii* on *Cyperus dofformis* and its potential host specificity among the genus *Cyperus*. *Journal of Plant Pathology and Microbiology*, S3: 002.
- Tariq, R.R., Razdn, V.K., Tiwari, A.K., Shanaz, E., Bhat, Z.A., Hassan, M.G. & Wani, T.A. (2012). Integrated management of wilt complex disease in bell pepper (*Capsicum annum*). *Journal of agricultural science*, 4(7):141-147.
- Tulipa, De and L.C. De (2019). Disease management in organic agriculture. *International Journal of Recent Scientific Research*, 10(07): 33458-33461.

- Valle, P.G., Luna, L.B., Belmont, R.M., Lopez, C.G. & Jimenez, G.S. (2013). Induction of resistance to *Sclerotium rolfsii* in different varieties of onion by inoculation with *Trichoderma asperellum*. *European Journal of Plant Pathology*, 138:223–229.
- Verma, M., Brar, K. S., Tyagi, R. D., Surampalli, R.Y. & Valero, J.R. (2007). Antagonist fungi, *Trichoderma* species: a panoply of biological control. *Biochemical Engineering Journal*, 37: 1-20.
- Vincent, J. M. (1947). Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, 159: 850.
- Walkley, A. & Black, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 37(1):29-38.
- Wang, Y., Duan, Y.B. & Zhou, M.G. (2015). Baseline sensitivity and efficacy of fluazinam in controlling *Sclerotinia* stem rot of rapeseed. *European Journal of Plant Pathology*, 144:337-343.
- Wavare, S. H., Gade, R. M. & Shitole, A. V. (2017). Effect of plant extracts, bio agents and fungicides against *Sclerotium rolfsii* causing collar rot in Chickpea. *Indian Journal of Pharmaceutical Sciences*, 79(4):513-520.
- Webber, H. J. (1931). The economic importance of apogamy in Citrus and Mangifera. *In Proceedings of the American Society for Horticultural Science*, 28: 57-61.
- Whiting, D., Card, A., Wilson, C. and Reeder, J. (2011). “Estimating soil texture: Sand, Silt or Clayey?” CMG Garden Notes 214. Colorado Master Gardeners. *Colorado State University Extension*. [www.ext.colostate.edu/mg/Gardennotes/214.html](http://www.ext.colostate.edu/mg/Gardennotes/214.html). pp. 214-219.

- Yaqub, F. & Shahzad, S. (2006). Effect of fungicides on *in vitro* growth of *Sclerotium rolfsii*. *Pakistan Journal of Botany*, 38(38):881-883.
- Zaghloul, R. A., Neweigy, N. A., Hanafy, E. A., & Khalifa, N. A. (2008). Effectiveness of bio-control agents against tomato soil borne pathogens. In *Third Environment Conference*, pp. 123-142.
- Zape, A.S., Gade, R.M., Singh, R. & Deshmukh, V.A. (2014). Efficacy of different antagonist against the *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium solani*. *The Bioscan: an International Quarterly Journal of Life Sciences*, 9(4): 1431-1434.



# APPENDIX

## APPENDIX I

---

### RESEARCH PAPER PUBLICATION

- Dwivedi, S.K. and **Prasad, G.** (2016). Integrated management of *Sclerotium rolfsii*: An overview. *European Journal of Biomedical and Pharmaceutical Sciences*. 3(11): 137-146. (ISSN: 2349-8870).
- **Prasad, G.** and Dwivedi, S.K. (2017). Efficacy of Some Bioagents Against *Sclerotium rolfsii* Sacc. Causing Root-Rot in *Zea mays* L. Crop. *European Journal of Biomedical and Pharmaceutical Sciences*. 4(7): 478-481. (ISSN: 2349-8870).
- **Prasad, G.,** Kumar, V. and Dwivedi, S.K. (2018). Antifungal activity of some selected medicinal plants against *Fusarium solani* causing wilt and rot in Pearl millet. *Asian Journal of Bio Science*. **13** (1): 21-27. (ISSN: 0976-8343).
- **Prasad, G.** and Dwivedi, S.K. (2018). Biological and Chemical Management of *Fusarium solani* Causing Wilt in Eggplants. *International Journal of Scientific Research and Review*. 7(4):1914-1925. (ISSN: 2279-0543).
- Deepa Kanaujiya, Vinay Kumar, S. K. Dwivedi and **Ganesh Prasad** (2019). Photobiosynthesis of Silver Nanoparticle Using Extract of *Aspergillus flavus* CR500: Its Characterization, Antifungal Activity and Mechanism against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Journal of Cluster Science*. <https://doi.org/10.1007/s10876-019-01709-2>. (ISSN: 1040-7278)
- **Prasad, Ganesh** and Dwivedi, S.K. (2020). Efficacy of Bioagents, Plant extracts and Chemical fungicides against root-rot disease in *Zea mays* caused by *Sclerotium rolfsii*. *Asia Pacific Journal of Multidisciplinary Research*. 8 (1): 47-55. (ISSN: 2350-7756).

## LIFE MEMBERSHIP

- **Prasad, G.** (2016). The Indian Science Congress Association, Kolkata; Membership No. L30010.

## National and International Conference Publication

- **Prasad, G.** and Dwivedi, S.K. (15-16 February, 2016). “Impact of Soil Pollution on Human Health”. Two-day International seminar on ‘Globalisation, Environment and Social Justice: Perspectives, Issues and Concerns’ organized by the Department of Sociology, BBAU, Lucknow in collaboration with ASEDS, Lucknow. (GESJ-2016):pp. 82-83.
- **Prasad, G.** and Dwivedi, S.K. (3-4 March, 2017). “Efficacy of two medicinal plant extracts against *Sclerotium rolfsii* causing root-rot in *Zea mays*.” Two-day 4<sup>th</sup> Lucknow Science Congress seminar organized by BBAU on ‘Science technology & Innovations for Sustainable development’ (4<sup>th</sup> LUSCON-2017):pp. 32.
- **Prasad, G.** and Dwivedi, S.K. (30-31 August, 2017). National Symposium on “IPRs in Agricultural Research” Jointly organized by Babasaheb Bhimrao Ambedkar University, Lucknow and U.P. Council of Agricultural Research, Lucknow. Participate.
- **Prasad, G.** and Dwivedi, S.K. (15-17 September, 2017). “Antifungal activity of two fungicides against *Sclerotium rolfsii* causing root-rot in *Zea mays*. Three day XXXX All India Botanical conference of ‘The Indian Botanical Society’ & National Symposium organized by Department of Botany, Punjabi university, Patiala on ‘Evaluation and Conservation of Plant Germplasm’. (XXXX IBS-2017):pp143.

- **Prasad, G.** and Dwivedi, S.K. (10-11 January, 2018). “Integrated management of *Sclerotium rolfsii* causing root-rot in *Zea mays* crop.” Two-day 1<sup>st</sup> North Indian Science Congress & International conference organized by BBAU on ‘Science and technology for Sustainable future. (1<sup>st</sup> NISC-2018):pp51 .
- **Prasad, G.** and Dwivedi, S.K. (23<sup>rd</sup> April, 2018). One day Workshop on “Socio-environmental Dimensions in the Rejuvenating River Gomti” Organized by the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow in association with Lokbharti. Participate.
- **Prasad, G.** and Dwivedi, S.K. (10<sup>th</sup> January, 2019). One day seminar on “Environmental sustainability: Present scenario and future aspects” organized by DES, BBAU. Participate.
- **Prasad, G.** and Dwivedi, S.K. (13<sup>th</sup> March, 2019). One day Workshop on “Environmental Hazards of Electronic Waste” Organized by the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow. Participate.

## APPENDIX II

1. Antagonistic activity of some bio-agents against mycelial growth of *Sclerotium rolfsii* on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	47.47619	6	7.912698	7.608047*	1.99E-06	2.218816
Within Groups	80.08333	77	1.040043			
Total	127.5595	83				

\*=Significant at 5% level

2. Antagonistic activity of some bio-agents against mycelial growth of *Sclerotium rolfsii* on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	498.5714	6	83.09524	3.148786*	0.008167	2.218817
Within Groups	2032	77	26.38961			
Total	2530.571	83				

\*=Significant at 5% level

3. Antagonistic activity of some bio-agents against mycelial growth of *Sclerotium rolfsii* on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	3919.31	6	653.2183	6.499684*	1.41E-05	2.218817
Within Groups	7738.5	77	100.5			
Total	11657.81	83				

\*=Significant at 5% level

4. Treatment of mycelial growth of *Sclerotium rolfsii* with Myclobutanil 10% WP at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	1581.267	4	395.3167	577.2323*	3.39E-44	2.539689
Within Groups	37.66667	55	0.684848			
Total	1618.933	59				

\*=Significant at 5% level

5. Treatment of mycelial growth of *Sclerotium rolfsii* with Myclobutanil 10% WP at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	15155.17	4	3788.792	1413.568*	1.01E-54	2.539689
Within Groups	147.4167	55	2.680303			
Total	15302.58	59				

\*=Significant at 5% level

6. Treatment of mycelial growth of *Sclerotium rolfsii* with Myclobutanil 10% WP at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	35815.27	4	8953.817	5202.041*	3.39E-70	2.539689
Within Groups	94.66667	55	1.721212			
Total	35909.93	59				

\*=Significant at 5% level

7. Treatment of mycelial growth of *Sclerotium rolfsii* with Conika 50% WP at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	1104.233	4	276.0583	366.5966*	6.13E-39	2.539689
Within Groups	41.41667	55	0.75303			
Total	1145.65	59				

\*=Significant at 5% level

8. Treatment of mycelial growth of *Sclerotium rolfsii* with Conika 50% WP at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	17952.17	4	4488.042	2514.522*	1.51E-61	2.539689
Within Groups	98.16667	55	1.784848			
Total	18050.33	59				

\*=Significant at 5% level

9. Treatment of mycelial growth of *Sclerotium rolfsii* with Conika 50% WP at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	46100.9	4	11525.23	19355.34*	7.28E-86	2.539689
Within Groups	32.75	55	0.595455			
Total	46133.65	59				

\*=Significant at 5% level

10. Treatment of mycelial growth of *Sclerotium rolfsii* with Carbendazim 50% WP at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	3259.333	4	814.8333	1532.165*	1.13E-55	2.539689
Within Groups	29.25	55	0.531818			
Total	3288.583	59				

\*=Significant at 5% level

11. Treatment of mycelial growth of *Sclerotium rolfisii* with Carbendazim 50% WP at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	25065.73	4	6266.433	13255.92*	2.39E-81	2.539689
Within Groups	26	55	0.472727			
Total	25091.73	59				

\*=Significant at 5% level

12. Treatment of mycelial growth of *Sclerotium rolfisii* with Carbendazim 50% WP at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	64257.4	4	16064.35	30120.66*	3.83E-91	2.539689
Within Groups	29.33333	55	0.533333			
Total	64286.73	59				

