BIOFUMIGATION FOR THE MANAGEMENT OF *Sclerotium rolfsii,* IN VEGETABLE COWPEA

by

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(2018-11-074)

THESIS

Submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM - 695 522

KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled "**Biofumigation for the management of *sclerotium rolfsii,* in vegetable cowpea**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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ACKNOWLEDGEMENT

First of all, I bow my head before the Almighty God for making me confident and optimistic throughout my journey and enabled me to complete the thesis work successfully on time.

With immense pleasure, I wish to express my sincere gratitude and indebtedness to ***Dr. Sajeena A.,*** *Assistant Professor (Plant Pathology), Integrated Farming System Research Station (IFSRS), Karamana and Chairperson of my Advisory Committee for her valuable suggestions, constant support, extreme patience and diligent assistance and co-operation throughout the investigation. This work would not have been possible without her valuable help and support. It was her sincerity, dedication and perfectionism which influenced me deeply to improve myself in all aspects. I feel proud of myself in confessing that it has been a unique privilege for me being one of her students.*

I am extremely thankful to ***Dr. Joy M.,*** *Associate Professor and Head, Department of Plant Pathology, College of Agriculture, Vellayani, and a member of Advisory Committee for the constant support, and valuable suggestions rendered throughout the period of research work and course of study.*

I am indebted to ***Dr. Jacob John,*** *Professor and Head, IFSRS, Karamana and member of Advisory Committee, for his valuable advice and whole hearted approach for the successful completion of the thesis.*

With great pleasure, I express my gratitude to ***Dr. Radhika.N S.*** *Assistant Professor, Department of Plant Pathology, College of Agriculture, Vellayani and a member of Advisory Committee for her encouragement, and support throughout the period of my research work.*

I am extremely thankful to my beloved teacher ***Dr. K, Umamaheshwaran****, Professor, Department of Plant Pathology, College of Agriculture, Vellayani for his sustained encouragement, constant support and passionate approach which made be optimistic throughout my work. No choice of words will suffice to express my deep sense of gratitude and respect to* ***Dr. Heera G, Dr. Sreeja S. V, Dr. Ayisha, Dr. Pramod,*** *Assistant Professor, Department of Plant Pathology for their help, encouragement and moral support. I also extend my thankfulness to all other teaching and non teaching staff of our department for their timely help and support during the lab work.*

I convey my heartfelt gratitude to ***Dr. B. Sudha, Dr. Meera A. V.*** *and* ***Sri. Krishnakumar G.*** *(IFSRS, Karamana) for their valuable suggestions and support throughout the course of work. I*

am greatly obliged to non teaching staff of IFSRS, for their constant support during my pot culture trials.

I duly acknowledge the encouragement, help, love and moral support by my dear class mates Arya, Anit, Athira, Aswathy, Chippy, Divya, Deena, Haritha and Veny. I am also indebted to express my thanks to my beloved seniors Deepthi chichi, Bincy chechi, Jyothi chechi, Safana chechi, Athira chechi, Deepa chechi, Bhavana akka, Shilpa chechi, Chandran cheta and Pavan anna for their hearted support throughout my research work. I extend my heartfelt thanks to dearest juniors for their support.

Words are inadequate to express the love, cooperation and caring extended by my roommates, Yasasvi and Divya, who stood with me during all hardships I passed through and kept me encouraged and happy throughout the course of work. At this moment I recall the love, care and support of my ever favourite friends Sunitha, Rachel, Yasasvi, Pooja, Vani, thirumala, Mounika, Tejasvi, Tara, Sinchana, Yashaswini, Menaka, Nita, Supriya, Himabindhu, Swathi, Mythri, Rahalya, Parvathi and Bhavana

I extend my gratitude to Kerala Agricultural University for the technical and financial assistance for completing my research work. I am also grateful to Advanced Research Centre for Plant Disease Diagnosis (ARCPDD), Department of Plant Pathology, College of Agriculture, Vellayani and IFSRS, Karamana for technical support.

Mere words cannot express my profound indebtness to my beloved Tatayya, Ammama, Dady, Amma who are my life time support system and also my family members Pedannana, Pedamma, Chinnana, Pinni, Chinnari akka, Srinu mavayya, Ganesh annaya, Jonica vadhina, rajini akka, Naga devi akka, Yamini, Bhargavi, Kishore and Pooja for their unconditional love and support bestowed on me. I once again express my sincere gratitude to all those who helped me in one way or another in the successful completion of this venture.

P. Tejasri

Peteti Teja Sri

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LIST OF ABBREVIATIONS

G	Gram
et al.	And other co workers
PDI	Per cent Disease Index
DI	Disease Incidence
viz.	Namely
%	Per cent
Cm	Centimetre
^{0}C	Degree Celsius
Mg	Micro gram
mL	Millilitre
Mg	Milligram
spp.	Species (several)
sp.	Species (single)
IDM	Integrated Disease Management
Mm	Millimetre
CRD	Completely Randomized Design
Kg	Kilogram
PDA	Potato Dextrose Agar
ITS	Internal Transcribed Spacer
Psi	Pound-force per square inch
DAP	Days after planting
var.	Variety
Fig.	Figure
DAI	Days After Inoculation
DAG	Days After Growth
NCBI	National Center for Biotechnology Information
KAU	Kerala Agricultural University
IFSRS	Integrated Farming System Research Station

q ha^{-1}	Quintals per hectare
T	Treatments
NS	Non-significant
CD	Critical Difference
SEm	Standard Error of the mean
SD	Standard Deviation
Cfu	Colony Forming Units

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Introduction

1. INTRODUCTION

Vegetable cowpea (*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* (L.) Verdc.) is one of the most popular vegetable crops cultivated in Kerala. It is susceptible to several diseases resulting in significant crop loss. The major soil borne diseases adversely affecting the crop include vascular wilt and basal swelling caused by *Fusarium oxysporum* (Omoigui *et al*., 2019) (30-100% yield loss), collar rot and web blight caused by *Rhizoctonia solani* (Kanakam *et al*., 2018) (65% yield loss) as well as seed decay and stem rot incited by *Pythium aphanidermatum* (Onuorah, 1973**)** (11% yield loss).The other diseases infecting the crop include cowpea aphid borne mosaic (Neya *et al*., 2015) (60% yield loss), leaf spot caused by *Cercospora* spp. (Omoigui *et al*., 2019) (40% yield loss), powdery mildew caused by *Erysiphe polygoni* (Amazue and Adewale, 2016) (25-50% yield loss), anthracnose caused by *Colletotrichum lindemuthianum* (Falade *et al*., 2017) (75% yield loss) and rust caused by *Uromyces phaseoli* (Anilkumar *et al*., 1989) (10-15 % yield loss).

A new and an emerging disease *viz*., basal stem rot and blight was observed to infect vegetable cowpea resulting in significant yield loss during the past few years in different districts of Kerala. The fungus was identified as *Sclerotium rolfsii* (Sajeena *et al*., 2014). The disease was manifested as wilting, yellowing, defoliation, stem rot and stem shredding thereby, ultimately resulting in complete death of the plants.

S. rolfsii has been identified as a major soil borne fungus causing southern blight of cowpea worldwide resulting in 53.4 per cent yield loss (Sharma *et al*., 2002). It has been reported to cause damping off as well as stem rot of cowpea resulting in 40 per cent yield loss in other parts of the country (Kossou *et al*., 2001; Ferry and Dukes, 2002). Dwivedi and Prasad (2016) reported *S. rolfsii* as a major threat to common bean resulting in 100 per cent pre emergence damping off with 4.4 to 100 per cent disease severity. However, in Kerala, it was not identified as a major pathogen of vegetable cowpea, until recently.