\*=Significant at 5% level

13. Treatment of mycelial growth of *Sclerotium rolfisii* with *Azadirachta indica* leaf extract at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	3272.1	4	818.025	851.5718*	9.53E-49	2.539689
Within Groups	52.83333	55	0.960606			
Total	3324.933	59				

\*=Significant at 5% level

14. Treatment of mycelial growth of *Sclerotium rolfisii* with *Azadirachta indica* leaf extract at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	25980.77	4	6495.192	3676.524*	4.6E-66	2.539689
Within Groups	97.16667	55	1.766667			
Total	26077.93	59				

\*=Significant at 5% level

15. Treatment of mycelial growth of *Sclerotium rolfisii* with *Azadirachta indica* leaf extract at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F- calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	45340.77	4	11335.19	11369.65*	1.62E-79	2.539689
Within Groups	54.83333	55	0.99697			
Total	45395.6	59				

\*=Significant at 5% level

16. Treatment of mycelial growth of *Sclerotium rolfsii* with *Ocimum tenuiflorum* leaf extract at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	6837.433	4	1709.358	2321.351*	1.34057E-60	2.539689
Within Groups	40.5	55	0.736364			
Total	6877.933	59				

\*=Significant at 5% level

17. Treatment of mycelial growth of *Sclerotium rolfsii* with *Ocimum tenuiflorum* leaf extract at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	39754.17	4	9938.542	6632.394*	4.33E-3	2.539689
Within Groups	82.41667	55	1.498485			
Total	39836.58	59				

\*=Significant at 5% level

18. Treatment of mycelial growth of *Sclerotium rolfsii* with *Ocimum tenuiflorum* leaf extract at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	65640.23	4	16410.06	13939.05*	6.02E-82	2.539689
Within Groups	64.75	55	1.177273			
Total	65704.98	59				

\*=Significant at 5% level

19. Treatment of mycelial growth of *Sclerotium rolfsii* with *Syzygium aromaticum* oil at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	8089.6	4	2022.4	667.7259*	6.76E-46	2.539689
Within Groups	166.5833	55	3.028788			
Total	8256.183	59				

\*=Significant at 5% level

20. Treatment of mycelial growth of *Sclerotium rolfsii* with *Syzygium aromaticum* oil at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	48027.1	4	12006.78	5652.262*	3.48E-71	2.539689
Within Groups	116.8333	55	2.124242			
Total	48143.93	59				

\*=Significant at 5% level

21. Treatment of mycelial growth of *Sclerotium rolfisii* with *Syzygium aromaticum* oil at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	91181.73	4	22795.43	14047.61*	4.86E-82	2.539689
Within Groups	89.25	55	1.622727			
Total	91270.98	59				

\*=Significant at 5% level

22. Treatment of mycelial growth of *Sclerotium rolfisii* with *Tinospora cordifolia* extract at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	14228.67	4	3557.167	11797.64*	5.88E-80	2.539689
Within Groups	16.58333	55	0.301515			
Total	14245.25	59				

\*=Significant at 5% level

23. Treatment of mycelial growth of *Sclerotium rolfisii* with *Tinospora cordifolia* extract at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	44228.23	4	11057.06	6435.325*	9.91E-73	2.539689
Within Groups	94.5	55	1.718182			
Total	44322.73	59				

\*=Significant at 5% level

24. Treatment of mycelial growth of *Sclerotium rolfisii* with *Tinospora cordifolia* extract at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	57320.27	4	14330.07	73889.41*	7.4E-102	2.539689
Within Groups	10.66667	55	0.193939			
Total	57330.93	59				

\*=Significant at 5% level

25. Treatment of mycelial growth of *Sclerotium rolfisii* with *Trachyspermum ammi* seeds powder at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	9249.667	4	2312.417	2465.582*	2.58E-61	2.539689
Within Groups	51.58333	55	0.937879			
Total	9301.25	59				

\*=Significant at 5% level

26. Treatment of mycelial growth of *Sclerotium rolfsii* with *Trachyspermum ammi* seeds powder at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	47183.07	4	11795.77	3687.923*	4.22E-66	2.539689
Within Groups	175.9167	55	3.198485			
Total	47358.98	59				

\*=Significant at 5% level

27. Treatment of mycelial growth of *Sclerotium rolfsii* with *Trachyspermum ammi* seeds powder at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	83832.07	4	20958.02	6278.843*	1.95E-72	2.539689
Within Groups	183.5833	55	3.337879			
Total	84015.65	59				

\*=Significant at 5% level

28. Treatment of mycelial growth of *Sclerotium rolfsii* with *A. flavus* metabolite at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	5784.6	4	1446.15	326.534*	1.3E-37	2.539689
Within Groups	243.5833	55	4.428788			
Total	6028.183	59				

\*=Significant at 5% level

29. Treatment of mycelial growth of *Sclerotium rolfsii* with *A. flavus* metabolite at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	9733.433	4	2433.358	2501.583*	1.74E-61	2.539689
Within Groups	53.5	55	0.972727			
Total	9786.933	59				

\*=Significant at 5% level

30. Treatment of mycelial growth of *Sclerotium rolfsii* with *A. flavus* metabolite at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	30924.93	4	7731.233	10719.78*	8.17E-79	2.539689
Within Groups	39.66667	55	0.721212			
Total	30964.6	59				

\*=Significant at 5% level

31. Treatment of mycelial growth of *Sclerotium rolfsii* with *A. niger* metabolite at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	8039.067	4	2009.767	453.1759*	2.19E-41	2.539689
Within Groups	243.9167	55	4.434848			
Total	8282.983	59				

\*=Significant at 5% level

32. Treatment of mycelial growth of *Sclerotium rolfsii* with *A. niger* metabolite at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	13228.93	4	3307.233	4409.644*	3.15E-68	2.539689
Within Groups	41.25	55	0.75			
Total	13270.18	59				

\*=Significant at 5% level

33. Treatment of mycelial growth of *Sclerotium rolfsii* with *A. niger* metabolite at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	39751.43	4	9937.858	7436.493*	1.87E-74	2.539689
Within Groups	73.5	55	1.336364			
Total	39824.93	59				

\*=Significant at 5% level

34. Treatment of mycelial growth of *Sclerotium rolfsii* with *T. viride* metabolite at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	7897.767	4	1974.442	379.4792*	2.46E-39	2.539689
Within Groups	286.1667	55	5.20303			
Total	8183.933	59				

\*=Significant at 5% level

35. Treatment of mycelial growth of *Sclerotium rolfsii* with *T. viride* metabolite at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	12778.67	4	3194.667	1902.96*	3.05E-58	2.539689
Within Groups	92.33333	55	1.678788			
Total	12871	59				

\*=Significant at 5% level

36. Treatment of mycelial growth of *Sclerotium rolfii* with *T. viride* metabolite at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	37363.77	4	9340.942	9484.648*	2.36E-77	2.539689
Within Groups	54.16667	55	0.984848			
Total	37417.93	59				

\*=Significant at 5% level

37. Treatment of mycelial growth of *Sclerotium rolfii* with *P.citrinum* metabolite at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	7863.9	4	1965.975	1570.876*	5.71E-56	2.539689
Within Groups	68.83333	55	1.251515			
Total	7932.733	59				

\*=Significant at 5% level

38. Treatment of mycelial growth of *Sclerotium rolfii* with *P.citrinum* metabolite at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	28982.57	4	7245.642	5659.318*	3.37E-71	2.539689
Within Groups	70.41667	55	1.280303			
Total	29052.98	59				

\*=Significant at 5% level

39. Treatment of mycelial growth of *Sclerotium rolfii* with *P.citrinum* metabolite at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	57314.17	4	14328.54	9049.605*	8.55E-77	2.539689
Within Groups	87.08333	55	1.583333			
Total	57401.25	59				

\*=Significant at 5% level

40. Treatment of mycelial growth of *Sclerotium rolfii* with *P.italicum* metabolite at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	7882.9	4	1970.725	1582.334*	4.68E-56	2.539689
Within Groups	68.5	55	1.245455			
Total	7951.4	59				

\*=Significant at 5% level

41. Treatment of mycelial growth of *Sclerotium rolfsii* with *P.italicum* metabolite at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	26842.9	4	6710.725	4642.64*	7.68E-69	2.539689
Within Groups	79.5	55	1.445455			
Total	26922.4	59				

\*=Significant at 5% level

42. Treatment of mycelial growth of *Sclerotium rolfsii* with *P.italicum* metabolite at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	52754.17	4	13188.54	7542.84*	1.27E-74	2.539689
Within Groups	96.16667	55	1.748485			
Total	52850.33	59				

\*=Significant at 5% level

43. Treatment of mycelial growth of *Sclerotium rolfsii* with *Penicillium* sp. metabolite at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	3112.767	4	778.1917	529.4912*	3.43E-43	2.539689
Within Groups	80.83333	55	1.469697			
Total	3193.6	59				

\*=Significant at 5% level

44. Treatment of mycelial growth of *Sclerotium rolfsii* with *Penicillium* sp. metabolite at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	15930.77	4	3982.692	3660.97*	5.16E-66	2.539689
Within Groups	59.83333	55	1.087879			
Total	15990.6	59				

\*=Significant at 5% level

45. Treatment of mycelial growth of *Sclerotium rolfsii* with *Penicillium* sp. metabolite at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	33116.4	4	8279.1	6324.313*	1.6E-72	2.539689
Within Groups	72	55	1.309091			
Total	33188.4	59				

\*=Significant at 5% level

46. Integrated management of mycelial growth of *Sclerotium rolfsii* with *Trichoderma viride* +Myclobutanil 10% WP at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	5740.267	4	1435.067	2045.667*	4.24E-59	2.539689
Within Groups	38.58333	55	0.701515			
Total	5778.85	59				

\*=Significant at 5% level

47. Integrated management of mycelial growth of *Sclerotium rolfsii* with *Trichoderma viride* +Myclobutanil 10% WP at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	28604.67	4	7151.167	15176.11*	5.82E-83	2.539689
Within Groups	25.91667	55	0.471212			
Total	28630.58	59				

\*=Significant at 5% level

48. Integrated management of mycelial growth of *Sclerotium rolfsii* with *Trichoderma viride* +Myclobutanil 10% WP at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	58838.27	4	14709.57	37339.67*	1.04E-93	2.539689
Within Groups	21.66667	55	0.393939			
Total	58859.93	59				

\*=Significant at 5% level

49. Integrated management of mycelial growth of *Sclerotium rolfsii* with *Ocimum tenuiflorum* +Myclobutanil 10% WP at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	12513.27	4	3128.317	9095.546*	7.44E-77	2.539689
Within Groups	18.91667	55	0.343939			
Total	12532.18	59				

\*=Significant at 5% level

50. Integrated management of mycelial growth of *Sclerotium rolfsii* with *Ocimum tenuiflorum* +Myclobutanil 10% WP at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	46458.57	4	11614.64	31676.3*	9.61E-92	2.539689
Within Groups	20.16667	55	0.366667			
Total	46478.73	59				

\*=Significant at 5% level

51. Integrated management of mycelial growth of *Sclerotium rolfisii* with *Ocimum tenuiflorum* +Myclobutanil 10% WP at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	84280.27	4	21070.07	73969.38*	7.2E-102	2.539689
Within Groups	15.66667	55	0.284848			
Total	84295.93	59				

\*=Significant at 5% level

52. Integrated management of mycelial growth of *Sclerotium rolfisii* with *Ocimum tenuiflorum* + *Trichoderma viride* at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	20150.6	4	5037.65	15757.58*	2.07E-83	2.539689
Within Groups	17.58333	55	0.319697			
Total	20168.18	59				

\*=Significant at 5% level

53. Integrated management of mycelial growth of *Sclerotium rolfisii* with *Ocimum tenuiflorum* + *Trichoderma viride* at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	69460.93	4	17365.23	35049.09*	5.95E-93	2.539689
Within Groups	27.25	55	0.495455			
Total	69488.18	59				

\*=Significant at 5% level

54. Integrated management of mycelial growth of *Sclerotium rolfisii* with *Ocimum tenuiflorum* + *Trichoderma viride* at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	100816.8	4	25204.19	93453.74*	1.2E-104	2.539689
Within Groups	14.83333	55	0.269697			
Total	100831.6	59				

\*=Significant at 5% level

55. Integrated management of mycelial growth of *Sclerotium rolfisii* with *Ocimum tenuiflorum* + *Trichoderma viride* + Myclobutanil 10% WP at different concentration on 1<sup>st</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	6583.433	4	1645.858	2080.97*	2.66E-59	2.539689
Within Groups	43.5	55	0.790909			
Total	6626.933	59				

\*=Significant at 5% level

56. Integrated management of mycelial growth of *Sclerotium rolfsii* with *Ocimum tenuiflorum* + *Trichoderma viride* + Myclobutanil 10%WP at different concentration on 2<sup>nd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	28070.27	4	7017.567	8788.603*	1.91E-76	2.539689
Within Groups	43.91667	55	0.798485			
Total	28114.18	59				

\*=Significant at 5% level

57. Integrated management of mycelial growth of *Sclerotium rolfsii* with *Ocimum tenuiflorum* + *Trichoderma viride* + Myclobutanil 10%WP at different concentration on 3<sup>rd</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	51851.43	4	12962.86	9443.142*	2.66E-77	2.539689
Within Groups	75.5	55	1.372727			
Total	51926.93	59				

\*=Significant at 5% level

58. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant without treatment (control) on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	6826.25	3	2275.417	1092.2*	8.65E-11	4.066181
Within Groups	16.666667	8	2.083333			
Total	6842.9167	11				

\*=Significant at 5% level

59. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Ocimum tenuiflorum* leaf extract at 1% V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	0	3	0	65535*	0	4.066181
Within Groups	0	8	0			
Total	0	11				

\*=Significant at 5% level

60. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Ocimum tenuiflorum* leaf extract at 2% V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	7214	3	2404.667	1154.24*	6.94E-11	4.066181
Within Groups	16.666667	8	2.083333			
Total	7230.6667	11				

\*=Significant at 5% level

61. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Ocimum tenuiflorum* leaf extract at 3% V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	9264.9167	3	3088.306	617.6611*	8.39E-10	4.066181
Within Groups	40	8	5			
Total	9304.9167	11				

\*=Significant at 5% level

62. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride* at 1% W/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	0	3	0	65535*	0	4.066181
Within Groups	0	8	0			
Total	0	11				

\*=Significant at 5% level

63. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride* at 2% W/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	7375.333	3	2458.444	737.5333*	4.14E-10	4.066181
Within Groups	26.66667	8	3.333333			
Total	7402	11				

\*=Significant at 5% level

64. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride* at 4% W/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	9726.917	3	3242.306	432.3074*	3.47E-09	4.066181
Within Groups	60	8	7.5			
Total	9786.917	11				

\*=Significant at 5% level

65. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with Myclobutanil 10% WP at 200ppm V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	0	3	0	65535*	0	4.066181
Within Groups	0	8	0			
Total	0	11				

\*=Significant at 5% level

66. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with Myclobutanil 10% WP at 600 ppm V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	6665.583	3	2221.861	701.6404*	5.05E-10	4.066181
Within Groups	25.33333	8	3.166667			
Total	6690.917	11				

\*=Significant at 5% level

67. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with Myclobutanil 10% WP at 1000 ppm V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	6569.667	3	2189.889	821.2083*	2.7E-10	4.066181
Within Groups	21.33333	8	2.666667			
Total	6591	11				

\*=Significant at 5% level

68. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant without treatment (control) on 50<sup>th</sup> day Integrated control

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	7532.25	3	2510.75	654.9783*	6.64E-10	4.066181
Within Groups	30.66667	8	3.833333			
Total	7562.917	11				

\*=Significant at 5% level

69. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride*+Myclobutanil 10% WP at 1% W/W+200 ppm V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	6623.583	3	2207.861	1019.013*	1.14E-10	4.066181
Within Groups	17.33333	8	2.166667			
Total	6640.917	11				

\*=Significant at 5% level

70. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride*+Myclobutanil 10% WP at 2% W/W+600 ppm V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	13847.58	3	4615.861	2769.517*	2.11E-12	4.066181
Within Groups	13.33333	8	1.666667			
Total	13860.92	11				

\*=Significant at 5% level

71. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride*+Myclobutanil 10% WP at 4% W/W+1000 ppm V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	15371.58	3	5123.861	1499.667*	2.44E-11	4.066181
Within Groups	27.33333	8	3.416667			
Total	15398.92	11				

\*=Significant at 5% level

72. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Ocimum tenuiflorum* + Myclobutanil 10% WP at 1% V/W+200 ppm V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	6833.583	3	2277.861	719.3246*	4.57E-10	4.066181
Within Groups	25.33333	8	3.166667			
Total	6858.917	11				

\*=Significant at 5% level

73. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Ocimum tenuiflorum* + Myclobutanil 10% WP at 2% V/W+600 ppm V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	12598.33	3	4199.444	969.1026*	1.39E-10	4.066181
Within Groups	34.66667	8	4.333333			
Total	12633	11				

\*=Significant at 5% level

74. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Ocimum tenuiflorum* + Myclobutanil 10% WP at 3% V/W+1000 ppm V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	17122.92	3	5707.639	951.2731*	1.5E-10	4.066181
Within Groups	48	8	6			
Total	17170.92	11				

\*=Significant at 5% level

75. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride*+*Ocimum tenuiflorum* at 1% W/W+1% V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	6797.667	3	2265.889	1132.944*	7.48E-11	4.066181
Within Groups	16	8	2			
Total	6813.667	11				

\*=Significant at 5% level

76. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride*+*Ocimum tenuiflorum* at 2% W/W+2% V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	12654.92	3	4218.306	2109.153*	6.25E-12	4.066181
Within Groups	16	8	2			
Total	12670.92	11				

\*=Significant at 5% level

77. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride*+*Ocimum tenuiflorum* at 4% W/W+3% V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	14486.92	3	4828.972	1207.243*	5.8E-11	4.066181
Within Groups	32	8	4			
Total	14518.92	11				

\*=Significant at 5% level

78. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride*+Myclobutanil 10% WP+*Ocimum tenuiflorum* at 1% W/W+200 ppm V/W+1% V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	6955	3	2318.333	479.6552*	2.29E-09	4.066181
Within Groups	38.66667	8	4.833333			
Total	6993.667	11				

\*=Significant at 5% level

79. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride*+Myclobutanil 10% WP+*Ocimum tenuiflorum* at 2% W/W+600 ppm V/W+2% V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	13705.67	3	4568.556	1827.422*	1.11E-11	4.066181
Within Groups	20	8	2.5			
Total	13725.67	11				

\*=Significant at 5% level

80. Shoot length, root length, fresh weight, and dry weight of *Zea mays* plant treated with *Trichoderma viride*+Myclobutanil 10% WP+*Ocimum tenuiflorum* at 4% W/W+1000 ppm V/W+3% V/W on 50<sup>th</sup> day

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F-calculated</i>	<i>P-value</i>	<i>F-tabular (5%)</i>
Between Groups	15877.67	3	5292.556	1411.348*	3.11E-11	4.066181
Within Groups	30	8	3.75			
Total	15907.67	11				

\*=Significant at 5% level



**EFFICACY OF SOME BIOAGENTS AGAINST *SCLEROTIUM ROLFSII* SACC.  
CAUSING ROOT-ROT IN *ZEA MAYS* L. CROP**

Ganesh Prasad and S. K. Dwivedi\*

Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow- 226025.

\*Corresponding Author: Dr. S. K. Dwivedi

Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow- 226025.

Article Received on 11/05/2017

Article Revised on 01/06/2017

Article Accepted on 22/06/2017

**ABSTRACT**

*Sclerotium rolfsii* is a saprotroph fungus with living plants. *Sclerotium rolfsii* is a soil-borne fungus which causes root-rot in *Zea mays* crop. *Zea mays* is a staple food in many regions of the world. It is a good nutritional crop which contains proteins, carbohydrates, fats and vitamins (B-complex, A, C & K). It maintains various organs of the body like hair, skin, digestive system, heart, liver, kidney and brain. *Zea mays* contain selenium element that helps proper function of the thyroid gland which maintains immune system of human body. *Sclerotium rolfsii* was isolated from infected maize from Barabanki district of Uttar-Pradesh. *Penicillium* spp., *Trichoderma viride*, *Aspergillus niger* and *Aspergillus flavus* were isolated from rhizospheric soil of maize. Bio-agents *Penicillium* sp. (30.20%, 35.82%, 44.22%), *Trichoderma viride* (3.21%, 23.48%, 26%), *Aspergillus niger* (22.43%, 40.98%, 49.02%) and *Aspergillus flavus* (25.18%, 27.96%, 34.39%) on 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> day inhibited the mycelial growth of *Sclerotium rolfsii* using under dual culture technique. *Penicillium* sp. and *A. niger* were more effective antagonists than *Trichoderma viride* and *Aspergillus flavus* against mycelial growth of *S. rolfsii* under *in vitro* condition.

**KEYWORD:** *Zea mays*, *Sclerotium rolfsii*, *Penicillium* spp., *Trichoderma viride*, *Aspergillus niger* and *Aspergillus flavus*.