Sclerotium rolfsii is a saprophytic soil borne fungal pathogen having a wide host range (Punja, 1985; Okabe *et al*, 2001; Billah *et al*., 2017). High levels of

variability, wide host range and facultative parasitic ability make it a fungus of great relevance. The survival rate of the fungus is also very high as it can survive as sclerotia in the soil up to and even, above seven years (Billah *et al*., 2017). Moreover, the fungus can cause wilting or stem rot or root rot resulting in plant death and complete yield loss. Thus, it is of utmost importance to undertake detailed study of the pathogen and the disease incited by it in vegetable cowpea.

The soil borne nature and facultative saprophytic ability of the fungus advocates development of integrated disease management strategies against the disease. Fungicides, soil fumigants, botanicals and biocontrol agents have been tested for their efficacy in the management of the disease (Dwivedi and Prasad, 2016). Studies confirmed that non judicious use of fungicides and soil fumigants resulted in environment pollution as well as health hazards. Hence, the present study was undertaken to develop an ecofriendly strategy for the management of *S. rolfsii*, an emerging soil borne fungus causing basal stem rot and blight of vegetable cowpea using various plants and oil cakes with the following objectives.

- Isolation of *S. rolfsii* from infected vegetable cowpea plants and pathogenicity testing
- *In vitro* screening of plants and oil cakes for their antifungal and biofumigation potential
- Compatibility studies of the effective biofumigants with *Trichoderma viride* (KAU)
- *In vivo* management studies using biofumigants and biocontrol agents
- Effect of biofumigation on soil fungal population dynamics

Review of Literature

2. REVIEW OF LITERATURE

Sclerotium rolfsii Sacc. (teleomorph *Athelia rolfsii* [Curzi] C. C. Tu. & Kimbr.) is one of the most destructive soil borne fungal pathogens worldwide with wide host range (Paul *et al*., 2017). Significant yield loss has been reported in all major crops by the fungus (Ferry and Dukes, 2002). Hence, detailed studies have to be undertaken on the pathogen as well as its management. Thus, the present study entitled "Biofumigation for the management of *Sclerotium rolfsii* in vegetable cowpea" was carried out with an objective to develop an eco-friendly strategy for the management of *S. rolfsii*, an emerging soil borne fungus of vegetable cowpea using the biofumigation potential of selected plants and oil cakes. The data pertaining to the importance of the fungus, disease caused, yield loss and *in vitro* as well as *in vivo* management trails have been reviewed and described in this chapter.

2.1. ETIOLOGY, SYMPTOMOTOLOGY AND ECONOMIC IMPORTANCE

S. rolfsii was first reported by Rolfs (1892) in tomato crop from Florida in USA. Shaw and Ajrekar (1915) for the first time isolated *S. rolfsii* in India from rotted potatoes. Ayock (1966) reported the 'H' like structure or clamp connections of the fungus. *S. rolfsii*, having wide host range in both agricultural and horticultural crops (Ferreira and Boley, 1992). Sclerotial bodies were mostly spherical and rarely irregular in shape and resembled the mustard seed, the size may vary from 0.1 to 3.0 mm (Naidu, 2000; Mohan *et al*., 2000; Anahosur, 2001).

Kamalakannan *et al.* (2007) observed the symptoms caused by *S. rolfsii* in stevia as yellowing and dropping of the leaves, along with wilting of plants and white silky mycelial growth at the collar region. The mycelial growth spread both upwards and downwards causing rotting of the infected tissue with characteristic mustard shaped sclerotial bodies. *S. rolfsii* in elephant foot yam initially resulted in rotting at collar region of stem where the plant ultimately toppled down (Singh *et al*., 2006). Kator *et al*. (2015) studied the *S. rolfsii* infection on tomato cultivars by artificial inoculation and reported the disease symptoms such as chlorosis, wilting, damping off, blighting and necrosis. Chandra *et al*. (2017) reported the *S. rolfsii* symptoms in sweet potato as

white dense mycelium along with sclerotia at collar region, resulting in quick rotting of roots and destruction of the crop in large areas of cultivation.

Tewari and Mukhopadhyay (2001) reported that even though chickpea wilt is a complex disease caused by several fungal pathogens but significant yield loss of 60-70 per cent was caused by *S. rolfsii*, *Rhizoctonia solani and Fusarium oxysporum f. sp. ciceri.* Fery and Dukes (2002) reported *S. rolfsii* infection in cowpea results in dry seed loss of 53.4 per cent.

Singh *et al*. (2003) did survey during 1999-2002 in betel vine growing tracts in (Uttar Pradesh) and reported that approximately 27 per cent crop was affected by *S. rolfsii* with 30 per cent disease severity. Gupta and Sharma (2004) observed 11-56 per cent yield loss of French bean due to crown rot caused by *S. rolfsii*. *S. rolfsii* in ragi reported to cause more than 50 per cent yield loss (Manu *et al*., 2012). Paparu *et al*. (2020) stated that stem rot of ground nut caused by *S. rolfsii* resulted in 80 per cent yield losses.

2.2. ISOLATION AND PATHOGENECITY

S. rolfsii can be isolated and purified from any part of the infected plant. De Tempe (1953) isolated *S. rolfsii* from the infected sunflower seeds using blotter technique. Ansari and Agnihotri (2000) isolated the fungus from lesions and sclerotia attached with the collar region of infected soybean plant. Anahosur (2001) isolated the pathogen from infected root and collar region of potato plant. Yaqub and Shahzad (2005) isolated and purified the *S. rolfsii* form the infected roots of mungbean and sunflower. Akram *et al*. (2007) isolated the pathogen from infected stem portion of chickpea near the soil line. Rakholiya and Jadeja (2011) made isolation of *S. rolfsii* from stem and pod of groundnut plant showing typical stem rot symptom.

Datar and Bindu (1974) tested the pathogenicity of *S. rolfsii* in sunflower crop by soil inoculation method. Rao and Prasad (1979) proved the pathogenicity of *S. rolfsii* by inoculating sand culture of pathogen in colocasia. Mishra and Bais (1987) used fungal culture grown on sand corn meal medium to test the pathogenicity of *S. rolfsii* on barley by mixing inoculum in upper 4-5 cm layer of soil.

Singh and Thapliyal (1998) tested the pathogenicity of *S. rolfsii* in soybean by seed dipping and soil infestation method by mixing soil with *S. rolfsii* culture raised on sorghum grain. Ansari and Agnihotri (2000) proved the pathogenicity of *S. rolfsii* in soybean by inoculating 5 mm mycelial disc of 7 days old culture at the collar region of the of the plant near soil line.

Rajalakshmi (2002) proved the pathogenicity of *S. rolfsii* in crossandra plant by placing 5 sclerotia per plant at collar region. Haral Patil and Raut (2008) tested the pathogenicity of *S. rolfsii* by placing mature sclerotia in poly bag containing 3 months old betel vine cuttings. Yaqub and Shahzad (2005) conducted pathogenicity of *S. rolfsii* in different crops by artificially infecting sterilized soil with one sclerotia per gram soil. Begum *et al*. (2011) confirmed the pathogenicity of *S. rolfsii* on 30 days old seedlings of potato by soil drenching with 7 days old fungal culture. Different isolates of *S. rolfsii* collected from different locations showed variation in pathogenicity in groundnut seedlings, one isolate resulted in high disease incidence and disease index; and rest of them caused medium and less disease incidence (Awasthi and Dasgupta, 2011). Mahato *et al*. (2017) described that different isolates of *S. rolfsii* exhibited morphological, cultural and pathogenic variability and also led to high, moderate and slow pathogenic reaction on artificial inoculation onto tomato seedlings. *S. rolfsii* with wide host range caused root rot and stem rot with significant white mycelial growth along with mustard shaped sclerotia on different crops – chilli, chickpea, groundnut, tomato, cowpea, pumpkin, pea, cotton and soybean (Billah *et al*., 2017).