**1. INTRODUCTION**

*Zea mays* is commonly called Makka, which is facultative short-day and large grain plant. It is mostly 2 to 3 meters in height, internodes occur every 15 to 20 centimeters, each leaf grows 70 to 100 cm in height every one internode. Male flower and female inflorescence are separate in the same plant. Maize is a C<sub>4</sub> plants grow in the temperate zone. *Zea mays* are a fastener food in many countries of the world. It is high-quality nutritional crop which includes proteins, carbohydrates, fats and vitamins (B-complex, A, C & K). It sustains various organs of the body like hair, skin, digestive system, heart, liver, kidney and brain. *Zea mays* restrain selenium element that helps proper function of the thyroid gland which maintains immune system of human body. *Zea mays* productivity inhibited through bacteria, pest and fungal diseases. The fungal diseases like root-rot caused by *Sclerotium rolfsii* at the lower portion of *Zea mays* crop. *Sclerotium rolfsii* is a saprotroph fungus with living plants. *Sclerotium rolfsii* firstly attacks host plants like corn at the soil line and rapidly moves to the root as it destroys plant tissues with oxalic acid and pectolytic enzymes. *Sclerotium rolfsii* is a soil-borne which occurs worldwide and infects more than 500 plant species.<sup>[1,12]</sup> *Sclerotium rolfsii* grow at 70% soil moisture of field capacity and at a temperature range between 25<sup>0</sup>C to 30<sup>0</sup>C.<sup>[11]</sup> In recent year, farmers

have used chemical fungicides in the field against soil-borne fungus which is more advantageous but the chemical fungicides are more dangerous for environmental pollution and threaten human being. Biological control of the pathogen neither creates environmental pollution nor human health. Use of fungal antagonists like *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viride* and *Penicillium* sp. are bio-control agents against several phytopathogens.

Nawar (2013)<sup>[9]</sup> has reported that *Aspergillus niger* and *Penicillium* sp. were most effective and inhibited the radial growth of *Sclerotium rolfsii* under *in vitro* condition. Parmar et al. (2015)<sup>[10]</sup> have concluded that *S. rolfsii* is a phytopathogenic fungus which infects crops and was controlled by *T. harzianum*, *T. hamantum*, *T. virens*, *T. viride*, *T. koningii*, *T. pseudokoningii* but *T. viride* (61%) inhibited maximum growth of *S. rolfsii* under *in vitro* condition.

**2. MATERIALS AND METHODS**

**2.1 Sample collection**

The samples were collected from infected agriculture field of maize crop from district Barabanki. The collection of infected maize crop of the rhizospheric region and non-rhizospheric were carried out in sterilized

polythene bags. The collected samples (infected part of crops and soil) were preserved for further studies.

### 2.2 Isolation and identification of pathogen

Infected maize plant parts collected from the agriculture field of district Barabanki were cut into small pieces of about 2-4 mm length and sterilized with 0.5% mercuric chloride solution for 30 seconds and washed in sterilized distilled water and dried at room temperature. The sections were placed onto potato dextrose agar plates and incubated at  $27 \pm 1^\circ\text{C}$  for 7 days. After 7<sup>th</sup> day full extend mycelia of *Sclerotium rolfsii* on PDA plate was observed and pure culture was maintained on PDA slant. The isolated *Sclerotium rolfsii* was identified on the basis of morphology and available literature.

### 2.3 Isolation and identification of antagonistic mycoflora

Antagonistic mycoflora have been isolated from rhizospheric soil of infected agriculture field of maize using serial dilution technique. The isolated antagonistic soil mycoflora were maintained on Potato dextrose agar Petriplate. Isolated antagonistic mycoflora were identified on the basis of morphology and available literature.

### 2.4 Dual culture technique

The isolated four antagonistic mycoflora viz. *Trichoderma viride*, *Aspergillus flavus*, *A. niger* and *Penicillium* sp., against the radial growth of *S. rolfsii* using dual culture technique under *in vitro* condition was studied. In dual culture technique, the PDA medium was prepared with standard protocol and autoclaved at  $121^\circ\text{C}$

on 15 lb for 15 minutes. After autoclaving PDA, cooled down PDA was poured in sterilized Petriplates. Five mm diameter block of *S. rolfsii* was inoculated one side and antagonist on opposite side of the same Petriplate as a treatment and one block of *S. rolfsii* was inoculated in the center of another Petriplate as control. The treatment and control plates were incubated at  $26 \pm 1^\circ\text{C}$  temperatures in an incubator. All treatments and control plates were set in triplicates. After incubation, on 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day the mycelial growth of *S. rolfsii* with control plate and mycelial growth of *S. rolfsii* with treatment plate was measured. The percent inhibition of *S. rolfsii* was calculated using following formula.

$$\text{Percent Inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

### 2.5 Data analysis

The data were analyzed statistically.

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation and identification of pathogen

*Sclerotium rolfsii* was isolated from infected maize plant on potato dextrose agar Petriplate within seven days after inoculation. The isolated pathogen produced cottony white mycelia on potato dextrose agar plate. After five days white mycelia changed into light brown and after seven days light brown mycelia changed into dark brown and become hard; and mustard seed like "sclerotia" are formed which is a characteristic feature of *S. rolfsii*.<sup>[4]</sup> *Sclerotium rolfsii* was identified on the basis of available literature.

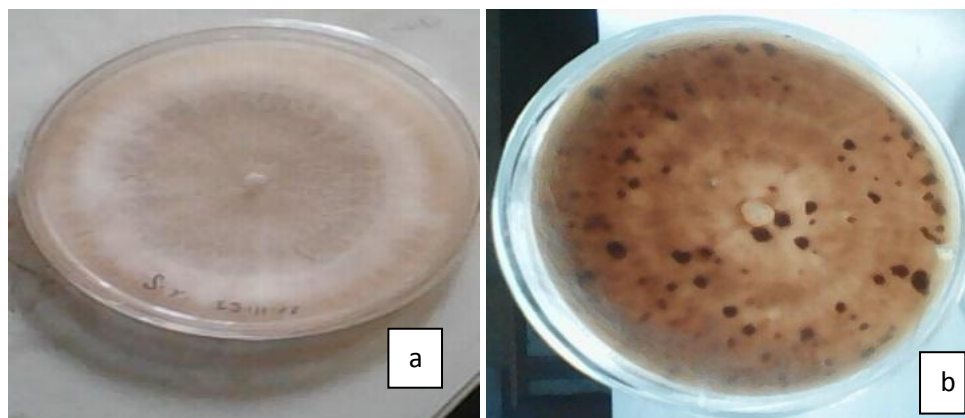


Fig. 1: a. Pure culture of *Sclerotium rolfsii* b. After 7<sup>th</sup> day old *Sclerotium rolfsii* formed sclerotia.

### 3.2 Isolation and identification of antagonistic mycoflora

The antagonistic mycoflora were isolated from various collected soil samples using serial dilution technique. These were isolated and identified as *Trichoderma viride*, *Aspergillus flavus*, *A. niger*, *Penicillium* sp.

### 3.3 Dual culture technique

The isolated four fungal species viz. *Trichoderma viride*, *Aspergillus flavus*, *A. niger* and *Penicillium* sp. were tested under dual culture method to find out their

antagonistic activity against *S. rolfsii* *in vitro*. It was observed that among the test fungi, *Penicillium* sp. and *Aspergillus niger* were most effective bioagent. *Penicillium* sp. inhibited the growth of pathogen by overgrowing the mycelium of *S. rolfsii*. It showed that *Penicillium* sp. has antagonistic effect against *S. rolfsii* and percentage inhibition was recorded as 30.20%, 35.82%, 44.22% at 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> day respectively (Table 1). In case of *A. niger* the percentage inhibition was found to be 22.43%, 40.98%, 49.03% (Table 1) followed by *T. viride* 3.21%, 23.48%, 26% (Table 1) and *A. flavus*

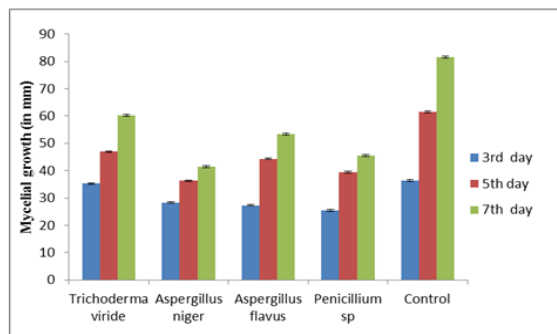
25.18%, 27.96%, 34.39% (Table 1) at 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day. The present *in vitro* study the *A. niger* and *Penicillium* sp. is more effective bioagent compared to another bioagent against *S. rolfisii*. *Trichoderma viride* was most effective inhibitor of the mycelial growth of *S. rolfisii* using dual culture technique. Karthikeyan et al. (2006)<sup>[8]</sup>; Saigan et al. (2008)<sup>[13]</sup>; Bosah et al. (2010)<sup>[3]</sup> reported that *Trichoderma viride*, *Aspergillus niger* and *Penicillium* species were most effective antagonists against the *S. rolfisii* causing stem rot in groundnut under *in vitro* condition. Zape et al. (2014)<sup>[14]</sup> observed that *Trichoderma viride* was most effective antagonist against inhibition of mycelial growth of *S. rolfisii* under *in vitro* condition using dual culture technique. *Aspergillus flavus* was least effective to inhibit the mycelium growth of *S. rolfisii*. Basumatary et al. (2015)<sup>[2]</sup> observed the similar

result against mycelium growth of *S. rolfisii*. *Trichoderma viride* and *T. harzianum* secreted antibiotics and hydrolytic enzymes which suppresses the plant pathogen.<sup>[2]</sup> *Trichoderma viride*, *A. flavus*, *A. niger* and *Penicillium italicum* were effective bioagent against inhibition of mycelium growth of *S. rolfisii* under *in vitro* condition.<sup>[6]</sup> *Trichoderma viride* effectively inhibits the growth of *S. rolfisii* at different pH value under *in vitro* condition and 77% inhibit at 37<sup>o</sup> C and pH 4.<sup>[7]</sup> *Trichoderma viride*, *Penicillium* sp. and *A. niger* were more significant inhibiting the radial growth of *S. rolfisii* causing collar rot, sclerotium wilt, stem-rot, charcoal rot, seedling blight, damping-off, foot-rot, stem blight and root-rot in maize, tomato, chilli, sunflower, cucumber, brinjal, soybean, groundnut, bean, watermelon and other crops.<sup>[5]</sup>

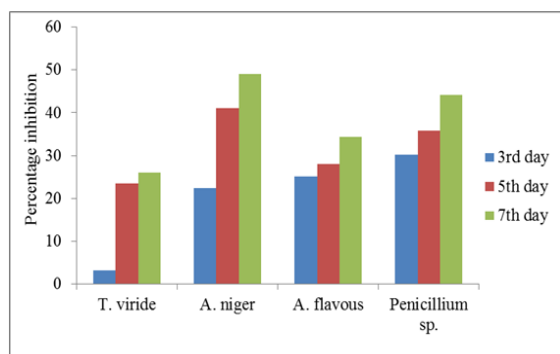
**Table 1: Percent inhibition of *S. rolfisii* by fungal antagonists using dual culture technique under *in vitro* condition**

Treatment	Incubation period (Day)					
	3 <sup>rd</sup>		5 <sup>th</sup>		7 <sup>th</sup>	
	* MG	% I	MG	% I***	MG	% I
<i>Trichoderma viride</i>	<sup>a</sup> 35.25±0.25	3.21	47±0.25**	23.48	60.25±0.25	26.00
<i>Aspergillus niger</i>	28.25±0.25	22.43	36.25±0.25	40.98	41.5±0.25	49.03
<i>Aspergillus flavus</i>	27.25±0.25	25.18	44.25±0.25	27.96	53.42±0.14	34.39
<i>Penicillium</i> sp.	25.42±0.38	30.20	39.42±0.38	35.82	45.42±0.38	44.22
Control	36.42±0.38	-	61.42±0.38	-	81.42±0.38	-

\*MG = Mycelial growth (in mm), \*\* Mean value ±SD, <sup>a</sup>Values are average of triplicate  
\*\*\* % I = Percent inhibition



**Fig 2: Mycelial growth (Mean ±SD) of *Sclerotium rolfisii* by using dual culture technique under *in vitro* condition.**



**Fig 3: Percent inhibition of *Sclerotium rolfisii* by fungal antagonists using dual culture technique under *in vitro* condition.**

#### 4. CONCLUSION

The management of soil borne diseases in maize with chemicals under field condition is price prohibitive, hazardous, loss of soil fertility and cause serious environmental pollution, so, use of biological control as eco-friendly management of *Sclerotium rolfisii* is an alternative best way. The present research article concluded that biological antagonists ie. *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma viride*, and *Penicillium* sp. inhibited mycelial growth of *S. rolfisii* under *in vitro* condition. *Aspergillus niger* and *Penicillium* sp. were more effective compared to *Aspergillus flavus* and *Trichoderma viride*.