2.3. CHARACTERIZATION OF SCLEROTIUM ROLFSII

2.3.1. Morphological Characterization

West (1961) described *S. rolfsii* as a fungus belonging to non-spore producing fungi group. It is a very fastly growing pathogen reaching 9 cm diameter within three days, where initially the fungal mycelium is silky white but gradually losses its luster. The small round mustard shaped sclerotia (coiling of vegetative mycelium), which serve as a resting structure is an outstanding morphological characteristic feature of the organism (Subramanian, 1964). Rao (2000) studied growth of the *S. rolfsii* as

characteristic white silky mycelium with significant number of mustard shaped sclerotia which were initially white colour later turn to chocolate brown of 0.5 to 2.5 mm in diameter. Anahosur (2001) reported that *S. rolfsii* was characterized with white fan/thread like mycelium with spherical or ellipsoidal sclerotia of 1-3 mm diameter. Rakholiya and Jadeja (2011) studied the morphological variation of thirty isolates of *S. rolfsii*, isolated from different groundnut cultivating tracts in Gujarat. Among them, most of the isolates produced white colonies except four with dull white colonies. Sclerotia of all the isolates were light to dark brown in colour whereas one isolate produced dark brown sclerotia; but all the isolates differed in their sclerotia size ranging from 1.0 to 1.7 mm. Waghunde *et al*. (2011) studied *S. rolfsii* isolated from finger millet and revealed that the mycelium was white coloured; covered 9 cm Petri plate within 3 to 4 days after inoculation and started forming sclerotia after 9 days. Sclerotial bodies were white in colour initially which turns brown at later stage. Chaurasia *et al*. (2014) observed the mycelial growth of *S. rolfsii* as white cottony, superficial dense hyphae which produced sclerotia within 4-5 days, as soft round white bodies, which were slowly turned to light brown and finally changed to dark brown and becomes relatively hard.

2.3.2. Molecular Characterization

Lyon and Lopezlavalle (2000) stated internal transcribed spacer (ITS) region of ribosomal DNA of the fungus can be targeted in PCR procedure. Once the targeted region of DNA is multiplied using PCR, the sequence of order of the four bases (designated by letters A, C, G, or T) in the DNA can be determined. The order of the bases in a given DNA is usually unique of an organism; and therefore acts like a signature to confirm its identity. Thus an unknown organism can be identified by size or sequence of a portion of its DNA. Genomic DNA of the fungus was isolated and internal transcribed region (ITS) of ribosomal DNA was amplified using the ITS1 and ITS4 universal primers. The rDNA sequence results showed 99 to 100 per cent similarity with reference sequence of AB075298.1 and JF966208.1 confirming the pathogen as *S. rolfsii* (Mahadevakumar *et al*., 2016)

2.4. FACTORS INFLUENCING GROWTH OF S. ROLFSII

Hari *et al*. (1991) reported mycelial growth and sclerotia production of *S. rolfsii* was best at pH 6. Singh and Gandhi (1991**)** observed maximum mortality of guar seedlings by *S. rolfsii* at pH 6.1 and an increase in pH over 8.4 significantly reduced the infection. Dey *et al*. (1992) reported maximum number of sclerotia was seen between pH 4-7. Mishra *et al*. (1996) recorded maximum mycelial growth and sclerotia formation at pH 6.5. Kulkarni and Kulkarni (1998) revealed that saprophytic activity of *S. rolfsii* was found to be maximum at pH 6.0. Tripathi and Khare (2006) reported that, pH of 5.0 and 6.0 was the optimum for mycelial and sclerotia production of *S. rolfsii*. Banyal *et al*. (2008) reported incidence of collar rot of tomato caused by *S. rolfsii* was more at pH 6 and also stated that cent per cent disease incidence was observed at pH 6.5 and minimum disease was seen at pH 8. Chaurasia *et al*. (2014) tested the different levels of pH on mycelial growth and sclerotia formation of *S. rolfsii*, and reported pH 5.0 was optimum for mycelial growth whereas pH 4.0 to 7.0 was optimum for the sclerotia formation. Zape *et al*. (2013) reported that even though *S. rolfsii* could grow in wide range of pH, 6.5 pH was optimum for maximum mycelial growth and sclerotia formation.

Lingaraju (1977) studied the effect of different moisture levels on *S. rolfsii* growth and reported the growth of the fungus was significantly high at 10 per cent soil moisture and the fungus did not survive when the soil moisture was raised to above 50 per cent. Nargund (1983) reported, survival and yield of wheat seedlings were considerably higher in the sick soil plots with 6 irrigations than those with 3 irrigations. Palakshappa (1986) evaluated the effect of different soil moisture levels on *S. rolfsii* causing foot rot of betelvine and reported the fungus survives considerably better at lower moisture levels between 20 and 40 than at higher levels of 60 and 70 per cent. Palakshappa *et al*. (1989) and Devi *et al*. (1999) stated *S. rolfsii* survived better at low soil moisture levels compared to high moisture. Banyal *et al*. (2008) reported that soil moisture level was inversely proportional to collar rot incidence and intensity.

Thiribhuvanamala *et al*. (2000) tested the effect of 3 inoculum levels *i.e.* 5, 10 and 15 sclerotia of *S. rolfsii* in tomato and reported that the increase in inoculum load

resulted in increased disease incidence of 72.5, 87.5 and 97.5 respectively. Sugha *et al*. (1993) also reported inoculum load and disease incidence by *S. rolfsii* in chickpea was directly proportional. Yaqub and Shahzad (2005) reported a negative correlation between the inoculum level of *S. rolfsii* and plant biometric characters. Muthukumar and Venkatesh (2013**)** tested the different inoculum levels and found the maximum incidence of collar rot at per cent inoculum, followed by 4 per cent. Thus, the results revealed that the disease incidence increased with increase in inoculum level.

2.5. *IN VITRO* INHIBITION OF S. ROLFSII

2.5.1. Fungicides

Dutta and Das (2002) reported that mancozeb was more effective in inhibiting both mycelium growth (76.5%) and sclerotia formation (98.6%) of *S. rolfsii*. Prabhu and Hiremanth (2003) screened both systemic and contact fungicides against *S. rolfsii* causing collar rot of cotton and revealed among contact fungicide mancozeb showed maximum percentage inhibition of both mycelium and sclerotia. Singh *et al*. (2004) tested the effect of different concentrations of six fungicides on the *S. rolfsii* isolated from betelvine. The results revealed that 3-thioallophanate and mancozeb shows cent per cent inhibition on both mycelial and sclerotia growth. Yaqub and Shahzad (2005) stated that mancozeb and mancozeb combination fungicides are effective in reducing both mycelial growth and sclerotia formation of *S. rolfsii*, whereas least inhibition of *S. rolfsii* was observed in carbendazim at different concentrations tested (Kulkarni, 2007). Manu *et al*. (2012) screened 14 fungicides and 5 bioagents *in vitro* against *S. rolfsii* inciting foot rot of ragi. Systemic fungicides *viz*., hexaconazole, propiconazole, difenconazole and vitavax were found effective, whereas contact fungicide, mancozeb results in cent per cent inhibition. Pandav *et al*. (2013) reported hexaconazole (0.1 %), propiconazole (0.1 %), mancozeb (0.1 % and 0.2 %) and captan (0.1 %) completely inhibited the mycelial growth and sclerotia formation of *S. rolfsii*. Carbendazim (0.1 %) resulted in only 31.11 per cent inhibition with 37 sclerotia formed over control**.** Mahato *et al*. (2014) screened systemic and contact fungicides; among them mancozeb resulted significant inhibition (90.66 %) whereas copper oxychloride had negligible

effect (20.17 %) on reduction of mycelial growth as well as sclerotia formation of *S. rolfsii*.