#### ACKNOWLEDGEMENT

The authors are thankful to Head, Department of Environmental Science, Lucknow for providing facilities; one of us (Ganesh Prasad) is grateful to BBAU, Lucknow for providing UGC Non-NET fellowship.

#### REFERENCES

1. Aycock R. Stem rot and other diseases caused by *Sclerotium rolfisii*. N.C. state univ. Tech. Bull., 1966; 174 - 202.
2. Basumatarg M, Dutta BK, Singha DM, Das N. Some *in vitro* observations on the biological control of *Sclerotium rolfisii*, a serious pathogen of various agricultural crop plants. IOSR Journal of Agriculture and veterinary science, 2015; 8(2): 87-94.

3. Bosah O, Igalek CA, Omorusi VI. *In vitro* microbial control of pathogenic *Sclerotium rolfsii*. International Journal of Agriculture & Biology, 2010; 12(3): 474-476.
4. Chaurasia S, Chaurasia AK, Chaurasia S, Chaurasia S. Pathological studies of *Sclerotium rolfsii* causing foot rot disease of Brinjal (*Solanum melongena*). Int. J. of Pharm. Life science, 2014; 5(1): 3257-3264.
5. Dwivedi SK, Prasad G. Integrated management of *sclerotium rolfsii*: an overview. European Journal of Biomedical and Pharmaceutical sciences, 2016; 3(11): 137-146.
6. Ekundayo EA, Boboye BE, Adetuyi FC. *In vitro* Antifungal Efficacies of Maize Associated Microorganisms. Advances in Microbiology, 2015; 5: 258-268.
7. Ekundayo EA, Ekundayo FO, Osinowo IA. Antifungal activities of *Trichoderma viride* and two fungicides in controlling diseases caused by *Sclerotium rolfsii* on tomato plants. Advance in Applied Science research, 2015; 6(3): 12-19.
8. Karthikeyan V, Sankaralingam A, Nakkeeran S. Biological control of groundnut stem rot caused by *Sclerotium rolfsii* (Sacc.), Archives of Phytopathology and Plant Protection, 2006; 39(3): 239-246.
9. Nawar LS. *In-vitro* efficacy of some fungicides, bioagents and culture filtrates of selected saprophytic fungi against *Sclerotium rolfsii*. Life Science Journal, 2013; 10(4): 2222-2228.
10. Parmar HJ, Hassan MM, Bodar NP, Umrania VV, Patel SV, Lakhani HN. *In vitro* antagonism between phytopathogenic fungi *Sclerotium rolfsii* and *Trichoderma* strains. Int J Appl Sci Biotechnol, 2015; 3(1): 16-19.
11. Pinheiro VDR, Seixas CDS, Godoy CV, Soares RM, Oliveira MCN, Almeida AMR. Development of *Sclerotium rolfsii*, sclerotia on soybean, corn and wheat straw, under different soil temperature and moisture contents. Pesq. agropec. Bras. Brasilia, 2010; 2010; 45(3): 332-334.
12. Punja ZK. The Biology, Ecology, and control of *Sclerotium rolfsii*. Annual review of Phytopathology, 1985; 23: 97-127.
13. Shaigan S, Seraji A, Moghaddam SAM. Identification and Investigation on Antagonistic Effect of *Trichoderma* sp. on Tea Seedlings White Foot and Root Rot (*Sclerotium rolfsii* Sacc.) *in vitro* Condition. Pakistan Journal of Biological Sciences, 2008; 11: 2346-2350.
14. Zape AS, Gade RM, Singh R, Deshmukh VA. efficacy of different antagonist against the *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium solani*. The Bioscan, 2014; 9(4): 1431-1434.

# Efficacy of Bioagents, Plant extracts and chemical fungicides against root rot disease in *Zea mays* caused by *Sclerotium rolfsii*

Asia Pacific Journal of  
Multidisciplinary Research

Vol. 8 No.1, 47-55

February 2020

P-ISSN 2350-7756

E-ISSN 2350-8442

www.apjmr.com

ASEAN Citation Index

Ganesh Prasad<sup>1</sup> & S. K. Dwivedi<sup>2</sup>

<sup>1</sup> Student Researcher, Department of Environmental Science,

<sup>2</sup> Dean, School of Environmental Sciences, Babasaheb Bhimrao Ambedkar

(A Central) University, Vidya Vihar, Raebareli Road, Lucknow (U.P.)

India

ganeshat86@gmail.com<sup>1</sup>, skdwivedibbau@gmail.com<sup>2</sup>

Date Received: September 19, 2019; Date Revised: February 5, 2020

**Abstract** – *Zea mays* is a nutritional crop and heavy source of carbohydrate. The root rot disease in *Zea mays* caused by *Sclerotium rolfsii*. This study was evaluated for management of *S. rolfsii* due to it is more dangerous soil borne fungus. It was controlled by two antagonists, two metabolites of bioagents, two chemical fungicides and three plants extracts at different concentration using poisoned food technique. Histopathological study the root rot disease infected part of plant cut section to show with SEM. The T.S. of root and stem was observed by SEM image showed the mycelia of *S. rolfsii* was scattered in root and stem of infected *Zea mays* plant. The culture filtrate of *Penicillium citrinum* and *P. italicum* was inhibited by 100% growth of *S. rolfsii* at 75% concentration. Carbendazim was more effective compared to conika at different concentration. In vitro efficacy, the extracts of plants viz. *Azadirachta indica*, *Syzygium aromaticum*, *Trachyspermum ammi* having antifungal activity and checked mycelial expansion of *S. rolfsii* under in vitro condition. *Syzygium aromaticum* and *Trachyspermum ammi* were inhibited by 100% at 400 ppm and 50% concentration respectively. The extract of medicinal plants *Syzygium aromaticum* and *Trachyspermum ammi* were most significantly effective compared to *A. indica* on 3rd day. Amongst all treatments were significantly and ecofriendly effective against mycelial expansion of *S. rolfsii* under in vitro study.

**Keywords** – Bioagents, Chemical fungicides, Plant extracts, *Sclerotium rolfsii*, SEM, *Zea mays*.

## INTRODUCTION

*Zea mays* seeds are nutritious and edible that serves as good source of carbohydrates, protein fat and vitamin. Betacarotene and selenium also found in *Zea mays* that helps in treatment of thyroid gland [1]. The antioxidant property found in corn seeds removed free radicals which is responsible for cellular damage and cancer [2]. Corn seeds are used for alcohol production and stem fibres for manufacturing of paper [3]. *Zea mays* crop is infected by bacteria, fungi and pest but fungal disease like root rot more dangerous. The root rot disease is caused by *Sclerotium rolfsii*. *Sclerotium rolfsii* is a Soil-borne fungus [4], [5], [6], [7], [8]. The infected *Zea mays* crop showed reduced growth and floppy and finally crop died. *Sclerotium rolfsii* infects many economically important plants at different stages of growth and development. Pathogen firstly infect lower portion of plant and after then it infect whole parts of the plant [12]. The cutinolytic enzymes viz., Pectinase and cellulase which degrade cuticle of plant were produced by *S.*

*rolfsii* [13]. The vascular bundles of infected part of plant became brownish [14].

*Sclerotium rolfsii* is also known as white mold and a dreaded soil-borne plant pathogen [11], symbiotic, parasite and saprophyte fungus which is responsible for causing various diseases on crops like collar-rot, sclerotium wilt, seedling blight, stem canker, bulb rot, crown blight, tuber rot, fruit rot, crown rot, systemic wilt or blight of whole plant, stem-rot, charcoal rot, foot-rot, stem blight and root-rot in more than 500 plants species including tomato, chilli, , maize, groundnut, sunflower, cucumber, brinjal, soybean, bean, watermelon etc [4], [5], [6], [7], [8]. It was first observed by Peter Henry Rolfs in the year 1892 on tomato plants with 70% losses [17]. Fungus produced numerous tiny round white *sclerotia* of the same size when immature and dark brown on mature stage [6], [9] and *sclerotia* diameters ranged 4-8 mm in size which is characteristic feature of *S. rolfsii*. *Sclerotium rolfsii* develops at soil moisture level (70%)

of field capacity and temperature ranged between 25°C to 30°C [10].

*Trichoderma viride*, *Aspergillus niger* and *Penicillium* species were most effective against *S. rolfisii* causing stem rot in groundnut under *in vitro* condition [24], [25]. *In vitro* efficacy *Trichoderma harzianum* contained antagonistic activity against mycelial growth of *S. rolfisii* [32]. The percent inhibition of mycelial expansion of *S. rolfisii* by five *Trichoderma* species viz *T. hamatum*, *T. koningii*, *T. harzianum*, *T. ressei* and *T. viride*, indicated as enhanced antagonists in sustainable agricultural and environmental protection [35]. *Trichoderma viride* contained antagonistic activity against mycelial expansion of *S. rolfisii* under *in vitro* condition [36].

Hexaconazole was most effective compared to Carbendazim, difenoconazole, mencozeb, chlorothalonil at 125 ppm, 250 ppm, 500 ppm concentration against *S. rolfisii* under *in vitro* condition [31]. Taj et al. [30] studied seven fungicides viz. Captan, Carbendazim, Chlorothalonil, Mancozeb, Tebuconazole, Tricyclazole and Trifloxystoburin against *Sclerotium rolfisii* and inhibited effectively mycelial expansion of *Sclerotium rolfisii* at 1000 ppm concentration.

Mahato et al. [33] studied about eight medicinal plant extracts viz. *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Andrographis paniculata*, *Curcuma longa*, *Catharanthus roseus*, *Ocimum sanctum* and *Zingiber officinale* against *S. rolfisii* at 5, 10 and 20% concentration under *in vitro* condition. *Aspergillus* spp., *Rhizopus* sp., *Penicillium* spp. and chemical fungicides including tolclofos methyl, thiram, carboxin, fludioxonil, azoxystrobin metalaxyl-M were inhibited mycelial expansion of *S. rolfisii* under *in vitro* condition at different levels [15]. The leaf extracts of plants viz. *Annona squamosa* Linn, *Brassica campestris* Linn and *Ocimum sanctum* Linn were more effective against *S. rolfisii* under *in vitro* condition at difference concentration [16].

The present study evaluated that the bioagents, plant extracts and chemical fungicides were most significantly effective at different concentration against *S. rolfisii*. This experiment may be effective in field condition and control the root rot disease in maize and other beneficial crop.

#### OBJECTIVES OF THE STUDY

Study on bioagents, plant extracts and chemical fungicides against root rot disease caused by *Sclerotium rolfisii* in *Zea mays* crop. The use of above treatments may be ecofriendly effective in the field condition.

## MATERIALS AND METHODS

### Sample collection

The samples were collected from an infected agriculture field of maize from district Barabanki. The infected parts of maize crop and their rhizospheric and non-rhizospheric soil were carried out in sterilized polythene bags separately. Some medicinal plants were collected from the BBAU campus and other areas of Lucknow. Some selected bioagents were isolated from collected soil samples. Chemical fungicides were procured from the local market of Lucknow.

### Separation and recognition of Pathogen:

*Sclerotium rolfisii* was isolated from infected root of *Zea mays*. The infected root of plant was washed and cut into small pieces (2-4 mm length) and sterilized with 0.5% mercuric chloride (HgCl<sub>2</sub>) solution for 30 seconds followed by washing in sterilized distilled water and left at room temperature for 5 minutes. Small sections were placed on PDA Petriplates and incubated at 27±1°C in an incubator for seven days. After seven days, the culture was maintained on PDA slant for further studied [17]. The fungus recognized by morphological study. Morphologically the pathogen bear hyphae grow state forward on the surface of the infected plant stem with white mycelium and scattered inside and outside of the infected stem nearby soil surface. The old hyphae produce many tiny rounds, white *sclerotia* of the same size (Fig.1) when immature and dark brown *sclerotia* on mature stage like mustard seed [6]. The *sclerotia* diameters are 4-8 mm which is characteristic feature of *S. rolfisii* [1].

### Antagonistic activity of some fungal species against *Sclerotium rolfisii*:

Antagonistic activity of *Penicillium citrinum* and *P. italicum* were evaluated against the expansion of *S. rolfisii* using dual culture technique under *in vitro* condition [18]. In dual culture technique, 5 mm diameter block of *S. rolfisii* was inoculated one side and antagonist on opposite side of the same PDA containing Petriplate as a treatment and one block of *S. rolfisii* inoculated in the center of another Petriplate as control. The treatments and control plate was incubated at 27±1°C temperature in an incubator. All treatments and control plates were set in triplicates. On 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day of incubation, the mycelial expansion of *S. rolfisii* in control as well as treatments plate was measured. Inhibition of *S. rolfisii* was calculated by following formula.

$$\text{Percent Inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

### Preparation of metabolite

The metabolite was extracted in PD broth. In this method firstly three blocks (5mm) of *Penicillium citrinum* and *Penicillium italicum* cut with 5mm diameter cork borer from the actively growing margin of 5 days old culture was inoculated separately in 500 ml conical flask containing 300 ml of potato dextrose broth [19]. After 15 days, cultures were filtered firstly by Whatman filter paper no 42 and then filtered by Vacuum Seitz filter. Finally the filtrates were stored in sterile conical flask at 4°C for further study.

### Management of *Sclerotium rolfii*

The culture filtrates of *Penicillium citrinum* and *Penicillium italicum*, chemical fungicides Carbendazim 50% WP and Conika 50% WP, plant extract *Azadirachta indica*, *Syzygium aromaticum* and *Trachyspermum ammi* were tested against mycelial expansion of *S. rolfii* using poisoned food technique [20]. According to this method, the *Penicillium citrinum*, *Penicillium italicum*, Carbendazim 50% WP, Conika 50% WP, *Azadirachta indica*, *Syzygium aromaticum* and *Trachyspermum ammi* at different concentration were added separately in PDA medium and poured into sterilized Petri plates in laminar flow. After solidification, cut 5 mm block of fresh culture of *S. rolfii* placed in centre of each Petri plates and incubated at 27±1°C. The mycelial expansion of *S. rolfii* was recorded on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day. Inhibition of mycelial expansion of *S. rolfii* was calculated by following formula [21].

$$\text{Inhibition Percent} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

### Histopathological study

The root rot disease incidence in *Zea mays* plant caused by *Sclerotium rolfii* and histological study by using SEM analysis. The samples were analyzed from USIC BBAU Lucknow. In this method, the infected part of *Zea mays* was washed under running water followed by distilled water and then cut T.S. of root & stem. These sectioned were fixed with 2.5% glutaraldehyde for 2-6 hours. The fixed sectioned were washed with phosphate buffer for 3-4 times followed by series of dehydration process in 30-100% ethanol and then kept in dry acetone. Samples were mounted on aluminium stubs with double sided carbon conductive tape and were coated with thin layer of palladium under a vacuum. The images of samples were observed under Scanning Electron Microscope (Jeol JSM-6490 LV).

### Data analysis

The data were expressed as Mean and analyzed statistically using one way ANOVA (analysis of variance), coefficient variance, standard error, standard deviation, DMRT and Least significant difference test ( $p \leq 0.05$ ) were applied.

### RESULTS AND DISCUSSIONS

Root rot disease causing soil borne fungus *Sclerotium rolfii* was controlled significantly ( $p \leq 0.05$ ) by using *Penicillium citrinum*, *Penicillium italicum*, Carbendazim 50% WP, Conika 50% WP, *Azadirachta indica*, *Syzygium aromaticum* and *Trachyspermum ammi* at different concentration on 3<sup>rd</sup> day incubation period under *in vitro* condition. *Penicillium citrinum* (20.39%) and *P. italicum* (17.14%) inhibited expansion of *S. rolfii* using dual culture method under *in vitro* condition (Table 1). Whereas culture filtrate *Penicillium citrinum* and *P. italicum* were inhibited 100% mycelial growth of *S. rolfii* at 75% v/v concentration while 84.40%, 82.38%, 84.51% and 71.52%, 70.94%, 83.35% growth inhibition was recorded at 10%, 25%, 50% v/v concentration respectively on 3<sup>rd</sup> day (Table 2&3).

At 600 ppm concentration, Conika 50% WP inhibited the growth by 70.57% compared to 100 ppm (1.99%) 200 ppm (7.99%) 400 ppm (57.86%) concentration (v/v) against mycelial expansion of *S. rolfii* on 3<sup>rd</sup> day of incubation period (Table 4). Carbendazim 50% WP was most effective and inhibited the expansion of *S. rolfii* by 84.19% at 6 ppm (v/v) concentration whereas at 1 ppm, 2 ppm, 4 ppm (v/v) concentration the expansion of *S. rolfii* inhibited by 17.08%, 75.20%, 81.38% respectively on 3<sup>rd</sup> day of incubation under *in vitro* condition (Table 5). Amongst these chemical fungicides, the Carbendazim 50% WP was most effective ( $p \leq 0.05$ ) compared to Conika 50% WP against mycelial growth of *S. rolfii* at different concentration on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day of incubation period.

*Azadirachta indica* inhibited maximum (79.65%) at 75% v/v concentration followed by 18.59%, 32.10%, 71.05% at 10%, 25%, 50% v/v concentration on 3<sup>rd</sup> day against *S. rolfii* (Table 6). On another hand, *Syzygium aromaticum* oil inhibited 100% growth of *S. rolfii* at 400 ppm v/v concentration while at 100 ppm, 200ppm v/v concentration it inhibited the growth by 13.23%, 79.65% of *S. rolfii* on 3<sup>rd</sup> day respectively (Table 7). *Trachyspermum ammi* seeds powder inhibited the growth by 100% at 50% v/v concentration followed by 22.11%, 31.73% at 10%, 25% v/v concentration against *S. rolfii* on 3<sup>rd</sup> day of incubation period under *in vitro* condition (Table 8). Among all tested medicinal plants,

*Syzygium aromaticum* and *Trachyspermum ammi* inhibited the growth by 100% at 400 ppm (v/v) and 50% (v/v) concentration respectively on 3<sup>rd</sup> day. *Syzygium aromaticum* oil and *Trachyspermum ammi* were significantly ( $p \leq 0.05$ ) effective compared to *Azadirachta indica* at different concentration on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day under *in vitro* condition. Histopathological study the T. S. of root & stem of infected *Zea mays* was analyzed with SEM which images showed the xylem vessels of root and stem were ruptured from *S. rolfssii* (Fig.2).

All antagonists contain inhibitory activity and to bind actively growing mycelial tip of the pathogen and inhibits the mycelial growth of *S. rolfssii* [22]. Madhavi and Bhattiprolu [29] tested two *Trichoderma* sp. viz. *T. viride*, *T. harzianum* against *S. rolfssii* using dual culture technique and reported the growth inhibition by 55.8%, 57.5% respectively. In a study nine antagonistic microorganisms were selected for their efficacy against *S. rolfssii* using dual culture technique under *in-vitro* condition, out of these, the maximum mycelial expansion of *S. rolfssii* reduced (63.33%) by *Trichoderma viride* [23]. Karthikeyan et al. [37] isolated three bioagents viz; *T. viride*, *T. harzianum* and *Pseudomonas fluorescens* and studied their effectiveness against growth of *S. rolfssii*. Out of these three bioagents, only *T. viride* inhibited 69.40% mycelial development and 62.50% sclerotial germination of *S. rolfssii*. *Trichoderma viride*, *Aspergillus niger* and *Penicillium* species were most effective against *S. rolfssii* causing stem rot in groundnut under *in vitro* condition [24], [25]. Ekundayo et al. [17] observed similar result in root-rot disease in *Zea mays* when treated with *Aspergillus flavus*, *A. niger*, *T. viride* and *Penicillium italicum* against mycelium expansion of *S. rolfssii* under *in vitro* condition. Curtis et al. [26] found that *R. solni* and *S. rolfssii* causing crown and root-rot in tomato plants significantly checked by biocontrol agent as eco-compatible farming. The metabolites of *Trichoderma viride* and *T. harzianum* inhibited collar rot disease incidence in sunflower caused by *S. rolfssii* and improved the crop yielding [27].