2.5.2. Biofumigants

Alice (1984), evaluated the antifungal properties of 31 selected plant extracts against *S. rolfsii* and reported that extracts of *Piper betele, Eucalyptus sp. Allium sativum, Mentha pierita, Azadiracta indica* could inhibit mycelial growth and sclerotia germination. Mahfuzul (1997) tested selected plant extracts like garlic (*A. sativum*), ginger (*Zingiber officinale*), dolkalmi (*Ipomoea fistulosa*), nisinda (*Vitex negundo*), and marigold (*Tagetes erecta*) against the chilli seed borne fungal pathogens; and reported that garlic was found to show significant inhibition in fungal growth. Kurucheve and Padmavathi (1997) reported that, garlic clove recorded the minimum mycelial growth under the *in vitro* condition. Prithiviraj *et al*. (1998**)** reported the antifungal properties of garlic against plant pathogens is due to antimicrobial components like allicin, E-and Z-ajoene, iso-E-10-devinylajoene etc., which are antibacterial and antifungal in nature. Islam *et al*. (2005) evaluated *in vitro* efficacy of plant extracts against *R. solani* and *S. rolfsii* infecting peanut and reported garlic extract could significantly inhibit mycelial growth of both the pathogens. Islam and Faruq (2012) reported seed treatment of some winter vegetables *viz*., cabbage, tomato and eggplant with extracts of neem leaf, garlic clove, allamonda leaf, ginger rhizome, kalijira seed, bel leaf, turmeric rhizome, kata mehedi leaf and onion bulb against damping-off significantly reduced the disease incidence over untreated control and significant result was seen with neem leaf extract followed by garlic clove extract. Kumar *et al*. (2012) tested the antifungal potential of effects of aqueous leaf extracts of garlic, onion, pearl millet, sunflower and sorghum at 5% concentration and found all the extracts were effective in reducing the mycelial growth and sclerotial germination of *S. rolfsii*. Darvin (2013) evaluated eight plant extracts *viz*., ashoka (*Polyalthia longifolia*), garlic (*A. sativum*), ginger (*Z. officinalis*), neem (*A. indica*), seetha phal (*Annonas squamosa*), tulasi (*Ocimum sanctum*), milk weed (*Calotropis gigantean*) and perwinkle (*Vinca rosea*) at 10 per cent concentration on growth of *S. rolfsii* isolated from groundnut and reported clove extract of garlic recorded lowest mycelial growth (0.0 cm) and highest per cent inhibition (PI) (100%) under *in vitro* condition. Dwivedi and Prasad (2016) tested and reported plant extracts

viz., garlic clove, ginger rhizome, neem leaf, neem seed oil, turmeric rhizome, onion bulb and biological control were more significant than chemical control for the successful management of *S. rolfsii*. Sahana *et al*. (2017) evaluated eight plant extracts *viz*., onion bulb extract, garlic clove extract, tulsi leaf extract, neem leaf extract and marigold leaf extracts, at 5, 10 and 15 per cent concentrations and found that highest inhibition percentage (100%) was showed by onion bulb and garlic clove extract followed by marigold leaf extract (90.78%), neem leaf extract (91.11%) and tulsi leaf extract (53%). Mahato *et al*. (2018) evaluated the efficacy of eight medicinal plant extracts *viz*., *Curcuma along, Z. officinale, A. indica, A. sativum, Catharanthus roseus, Andrographis paniculata, Allium cepa and O. sanctum* against *S. rolfsii* mycelial growth and sclerotia formation by poisoned food technique at three concentrations (5, 10 and 20%) under *in vitro* condition and found maximum inhibition (84.89 %) of mycelial growth and sclerotium production of *S. rolfsii* was showed by *A. sativum* followed *A. indica* (80.86%).

2.5.3. Oil Cakes

Gautam and Kolte (1978) reported neem cake and mustard cake were equally effective in reducing the mycelial growth and sclerotial formation of *S. rolfsii* whereas castor cake is comparatively inferior. Harender Raj *et al*. (1996) tested the efficacy of three oil cakes such as (groundnut, mustard and sesamum) and stated that mustard cake at 2 per cent was effective in reducing fungal pathogen population. Chattopadhyay *et al*. (1999) reported, mustard cake amendment found to be most effective in inhibiting all three soil borne fungal pathogens (*M. phaseolina, F. oxysporum and S. rolfsii*) of chickpea followed by green manure and farm yard manure. Upmanyu *et al*. (2002) stated cotton, mustard and neem cakes were considerably effective in cent per cent inhibition of *S. rolfsii* growth. Jha *et al*. (2007) studied *in vitro* effect of organic amendment extracts (each @ 10 %) on mycelial growth of *Sclerotium* in *Phaseolus vulgaris* and reported that among the amendment extracts tested, maximum mycelial growth inhibition was recorded in saw dust extract (48.88 %) followed by castor cake extract (27.41 %), mustard cake extract (18.15 %) and neem cake extract (18.15 %). Fayzalla *et al*. (2009) explored biofumigant effect of mustard oil cake in the management of soil-borne pathogens such as *R. solani, M. phaseolina* and *S. rolfsii*

causing damping off, root rot and wilt diseases of soybean under *in vitro*; and recorded 92.2% inhibition of *R. solani* at 25 mg/Petri dish. Meena *et al*. (2014) tested the inhibitory effect of neem cake and mustard cake on *M. phaseolina* and reported maximum mycelial growth inhibition (19.42%) with mustard cake extract at 20% followed by 15% and 10%. Aparna and Girija (2018**)** reported mustard oil cake have effective bio-fumigant action on the pathogen at 10 per cent concentration.

2.6. COMPATIBILITY OF BIOFUMIGANTS WITH TRICHODERMA SPP

Sanchi *et al*. (2004) reported *Sclerotinia sclerotiorum* and *Sclerotinia minor* were more sensitive than *Trichoderma harzianum*-T39 to isothiocyanates released from sinigrin and also stated T39 was able grow even at the highest concentration glucosinolate (5 µmoles). Several studies proved that *Trichoderma* spp were compatible with botanicals including garlic, onion etc. and their combination was successful in inhibiting several soil borne phytopathogens (Ashwani *et al*., 2012; Bheemaraya, *et al*., 2012; Mukesh, 2014).

Lazzeri *et al.* (2013) reported, biofumigation can be integrated with biological control agents for synergic effect to control soil-borne fungal pathogens (*Pythium, Rhizoctonia, Sclerotinia*) because of the less sensitivity of *Trichoderma* to isothiocyanates, in fact, the integration will maximize the effectiveness of both treatments. Mukesh, (2014) studied the compatibility of neem, onion and garlic extracts with seven *Trichoderma* spp. and reported lower concentrations of these biofumigants were highly compatible with all the isolates and slight inhibition was seen at 15 per cent concentration. In fact only *T. harzianum*-T39 is able to grow in 5 µmoles glucosinolate per plate, the highest concentration tested.

2.7. *IN VIVO* MANAGEMENT STUDIES WITH BIOFUMIGANTS AND BIOCONTROL AGENTS

Generally soil-borne fungal pathogens have traditionally been controlled by soil fumigation with the synthetic compounds, methyl bromide and methyl isothiocynate (Kirkegaard and Matthiessen, 2004). Control of soil-borne pathogens based on naturally produced compounds extracted from plants has been termed as biofumigation

(Kirkegaard and Matthiessen, 2004) and the compounds most extensively investigated are the glucosinolates and their hydrolysis products such as isothiocynates, which are actual biocidal compounds. Substituting the synthetic carcinogenic fumigants with plant based isothiocyanates has recently gained much interest, especially after the ban of methyl bromide in 2006 (according to the Montreal protocol) because of its negative impact on the ozone layer.

2.7.1. Disease Suppression

Sivaprakash *et al*. (2011) studied the antifungal activity of different plant extracts against *Pythium aphanidermatum* inciting damping-off disease in tomato and revealed A. sativum (10%) and *A. cepa* var. *aggregatum* (20%), leaves of *Lawsonia inermis, Piper betle* (20% each), *Eucalyptus globulus* (40%) and *Vitex negundo* (40%) recorded cent per cent disease suppression. Zope *et al*. (2014) reported soil application of Kali haldi (root), Bawchi (seed) and Ashwagandha (leaf), Ashwagandha treatment (5%) showed minimum total incidence of collar rot (20.00%), root rot (23.33%) and wilt (16.67%) over control (100, 96.67 and 96.67%) respectively. Mahato (2018) tested *in vivo* efficacy of eight medicinal plant extracts *viz*. *Andrographis paniculata, Z. officinale, A. indica, A. sativum, Curcuma longa, C. roseus, A. cepa* and *O. sanctum* were evaluated against *S. rolfsii* inciting collar rot on tomato. Among all the extracts tested at 20 % concentration, *A. indica* shows 67.26 % reduction in disease incidence followed by *A. sativum* (62.65%). Awad (2016) tested *in vivo* the antifungal potential of six plant extracts and reported garlic extract at one per cent recorded only 29.25% disease incidence compared to the control (88.16%). Utobo (2016) reported three extracts, i.e. ginger rhizomes, garlic bulbs, and neem leaves tested at three different concentrations (4, 8, and 12%), and revealed that cucumber seeds treated with all these plant extracts at given concentrations significantly reduced the pre-and post-emergence damping-off and also weekly sprays of plant extracts significantly suppressed the incidence and severity of downy mildew. Wavare *et al*. (2017) reported 4 per cent marigold flower extract was found to reduce collar rot disease incidence of chickpea by 70.56 per cent.

Garrett (1956) defined biological control as the control of the disease by the application of biological agents to a host plant that prevents the development of disease by a pathogen where the biocontrol agents are usually bacterial or fungal strains isolated from endosphere or rhizospere. Fungi of the genus *Trichoderma* spp. are the most promising biocontrol agents of many plant pathogenic fungi (Papavizas, 1985; Chet *et al*., 1981). The hyphae of *Trichoderma* spp. have been known to parasitize many pathogenic fungi *viz*., *Sclerotium, Sclerotinia, Rhizoctonia, Helmenthosporium, Fusarium, Arimilaria, Colletotrichum, Rhizopus, Fusicladium, Botrytis* etc., (Chet *et al*., 1981; Wolfechechel and Jenson, 1992; Kumar and Mukherji, 1996; Bandyopadhyaya *et al*., 2001). The suggested mechanisms for biocontrol are antibiosis, lysis, competition and mycoparasitism (Cook and Baker, 1983; Hadar *et al.*, 1984). All these mechanisms may act singly or in combination for successful control of the pathogen.

Weindling (1932) was first to show that, *Trichoderma viride* pers. Fr. is a common promising saprophytic fungus able to parasitize the mycelia and sclerotia of *S. rolfsii* due to the secretion of an antibiotic called gliotoxin which was later identified as 'viridin' (Brain *et al*, 1946). Swathi *et al*. (2015) stated volatile metabolites of Tv5 isolate of *Trichoderma viride* shows 54.6 per cent inhibition over *S. rolfsii*. *Trichoderma viride* was most effective (91.31%) to reduce mycelia growth and sclerotia formation of *S. rolfsii* followed by *Trichoderma harzianum* (84.92%) and *Trichoderma virens* (84.29%) (Kushwaha *et al*., 2018).

2.7.2. Biometric Characters

Bhagat (2014) evaluated the effects of cowdung, rabbit manure and poultry manures on growth of tomato seedlings to observe growth promotion and percentage increase of shoot length in tomato seedlings and reported that among the three treatments, cow dung recorded better growth of tomato seedlings than that of rabbit manure and poultry manure.

Studies revealed that sulfur-containing compounds of garlic and garlic creeper can improve bud break in grapevines (Kubota *et al*., 2003; Potjanapimon *et al*., 2008;

Vargas-Arispuro *et al*., 2008; Botelho *et al*., 2010). Garlic and onion bulb extracts increased the floral bud break percentage, fruit set and fruit yield of the apple trees due accumulation of total indoles (precursors for auxin synthesis) (Perussi *et al*., 2010). Allelochemicals isolated from garlic seedlings and root exudates promoted growth of the receiver plants (Zhou *et al*., 2011). The allelopathic efficacy of sulphur compounds containing plant extracts has been found to alter the ecology of the cropping systems by improving soil conditions which ultimately results in improved growth of receiver plants (Ahmad *et al*., 2013). Cheng *et al*. (2016) reported diallyl disulphide from garlic significantly promoted root growth, cell size, mitotic activity of meristem and increased shoot length in tomato seedlings.

Garlic contains beneficial chemical ingredients such as enzymes, vitamins, flavonoids, organosulfur compounds *viz.*, allicin, diallyl-disulfide and thiosulfinates which have strong antimicrobial potential, growth promotion activity and alleolopathic effect (Bhuiyan *et al*., 2015). Hayat *et al*. (2018) reported application of aqueous extract of garlic as foliar spray or fertigation, resulted in increased plant height, leaf area, stem diameter, plant fresh and dry weight in addition to increased activity of superoxide dismutase (SOD) and peroxidase (POD). El-Rokiek *et al*. (2019) reported shoot length, number of branches and fresh and dry weight of quinoa plant significantly increased with garlic clove extract (15%) and Eucalyptus leaf extract (10%) and also revealed garlic extract and eucalyptus extract spray on quinoa plants caused significant increases in chlorophyll a, chlorophyll b and carotenoids over untreated control.

Kleifeld and Chet (1992) revealed application of *T. harzianum* resulted in increased pepper seed germination, leaf area, and length of the pepper seedlings, the emergence of bean, cucumber, pepper, radish and tomato seedlings, and the dry weight of cucumber plants. There are other studies also reporting *Trichoderma* strains significantly increased the fresh weight of shoots and roots as well as root length in peas (Naseby *et al*., 2000) and the dry weight of shoots in corn (Va squez *et al*., 2000). In cucumber plants, there was an 80 per cent increase in root area and 75 per cent increase in root length, shoot length by 45%, dry weight by 80%, and leaf area by 80% (Yedidia *et al*., 2001). Pan *et al*. (2009) reported seed treatment and soil application of *T. harzianum* at 5 kg/plot resulted reduction in collar rot incidence in groundnut (17%)

as well as more leaves and pods/plant. Rakholiya and Jadeja (2011) evaluated various fungicides and bioagents as seed treatment for the management of *S. rolfsii* in groundnut under field conditions; and reported there is significantly least disease incidence (37.43%) and high yield (1464 kg/ha) by seed treatment with *T. harzianum* @ 10 g/kg seed. Hermosa *et al*. (2012) reported many of the *Trichoderma* strains are known to have direct interaction with the roots of plants, thus increasing the growth potential, disease resistance, and abiotic stress tolerance. Stewart and Hill (2014) reported that *Trichoderma* helped in plant growth promotion by enhanced nutrient uptake, increased carbohydrate metabolism, photosynthesis, and phytohormone synthesis.

2.8. BIOFUMIGATION AND SOIL FUNGAL DYNAMICS

Lazzeri and Manici (2001) reported incorporation of fresh tissues of Capparidacea family members supressed *Pythium* sp and also acts as green manure. Mustard and turnip when used as a biofumigants found to suppress weeds and soilborne pests (Vaughn *et al*., 2005). Plants containing glucosinolates when used as a cover crops shows suppression of the soil-borne diseases in potato cropping systems (Snapp *et al*., 2007). In a field experiment, *Pisum sativum, Brassica oleracea* and *Sorghum vulgare* were incorporated into soil which resulted in more or less similar suppressive effects on the *Verticillium dahlia* population (Ochiai *et al*., 2008). Exposure of soil to 100 µl ITC (expand it) for 4 and 7 days resulted in the mortality of 50 and 100 per cent respectively against *S. rolfsii*. No viable sclerotia of the fungus was detected to the depth of 10 cm in the air dried, moist or wet soil fumigated for 7 days (Dhingra *et al*., 2013). Meng *et al*. (2018) reported soil biofumigation with Brassicaceae family members had been found to suppress soil-borne pathogens and changed the structure of the soil microbial community. The suppressive effect may be due to the fungicidal and bactericidal effect of their glucosinolate hydrolysis products. Hansen *et al*. (2019) reported Winter canola associated cropping system contained significantly less microbial biomass which may be due to biocidal secondary metabolites (ITC) of canola resulting in the decline of soil microbial biomass. These results demonstrated the relationship between soil microbial community composition and crop productivity.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled “Biofumigation for the management of *Sclerotium rolfsii* in vegetable cowpea” was conducted during 2018-2020. The laboratory and pot culture studies were undertaken at Integrated Farming System Research Station (IFSRS), Karamana and Department of Plant Pathology, College of Agriculture, Vellayani. Six isolates of *S. rolfsii* were isolated from vegetable cowpea and the most virulent isolate was selected for further *in vitro* and *in vivo* management studies. Selected plant extracts and oil cakes were tested for their biofumigation and antifungal potential against the fungus. The details of the materials used and the methodology followed are described in this chapter.