Chaurasia et al. [28] tested nine chemical fungicides viz., Bavistin, Brassicol, Captan, Dithane M-45, DM-145, Fytolan, Parasan, Manzate, Sulfex against *S. rolfssii* under *in vitro* condition and reported that all fungicides had adverse effect on the expansion of *S. rolfssii* and inhibited the growth by 100% at 0.1% concentration. In a study, carbendazim inhibited maximum growth of *S. rolfssii* by 93.7% at 0.2% concentration under *in vitro* condition [29]. Taj et al. [30] studied seven fungicides viz. Captan, Carbendazim, Chlorothalonil, Mancozeb, Tebuconazole, Tricyclazole and Trifloxystoburin against *Sclerotium rolfssii* and inhibited effectively mycelial expansion of *Sclerotium rolfssii* at 1000 ppm concentration. Hexaconazole was most effective compared to Carbendazim, difenoconazole, mencozeb, chlorothalonil at 125 ppm, 250 ppm, 500 ppm concentration against *S. rolfssii* as a similar result to be found *in vitro* condition [31]. Wavare et al. [32] evaluated that chemical fungicide Carbendazim checked mycelial expansion of *S. rolfssii* by 100% as a similar result to be found.

Mahato et al. [33] studied about eight medicinal plant extracts viz. *Azadirachta indica*, *Allium cepa*, *Allium sativum*, *Andrographis paniculata*, *Catharanthus roseus*, *Curcuma longa*, *Ocimum sanctum* and *Zingiber officinale* against *S. rolfssii* at 5, 10 and 20% concentration under *in vitro* condition. They reported that *Allium sativum* showed maximum inhibition (84.89%) of *S. rolfssii* at 20% concentration followed by *Azadirachta indica* (80.86%), while *Ocimum sanctum* inhibited the growth by 53.47%. Gupta, et al.[34] evaluated the efficacy of some plants extracts like ashok (*Polyalthia longifolia*), bhang (*Cannabis sativa*), clerodendron (*Clerodendrum inerme*), eucalyptus (*Eucalyptus globulus*), garlic, ginger, lantana (*Lantana camara*), karanj (*Pongamia pinnata*), madar (*Calotropis gigantea*), marigold (*Tagetes erecta*), mehendi (*Lawsonia inermis*), neem (*Azadirachta indica*), onion, parthenium (*Parthenium hysterophorus*), sadabahar (*Catharanthus roseus*), tulsi (*Ocimum tenuiflorum*) against *S. rolfssii* causing collar rot of chickpea at 5 and 10% concentration under *in vitro* and found that garlic extract inhibited growth of *S. rolfssii* by 100% at 10% concentration on 42 hours compared to neem (97.0%), ginger (95.8%), marigold (95.6%), lantana (87.1%), madar (79.9%), ashok (77.4%), parthenium (70.8%), clerodendron (66.3%), eucalyptus (61.9%) and onion (55.9%).

**Table 1. Antagonistic activity of some bio-agents against mycelial growth (in mm) of *Sclerotium rolfssii* using dual culture technique under *in vitro* condition**

Treatment	1st day	2nd day	3rd day	CV%
Control	32.58±1.24 (0.00)	63.08±0.79 (0.00)	84.58±0.67 (0.00)	36.03
<i>P. citrinum</i>	31.33±1.07 (3.83)	58.33±3.20 (7.63)	67.33±7.64 (20.39)	30.95
<i>P. italicum</i>	32.17±1.03 (1.26)	60.25±4.05 (4.49)	70.08±9.43 (17.14)	31.9
F-cal	7.608*	3.148*	6.499*	-

Mean ±SD, Bracket data represent % inhibition, CV%= Coefficient of variation F-cal= F-calculated value at 5% level

\*=Significant at  $p=0.05$

**Table 2. In vitro evaluation of Toxicity of culture filtrate of *Penicillium citrinum* at different concentration and incubation period against mycelial growth (in mm) of *Sclerotium rolfsii***

Treatment	1st day	2nd day	3rd day	CV%
Control	35.66±0.88 (0.00)	63.75±0.96 (0.00)	86.58±1.56 (0.00)	34.09
10%	13.5±1.44 (62.14)	13.5±1.44 (78.82)	13.5±1.44 (84.40)	10.37
25%	15.25±1.28 (57.23)	15.25±1.28 (76.07)	15.25±1.28 82.38	8.19
50%	13.41±1.31 (62.39)	13.41±1.31 (76.96)	13.41±1.31 (84.51)	9.47
75%	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
F-cal	1570.875**	5659.317**	9049.605**	-

Mean±SD, Bracket data represent % inhibition, CV%= Coefficient of variation F-cal= F-calculated value at 5% level

\*\*=Highly Significant at p=0.05

**Table 3. The culture filtrate toxicity of *Penicillium italicum* at different concentration and incubation period under in vitro condition against mycelial growth (in mm) of *Sclerotium rolfsii***

Treatment	1st day	2nd day	3rd day	CV%
Control	35.66±0.88 (0.00)	63.75±0.96 (0.00)	86.58±1.56 (0.00)	34.09
10%	18.66±1.23 (47.67)	24.66±1.37 (61.32)	24.66±1.37 (71.52)	13.85
25%	20.75±1.42 (41.)	25.16±1.58 (60.53)	25.16±1.58 (70.94)	10.89
50%	14.41±1.37 (59.59)	14.41±1.37 (77.39)	14.41±1.37 (83.35)	9.22
75%	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
F-cal	1582.333**	4642.639**	7542.84**	-

Mean±SD, Bracket data represent % inhibition, CV%= Coefficient of variation F-cal= F-calculated value at 5% level

\*\*=Highly Significant at p=0.05

**Table 4. Effect of Conika 50% WP against mycelial growth (in mm) of *Sclerotium rolfsii* at different concentration and incubation period under in vitro condition**

Treatment	1st day	2nd day	3rd day	CV%
Control	30.08±0.90 (0.00)	65.42±0.51 (0.00)	91.75±0.62 (0.00)	41.06
100ppm	29.58±0.51 (1.66)	65.33±0.49 (0.14)	89.92±0.51 (1.99)	40.77
200ppm	27.92±0.51 (7.18)	60.83±2.69 (7.02)	84.42±0.90 (7.99)	40.8
400ppm	22.5±0.67 (25.19)	30.58±0.90 (53.25)	38.66±1.07 (57.86)	22.07
600ppm	19.16±1.40 (36.30)	27±0.60 (58.73)	27±0.60 (70.57)	15.79
F-cal	366.596**	2514.522**	19355.34**	-

Mean±SD, Bracket data represent % inhibition, CV%= Coefficient of variation

F-cal= F-calculated value at 5% level

\*\*=Highly Significant at p=0.05

**Table 5. Effect of Carbendazim 50% WP against mycelial growth (in mm) of *Sclerotium rolfii* at different concentration and incubation period under *in vitro* condition**

Treatment	1st day	2nd day	3rd day	CV%
Control	30.08±0.90 (0.00)	65.42±0.51 (0.00)	91.75±0.62 (0.00)	41.06
1ppm	26.42±0.79 (12.17)	51.58±0.67 (21.15)	76.08±0.79 (17.08)	40.05
2ppm	18.92±0.67 (37.10)	22.75±0.75 (65.22)	22.75±0.75 (75.20)	9.12
4ppm	15.25±0.62 (49.30)	17.08±0.66 (73.89)	17.08±0.67 (81.38)	6.55
6ppm	9.75±0.62 (67.59)	14.5±0.79 (77.83)	14.5±0.79 (84.19)	18.43
F-cal	1532.165**	13255.92**	30120.66**	-

Mean±SD, Bracket data represent % inhibition, CV%= Coefficient of variation F-cal= F-calculated value at 5% level

\*\*=Highly Significant at p=0.05

**Table 6. Effect of *Azadirachta indica* leaf extract at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfii* over control**

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±1.11 (0.00)	70.5±1.88 (0.00)	90.08±0.99 (0.00)	40.14
10%	28.25±0.96 (5.29)	56.91±1.44 (19.27)	73.33±1.07 (18.59)	35.81
25%	22±0.95 (24.25)	45.16±0.93 (35.94)	61.16±1.11 (32.10)	38.18
50%	14.58±0.90 (51.12)	22.33±1.30 (68.32)	26.08±0.90 (71.05)	23.61
75%	11±0.95 (63.12)	14.91±0.79 (78.85)	18.33±0.88 (79.65)	21.35
F-cal	851.571**	3676.523**	11369.65**	-

Mean±SD, Bracket data represent % inhibition, CV%= Coefficient of variation F-cal= F-calculated value at 5% level

\*\*=Highly Significant at p=0.05

**Table 7. Effect of *Syzygium aromaticum* oil at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfii* over control**

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±1.11 (0.00)	70.5±1.88 (0.00)	90.08±0.99 (0.00)	40.14
100ppm	20.25±3.51 (32.11)	49.58±2.53 (29.67)	78.16±1.74 (13.23)	48.89
200ppm	11.33±1.23 (62.02)	15.08±0.79 (78.61)	18.33±2.01 (79.65)	21.52
400ppm	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
600ppm	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
F-cal	667.725**	5652.262**	14047.61**	-

Mean±SD, Bracket data represent % inhibition, CV%= Coefficient of variation F-cal= F-calculated value at 5% level

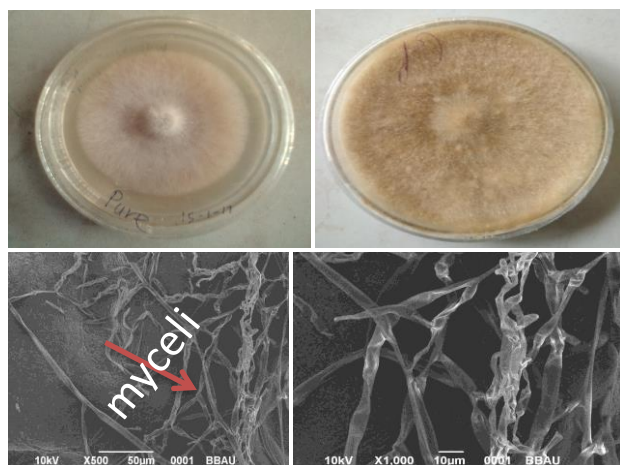
\*\*=Highly Significant at p=0.05

**Table 8. Effect of *Trachyspermum ammi* seeds powder at different concentration and incubation period under *in vitro* condition against mycelial growth (in mm) of *Sclerotium rolfsii* over control**

Treatment	1st day	2nd day	3rd day	CV%
Control	29.83±1.11 (0.00)	70.5±1.88 (0.00)	90.08±0.99 (0.00)	40.14
10%	23.41±1.16 (21.52)	49.75±1.60 (29.43)	70.16±3.58 (22.11)	40.9
25%	20.5±1.44 (31.27)	41.33±3.14 (41.37)	61.5±1.67 (31.73)	41.62
50%	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
75%	0±0.0 (100)	0±0.0 (100)	0±0.0 (100)	0
<b>F-cal</b>	2465.581**	3687.923**	6278.842**	-

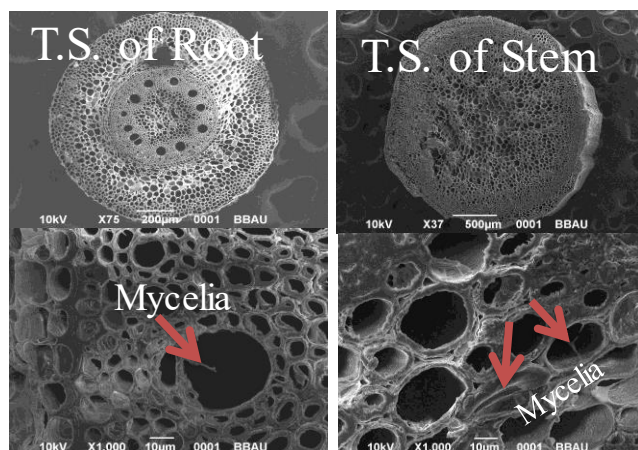
Mean ±SD, Bracket data represent % inhibition, CV%= Coefficient of variation F-cal= F-calculated value at 5% level

\*\*=Highly Significant at p=0.05



**Fig.1: Petriplates culture and Scanning Electron Microscope (SEM) Images of *Sclerotium rolfsii***

**Fig.2: SEM Images of T.S. of Root and Stem of root rot disease incidence in *Zea mays* caused by *Sclerotium rolfsii***



*Azadirachta indica* against *S. rolfsii* on 3<sup>rd</sup> day. The selected above bioagents, plant extracts and chemical fungicides having anti-pathogenic activity which inhibited significantly against *Sclerotium rolfsii*. The SEM image showed that hyphae of *S. rolfsii* was entering in the xylem vessels of the maize and blocked their vessels. The authors recommended that the *Penicillium citrinum*, *P. italicum*, Carbendazim 50%, *Syzygium aromaticum*, *Trachyspermum ammi* were effectively inhibited mycelial growth of *S. rolfsii* and also it may be effectiveness in the field.