3.1. ISOLATION OF BASAL STEM ROT AND BLIGHT PATHOGEN OF VEGETABLE COWPEA

3.1.1. Collection of Infected Plant Samples

Basal stem rot and blight disease affected vegetable cowpea plant samples were collected from different vegetable cultivating areas. Thus, six isolates *viz*., Sr_1 (from Pathanamthitta in var. Gitika), Sr_2 (from Karamana in var. Vellayani Jyothika), Sr_3 (from Vellayani in var. Vellayani Jyothika), Sr_4 (from Indian Institute of Vegetable Research, Varanasi in var. Kashi Kanchan), Sr_5 (from Karamana in var. Gitika) and Sr_6 (from Karamana in var. NS 621) of *S. rolfsii* were collected. Observations on the stage of the crop affected, nature of the disease symptoms and the presence or absence of sclerotia on the infected plants were recorded.

3.1.2. Isolation and Purification of the Pathogen Isolates

Six isolates of *S. rolfsii* were isolated from basal stem rot and blight disease affected vegetable cowpea plants using the procedure described by Rangaswami (1958). Infected parts along with healthy portions of the plant samples were separately chopped into small pieces. These pieces were washed with sterile water and surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) for one min followed by three washings with sterile water. Excess moisture was removed using sterilized blotting

paper and the bits were aseptically transferred to potato dextrose agar (PDA) medium which was melted and poured in sterilized Petri plates. These plates were incubated at room temperature (28 ± 1ºC). The mycelial growth of each isolate developed from the bits was transferred to Petri plates containing fresh PDA medium. The fungal isolates were subcultured on PDA slants and incubated at 28±1°C (Aneja, 2003). The pure culture of the fungal isolates were maintained separately in refrigerator at 5°C. Subsequently, virulence rating under *in vitro* and *in vivo* conditions were undertaken to identify the most virulent isolate among the six fungal isolates collected.

3.1.3. Virulence Rating of the Isolates

Virulence rating was undertaken to identify the most virulent isolate of the fungus. This was performed to study the symptomatology and growth characters of the different isolates of *S. rolfsii.*

3.1.3.1. Symptomatology of Isolates in Vegetable Cowpea on Artificial Inoculation

In vivo study was conducted using stem inoculation method as described by Inacio *et al*. (2017). Two week old vegetable cowpea seedlings (var. Gitika) were artificially inoculated at the basal part of the stem using seven day old five 5 mm mycelial discs of each of the six fungal isolates. The inoculated seedlings were enclosed and maintained in perforated polythene covers to provide adequate humidity for the disease development. Various observations including days for lesion initiation, lesion size and number of sclerotia produced on the seedlings inoculated with each isolate of the fungus were recorded.

3.1.3.2. Mycelial and Slerotial Characters of Isolates

All the six isolates were grown in PDA media. The mycelial characters including growth of mycelia at different durations, days for completing mycelial growth, colour and colony pattern were recorded. The various sclerotial character *viz*., presence or absence of sclerotia, days for sclerotial initiation, number of sclerotia on 15^{th} day after inoculation (DAI) as well as the shape, colour, arrangement and degree

of formation of sclerotia were recorded. The degree of formation of sclerotia was described as per the procedure developed by Chaurasia *et al*. (2013).

3.1.3.3. Selection of the Most Virulent Isolate of S. rolfsii

The most virulent isolate of *S. rolfsii* among the six isolates was selected based on the mycelial and sclerotial characters revealed from *in vitro* studies as well as based on the development of lesions and sclerotia from *in vivo* studies.

3.1.4. Pathogenicity Studies

3.1.4.1. Soil Inoculation

Soil inoculation method as described by Pande *et al*. (1994) was followed to perform the pathogenicity test. The inoculum of *S. rolfsii* (Sr_1 isolate) multiplied on sand oats medium was mixed with the top 5-6 cm layer of sterilized soil at the rate of two per cent (weight basis) inoculum level and the mixed soil was moistened with sterile water. Sterilized soil without pathogen inoculum was maintained as un-inoculated control. Two week old vegetable cowpea seedlings (var. Gitika) were transplanted to the inoculum containing soil and the pots were watered daily. The artificially inoculated plants were enclosed in perforated polythene covers for disease development. The plants were observed for the appearance of disease symptoms. The pathogen was re-isolated when the seedlings exhibited characteristic symptoms of basal stem rot and blight disease caused by *S. rolfsii*. The days taken for symptom development as well as the nature of symptoms were recorded.

3.1.4.2. Stem Inoculation

Stem inoculation method was undertaken as per the procedure developed by Inacio *et al*. (2017). Seven day old, five mm mycelial discs of the virulent isolate (Sr_1) of *S. rolfsii* was placed at the basal stem portion of two week old vegetable cowpea seedlings (var. Gitika) and the inoculated portions were covered with moistened cotton. The seedlings were maintained and the symptom development was observed as per the procedure followed in 3.1.4.1.

3.1.4.3. Leaf Inoculation

Leaf inoculation method was followed as per the methodology described by Kull *et al*. (2003). Healthy leaves from vegetable cowpea plants (var. Gitika) were detached, washed with sterile water and surface sterilized with 90 per cent alcohol. The leaves were inoculated with seven day old, five mm mycelial discs of the virulent isolate (Sr_1) of the fungus. The inoculated leaves were enclosed and maintained in perforated poly propylene covers for symptom development. The days for symptom development and the nature of the symptoms were recorded following the methodology as per 3.1.4.1.

3.2. PATHOGEN CHARACTERIZATION

The various morphological and cultural characteristics of the virulent isolate (Sr_1) of *S. rolfsii* was studied in detail.

3.2.1. Morphological Characters

The morphological characters of the isolate including nature of mycelium, nature of cytoplasm and clamp connections were studied by preparing slides of the fungus using cotton blue stain. The sclerotia were smashed and observed under microscope. The slides were observed at 1000X magnification.

3.2.2. Cultural Characters

The virulent isolate (Sr_1) of the fungus was grown on PDA medium. 15 ml of the medium was poured into sterile Petri plates for solidification under aseptic conditions and five mm mycelial discs of seven day old fungal culture was inoculated at the centre of the Petri plates. The Petri plates were incubated at room temperature (28 ± 1^0C). Observations on mycelial characters such as mycelial growth (diameter in cm), colour, colony pattern as well as various sclerotial characters including days for sclerotial initiation, number of sclerotia produced on 15 DAI, its shape, colour, arrangement in Petri plate and degree of formation were recorded.

3.2.3. Identification of the Fungus

The identification of the fungus was done based on its morphology, cultural characters as well as by molecular characterization.

3.2.3.1. Morphological and Cultural Identification

The morphological and cultural identification were done based on mycelial and sclerotial characteristics both under microscope and on PDA medium (Tanimu *et al.*, 2018)

3.2.3.2. Molecular Identification

The molecular identification of the fungus was done based on the internal transcribed spacer (ITS) region of rDNA of the fungus. The DNA was extracted using NucleoSpin® Plant II Kit. The ITS region was amplified using ITS1/ITS4 specific primers by polymerase chain reaction. Big Dye Terminator v3.1 (White *et al*., 1990) was used to undertake DNA sequencing. The resulting sequence was blasted in National Centre for Biotechnology Information (NCBI) database to reveal the percentage identity with all known deposited fungal sequences.

3.2.4. Standardization of Optimum pH for the Growth of *S. rolfsii*

A study was undertaken to identify the optimum pH which favour the maximum mycelial growth and sclerotial production of *S. rolfsii*. PDA medium with different levels of pH *viz*., 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 were prepared by adjusting the pH with HCl and NaOH. The calibration of pH was made using a pH metre. Fifteen ml molten PDA medium having different levels of pH were poured separately into Petri plates and allowed to solidify. Seven day old, five mm mycelial discs of *S. rolfsii* were placed on the medium. The inoculated plates were incubated at room temperature (28 ± 1^0C). The mycelial growth (diameter in cm) was recorded at every 24 h interval upto four days. The number of sclerotia produced per plate was recorded at 15 DAI (Tanimu *et al.*, 2018).

3.2.5. Standardization of Optimum Moisture Percentage for the Growth of *S. rolfsii*

The standardization of moisture percentage for the optimum growth of the fungus was performed as per the methodology described by Maiti and Sen (1988). Sterilized soil was filled in plastic cups (400 ml capacity). The inoculum of the fungus multiplied on sand oats medium was added into the soil and different levels of moisture *viz*., 35, 45, 50, 60, 70 and 80 per cent were maintained by adding the required quantity of sterile, distilled water at every 24 h interval. The moisture percentage at each level was calibrated using a soil moisture meter. Two week old vegetable cowpea seedlings (var. Gitika) were transplanted into the soil maintained at each level of moisture. Each treatment was replicated thrice. The observations on the initiation of basal stem rot and blight disease symptoms in the seedlings were recorded at each moisture level.

3.2.6. Standardization of Inoculum Level of the Fungus for Disease Development

Soil was sterilized in an autoclave at 121^0C temperature and 15 psi pressure for 15-20 min for two consecutive days. The sterilized soil was filled in plastic cups of 300 g capacity. Different concentrations (1, 2, 3, 5, 7, 9 and 11%) of the inoculum of *S. rolfsii* multiplied on sand oats medium for two weeks were added as a layer on the top of soil taken in each cup. The inoculum levels were prepared as per the methodology described by Yaqub and Shahzad (2005). One gram of the inoculum was added to 100 g soil to prepare one per cent inoculum amended soil. The same procedure was followed to prepare all other concentrations (2, 3, 5, 7, 9 and 11%). Cups filled with sterilized soil alone without any fungal inoculum was maintained as the control. The cups were incubated at room temperature (28 ± 1^0C) for one day. After 24 h, two week old, vegetable cowpea seedlings (var. Gitika) were transplanted into the cups. The number of days taken for the seedlings to exhibit the disease symptoms was recorded separately in each treatment.

3.2.7. Screening of Fungicides for the Inhibition of Growth of *S. rolfsii*

Five fungicides, *viz*., carbendazim (0.1%), wettable sulphur powder (0.25%), copper oxychloride (0.3%), mancozeb (0.3%) as well as the combination of mancozeb and carbendazim (0.20%) were screened to test their potential in inhibiting the mycelial growth of the fungus. Poisoned food technique (Nene and Thapliyal, 1993) was followed for the evaluation of the selected fungicides *in vitro*. Seven day old, five mm discs of the pathogen was placed at the centre of Petri plates containing 15 ml of PDA medium poisoned with each fungicide. Petri plates containing mycelial discs of the pathogen placed on PDA medium unamended with fungicides served as the control. The plates were incubated at room temperature (28 ± 1^0C). The mycelial growth (diameter in cm) of the fungus in each treatment was recorded when there was complete growth of the fungus in the control plates. The percentage inhibition of mycelial growth was calculated as per the procedure developed by Vincent (1947).

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

Where, I= Percentage inhibition of the mycelial growth (diameter in cm) of *S. rolfsii*

C= Mycelial growth (diameter in cm) of *S. rolfsii* in untreated control

T= Mycelial growth (diameter in cm) of *S. rolfsii* in the various treatments

3.3. *IN VITRO* MANAGEMENT STUDIES

Selected plant extracts and oil cakes were tested for their biofumigation and antifungal potential in inhibiting the mycelial growth and mycelial regeneration from sclerotia following paired plate technique (Prasad *et al*., 2018) and poisoned food technique (Nene and Thapliyal, 1993). Confined container technique was also performed in which the mycelial regeneration from sclerotia was checked at weekly intervals for a duration of one month to assess the biofumigation potential of the plant extracts and oil cakes in inhibiting the sclerotial germination of the fungus.

3.3.1. Assessing the Antifungal Potential of Plant Extracts and Oil cakes against Mycelia and Sclerotia of *S. rolfsii*

Table 1. Treatments selected for *in vitro* inhibition assay of *S. rolfsii*

Treatments	Description
T_1	Mustard (whole plant, except roots)
T_2	Cabbage (whole plant, except roots)
T_3	Garlic creeper (Leaves)
T_4	Castor (Leaves)
T_5	Cauliflower (whole plant, except roots)
T_6	Onion and garlic (bulbs)
T_7	Garlic (bulbs)
T_8	Onion (bulbs)
T_9	Castor cake (powdered cake)
T_{10}	Mustard cake (powdered cake)
T_{11}	Pongamia cake (powdered cake)
T_{12}	Untreated control

3.3.1.1. Antifungal Potential of Plant Extracts against S. rolfsii

The plant samples (mustard, cabbage, cauliflower, onion, garlic, garlic creeper and castor) were collected and washed thoroughly. The extracts were prepared as per the method described by Paul and Sharma (2002). One hundred g of each plant sample was weighed and ground with equal amount of sterile distilled water in 1:1 ratio (w/v basis). The macerated plant sample was filtered using muslin cloth and centrifuged at 6000 rpm for 25 min. The supernatant was collected and filtered through Whatman No. 42 filter paper. The resultant extract was maintained as the standard (100 per cent concentration). Fifty ml molten double strength PDA medium was mixed with one ml

of the plant extract which was diluted with 49 ml of sterile distilled water. The whole solution was taken in 250 ml conical flask. This resulted in the preparation of one per cent plant extract amended poisoned medium. The same procedure was followed to prepare 5, 10, 15, 20 and 25 per cent plant extract amended poisoned media. The conical flasks were rotated clock wise and anticlock wise so that the contents got thoroughly mixed and 15 ml of each concentration of the plant extract was poured into the Petri plates and allowed to solidify. Seven day-old five mm mycelial discs and sclerotia of *S. rolfsii* were placed separately on the centre of PDA medium poisoned with each of the plant extract concentration to assess the concentration which resulted in the inhibition of both the growth of the mycelia and the mycelial regeneration from sclerotia of the fungus. The untreated control contained the mycelial disc of the fungus and its sclerotia inoculated separately on PDA medium alone. All the treatments were replicated thrice. The plates were incubated at room temperature (28 ± 1^0C). The mycelial growth (diameter in cm) of *S. rolfsii* was measured in all the plates when there was complete mycelial growth in untreated control plates. The percentage suppression of mycelial was calculated as in 3.2.7.