#### ACKNOWLEDGEMENT

The authors are thankful to BBA University for providing UGC Non-NET fellowship as a financial support.

#### REFERENCES

- [1] Prasad, G. & Dwivedi, S. K.(2017). Efficacy of some bioagents against *sclerotium rolfsii* sacc. causing root-rot

- in *zea mays* l. crop. European Journal of Biomedical and Pharmaceutical Sciences, 4(7), 478-481.
- [2] Mbah, C.J., Orabueze, I. & Okorie, N.H. (2019). Antioxidants Properties of Natural and Synthetic Chemical Compounds: Therapeutic Effects on Biological System". Acta Scientific Pharmaceutical Sciences, 3(6), 28-42.
- [3] Kumar, D.& Jhariya, A.N. (2013). Nutritional, Medicinal and Economical importance of Corn: A Mini Review. *Research Journal of Pharmaceutical Sciences*, 2(7), 7-8.
- [4] Dwivedi, S.K. & Prasad, G. (2016). Integrated management of *sclerotium rolfsii*: an overview. European Journal of Biomedical and Pharmaceutical Sciences, 3(11), 137-146.
- [5] Gopalakrishnan, S., Beale, M.H., Ward, J.L. & Strange, R.N. (2005). Chickpea wilt: identification and toxicity of methyl fusarubin from *Fusarium acutatum*. *Phytochemistry*, 66, 1536-1539.
- [6] Kwon, J.H. & Park, C.S. (2002). Stem rot of tomato caused by *Sclerotium rolfsii* in Korea. *Microbiology*, 30(4), 244-246.
- [7] Aycock, R. (1966). Stem rot and other diseases caused by *Sclerotium rolfsii*. N.C. state univ. Tech. Bull., 174, 202.
- [8] Punja, Z.K. (1985). The Biology, Ecology and control of *Sclerotium rolfsii*. *Annual review of Phytopathology*, 23, 97-127.
- [9] Han, K.S., Lee, S.C., Lee, J.S., Soh, J.W. & Kim, S. (2012). First Report of *Sclerotium* Rot on *Cymbidium* Orchids Caused by *Sclerotium rolfsii* in Korea. *Mycobiology*, 40(4), 263-264.
- [10] Pinheiro, V.D.R., Seixas, C.D.S. Godoy, C.V., Soares, R.M., Oliveira, M.C.N. & Almeida, A.M.R. (2010). Development of *Sclerotium rolfsii*, sclerotia on soybean, corn and wheat straw, under different soil temperature and moisture contents. *Pesq. agropec. Bras. Brasilia*, 45(3), 332-334.
- [11] Sacchi, S., Maurya, S. & Choudhary, A.K. (2016). Antifungal Efficacy of Garlic and Ginger against *Sclerotium rolfsii*. *International Journal of Agricultural Science and Research*, 6(6), 419-424.
- [12] Mahadevakumar, S., Yadav, V., Tejaswini, G. S. & Janardhana, G. R. (2016). Morphological and molecular characterization of *Sclerotium rolfsii* associated with fruit rot of Cucurbita maxima. *European Journal of Plant Pathology*, 145, 215–219.
- [13] Linskens, H.F. & Haage, P. (1963). Cutinase-Nachweis in phytopathogenen Pilzen. *Journal of phytopathology*, 48 (3), 306-311.
- [14] Siddique, M.N.A., Ahmmed, A.N.F., Jahan, N., Mazumder, M.G.H. & Islam, M.R. (2018). Management of Foot and Root Rot Disease of Eggplant (*Solanum melongena* L.) Caused by *Sclerotium rolfsii* under *In Vivo* Condition. *The Agriculturists*, 16(1), 78-86.
- [15] Akgul, D.S., Ozgonen, H. & Erkilic, A. (2011). The effects of seed treatments with fungicides on stem rot caused by *Sclerotium rolfsii* sacc., in peanut. *Pak. J. Bot.*, 43(6), 2991-2996.
- [16] Quadri U. & Fatima S. (2016). Screening of Medicinal Plants against *Sclerotium rolfsii* Isolated from Root Rot of Chili. *International Journal of Innovative Research in Science, Engineering and Technology*, 5(5), 8082-8088.
- [17] Ekundayo, E.A., Boboye, B.E. & Adetuyi, F.C. (2015). *In vitro* Antifungal Efficacies of Maize Associated Microorganisms. *Advances in Microbiology*, 5, 258-268.
- [18] Huang, H.C. & Hoes, J.A. (1976). Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minitans*. *Can. J. Bot.*, 54, 406-410.
- [19] Sennoi, R., Singkham, N., Jogloy, S., Boonlue, S., Saksirirat, W., Kesmla, T., & Patanothai, A. (2013). Biological control of southern stem rot caused by *Sclerotium rolfsii* using *Trichoderma harzianum* and arbuscular mycorrhizal fungi on *Jerusalem artichoke* (*Helianthus tuberosus* L.). *Crop Protection*, 54, 148-153.
- [20] Grover, R.K. & Moore, J.D. (1962) Toximetric Studies of Fungicides against the Brown Rot Organisms, *Sclerotinia fructicola* and *S. laxa*. *Phytopathology*, 52, 876-879.
- [21] Vincent, J. M. (1947). Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, 159, 850.
- [22] Anees, M., Tronsmo, A., Edel-Hermann, H., Raud, L.G. & Steinberg, C., (2010). Characterization of field isolates of *Trichoderma* antagonistic against *Rhizoctonia solani*. *Fungal. Biol.*, 114 (9), 691–701.
- [23] Nagamma, G. & Nagaraja, A. (2015). Efficacy of biocontrol agents against *Sclerotium rolfsii* causing collar rot disease of chickpea, under *in vitro* conditions. *International Journal of Plant Protection*, 8(2), 222-227.
- [24] Shaigan S., Seraji A. & Moghaddam S.A.M. (2008). Identification and Investigation on Antagonistic Effect of *Trichoderma* sp. on Tea Seedlings White Foot and Root Rot (*Sclerotium rolfsii* Sacc.) *in vitro* Condition. *Pakistan Journal of Biological Sciences*, 11, 2346-2350.
- [25] Bosah, O., Igalek, C.A. & Omorusi, V.I. (2010). *In vitro* microbial control of pathogenic *Sclerotium rolfsii*. *International Journal of Agriculture & Biology*, 12(3), 474-476.
- [26] Curtis, F.D., Lima, G., Vitullo, D., & Cicco, V.D. (2010). Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering antagonistic bacteria through a drip irrigation system. *Crop Protection*, 29, 663–670.
- [27] Maji, A. & Nath, R. (2016). Management through Eco-Friendly Approach of Collar Rot Disease of Sunflower Caused By *Sclerotium rolfsii* Sacc in Sundarbans of West Bengal. *International Journal of Agricultural Science and Research (Ijasr)*, 6 (4), 9-12.
- [28] Chaurasia, A.K., Chaurasia, S., Chaurasia, S. & Chaurasia, S. (2014). *In vitro* efficacy of fungicides against the growth of foot-rot pathogen (*Sclerotium rolfsii* sacc.) of Brinjal. *International Journal of Current Microbiology and Applied Science*, 3(12), 477-485.

- [29] Madhavi, G.B. & Bhattiprolu, S.L. (2011). Integrated disease management of dry root rot of chilli incited by *Sclerotium rolfsii* (Sacc.). *International Journal of Plant, Animal and Environmental Sciences*, 1(2), 31-37.
- [30] Taj, A., Kumar, N.K. & Kumar, V.B.S. (2014). Fungicidal Evaluation and Physiological Studies of *Sclerotium rolfsii* Causing Collar Root of Capsicum. *Environment & Ecology*, 32 (2A), 758—761.
- [31] Sharma, R.K. & Dhruj, I.U. (2018). *In vitro* Evaluation of Some Fungicides against Indian Isolates of *Sclerotium rolfsii* Sacc. [Teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough]. *International Journal of Current Microbiology and Applied Sciences*, 7(11), 1561-1586.
- [32] Wavare, S. H., Gade, R. M. & Shitole A. V. (2017). Effect of Plant Extracts, Bio Agents and Fungicides against *Sclerotium rolfsii* Causing Collar Rot in Chickpea. *Indian Journal of Pharmaceutical Sciences*, 79(4), 513-520.
- [33] Mahato, A., Biswas, M.K. & Patra, S. (2018). Efficacy of Medicinal Plant Extracts Against Collar Rot of Tomato Caused by *Sclerotium rolfsii* (Sacc.). *International Journal of Microbiology Research*, 10(5), 1224-1227.
- [34] Gupta, R. P., Singh, S. K., Mishra, R. S. & Gupta, S. (2012). Efficacy of botanicals against *Sclerotium rolfsii* causing collar rot of Chick pea. *Annals of Plant Protection Sciences*, 20(2), 486-487.
- [35] Bagwan, N.B. (2011). Evaluation of biocontrol potential of *Trichoderma* species against *Sclerotium rolfsii*, *Aspergillus niger* and *Aspergillus flavus*. *Internat. J. Pl. Protec.* 4(1), 107-111.
- [36] Zape AS, Gade RM, Singh R, Deshmukh VA. efficacy of different antagonist against the *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium solani*. *The Bioscan*, 2014; 9(4): 1431-1434.
- [37] Kapadiya, H.J. and Moradiya, A.M. (2017). Management of groundnut disease through different organic practices and compared with chemical fungicide. *International Journal of Chemical Studies*. 5(6): 690-693.

#### COPYRIGHTS

Copyright of this article is retained by the author/s, with first publication rights granted to APJMR. This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4>).