3.3.1.2. Antifungal Potential of Oil Cakes against S. rolfsii

The extracts of the three oil cakes (mustard, pongamia and castor cakes) were prepared as mentioned in 3.3.1.1. and filtered through bacterial filters (0.22 µm). To prepare one per cent concentration of the poisoned media, 50 ml melted double strength PDA was mixed with 1 ml oil cake extract diluted with 49 ml sterile distilled water and was taken in 250 ml conical flask. The same procedure was followed to prepare 5, 10,15, 20 and 25 per cent oil cake amended poisoned media. The conical flasks were rotated clock wise and anticlock wise until the contents were thoroughly mixed. Fifteen ml of each concentration was poured into the Petri plates and allowed to solidify. Seven day-old five mm mycelial discs of *S. rolfsii* and sclerotia was placed separately at the centre of PDA medium poisoned with each concentration of the oil cake extract. The untreated control contained the mycelial disc and sclerotia of the fungus inoculated separately on PDA medium alone. The treatments were replicated thrice. All the plates were incubated at room temperature (28 ± 1^0C). The mycelial growth (diameter in cm) of *S. rolfsii* was measured in all the plates when there was complete mycelial growth in

untreated control plates. The percentage suppression of mycelial growth was calculated as in 3.2.7.

3.3.2. Biofumigation Potential of Plant Extracts and Oil Cakes against Mycelia and Sclerotia

3.3.2.1. Biofumigation Potential of Plant Extracts against S. rolfsii

Fresh plant samples (mustard, cabbage, garlic creeper, castor, cauliflower, combination of garlic and onion bulbs, garlic bulbs and onion bulb separately) were collected and washed with tap water to remove dirt. One hundred g of the samples were weighed and macerated with equal amount of sterile distilled water (w/v basis) as per the methodology described by (Charron and Sams, 1999). Two equal sized, bottom lids of Petri plates were selected. 15 ml of PDA medium was poured in the upper plate and allowed to solidify. To assess the biofumigation potential against fungal mycelium, seven day-old five mm mycelial discs of *S. rolfsii* was placed on the PDA medium whereas, to assess the inhibition of regeneration of mycelia from sclerotia, a single sclerotium was placed on the upper lid. 1, 5, 10, 15, 20 and 25 g of the macerated plant samples were kept separately in the lower plate. The plates were closed and sealed at the joining portion with parafilm to prevent the loss of any fumes. The untreated control contained the mycelial disc of the fungus and sclerotia inoculated on PDA separately on the upper lid and the lower lid without any macerated plant samples. All the plates were incubated at room temperature (28 ± 1^0C). The mycelial growth (diameter in cm) of *S. rolfsii* was measured in all the plates, when there was complete mycelial growth in untreated control plates. The percentage suppression of mycelial growth was calculated as per 3.2.7.

3.3.2.2. Biofumigation Potential of Oil Cakes against S. rolfsii

Good quality, fresh samples of oil cakes (mustard, castor and pongamia) were dried in shade for 2-3 days, and ground into powder with pestle and mortor. The powdered oil cakes were soaked in sterile water in the 2:1 ratio (w/v). One day after soaking, the oil cakes were taken in muslin cloth and squeezed to collect the extract in

beakers separately. The collected extracts were centrifuged at 5000 rpm for ten min as per the methodology given by Bhadrasree (2007). Two equal sized, bottom lids of Petri plates were selected. 15 ml of PDA medium was poured in the upper plate and allowed to solidify. Seven day old, five mm mycelial discs as well as sclerotia of *S. rolfsii* were separately placed on the PDA medium to assess the biofumigation potential of oil cakes in inhibiting the mycelial and sclerotial germination of the fungus. The centrifuged oil cake extracts (1, 5, 10, 15, 20 and 25ml) were poured separately to the lower plate. The plates were closed and sealed at their joining portion with parafilm to prevent the escape of any fumes produced from the extracts. The untreated control contained the mycelial disc and sclerotia of the fungus inoculated separately on PDA medium on the upper lid and the lower lid without any oil cakes extracts.All the plates were incubated at room temperature (28 ± 1^0C). The mycelial growth (diameter in cm) and mycelial regeneration from sclerotia of *S. rolfsii* was measured in all the plates when there was complete mycelial growth in untreated control plates. The percentage suppression of mycelial growth was calculated as in 3.2.7.

3.3.3. Assessment of Inhibition of Sclerotia by Confined Container Technique

The sclerotium is the vegetative resting structure of *S. rolfsii*, which aids in the survival of the fungus in soil for a longer period of time. Thus, the fungus can remain in a dormant stage under unfavourable conditions as sclerotia and can get revived during the favourable conditions to incite infection. Hence, it is of utmost importance that the sclerotia need to be made unviable to prevent subsequent infections. Thus, confined container technique was undertaken to assess the biofumigation potential of plant extracts as well as oil cakes in reducing the viability of the sclerotia.

The soil biofumigation potential of both the plant extracts and oil cakes was tested at five per cent concentration to assess the inhibition of the mycelial regeneration from sclerotia using confined container technique under *in vitro* conditions as per the methodology described by Dhingra *et al*. (2013).

Garden soil was collected and sterilized in autoclave at 121^0C temperature and 15 psi pressure for 15-20 min for two consecutive days. The treatments for assessing

the biofumigation potential included all the eight plant extracts and the three oil cakes. Plastic containers with lids were taken and filled with autoclaved soil upto half of its capacity. Each treatment was added as a layer into this soil filled in the plastic container and sprinkled with sterile distilled water. Another layer of sterilized soil was added above the treatment tested for its biofumigant potential. Sclerotia from fourteen day-old fungal culture of *S. rolfsii* was placed on sterilized muslin cloth at the rate of five per bag and tied as a cloth bag. These sclerotia enclosed cloth bags were placed on the top of sterilized soil layer taken in the plastic containers at the rate of three per container. The untreated control contained the sclerotia bags placed on the top of sterilized soil without any biofumigant material. All the plastic containers were sealed with klin film tightly to prevent the escape of any fumes and incubated at room temperature (28 ± 1^0C) for three weeks duration. The biofumigation potential of the plant extracts and oil cakes on the mycelial regeneration from sclerotia was assessed by inoculating the sclerotia incubated in muslin cloth in each treatment separately on to fresh PDA medium at weekly intervals upto three weeks. The sclerotia incubated in sterilized soil without any biofumigant treatment was maintained as the untreated control. The inhibition of the mycelial regeneration from sclerotia of each treatment was calculated as in 3.2.7.

3.3.4. Selection of Best Treatments

The best treatments from the plant extracts and oil cakes were selected based on their biofumigation and antifungal potential.

3.3.4.1. In vitro Studies to Identify the Effective, Lowest Concentration of the Best Treatments

The lowest effective concentrations of two best effective treatments were identified under *in vitro* conditions.

3.4. COMPATIBILITY OF *TRICHODERMAVIRIDE* (KAU ISOLATE) WITH BEST TWO TREATMENTS *IN VITRO*

The compatibility of the two effective treatments identified from 3.3 was tested with the biocontrol agent *viz*., *T. viride* (KAU isolate) under *in vitro* conditions.

3.4.1. Compatibility Based on Antifungal Potential

The compatibility of the two best treatments and *T. viride* was tested based on their antifungal potential using poisoned food technique as in 3.3.1.1.

3.4.2. Compatibility Based on Biofumigation Potential

The compatibility between the two best treatments and *T. viride* was tested based on their biofumigation potential using paired plate technique as in 3.3.2.1.

3.5. *IN VIVO* MANAGEMENT STUDIES

A pot culture study was undertaken at IFSRS, Karamana to test the biofumigation potential of the two best treatments selected from *in vitro* studies for the management of basal stem rot and blight disease of vegetable cowpea with nine treatments replicated thrice. The KAU variety of vegetable cowpea *viz*., Gitika was used for the study. The crop was maintained as per the Package of Practices recommendations of Kerala Agricultural University (KAU).

3.5.1. Treatments Selected for *in vivo* Studies

The nine treatments tested for their biofumigation potential against the disease is detailed in Table 2.

Table 2. Treatments selected for the *in vivo* management studies of *S. rolfsii*

Treatments	Description
T_1	Garlic bulb (3g/kg soil) + soil plastering with cowdung slurry for two weeks (basal application)
T_2	Garlic creeper leaves (25 g/kg soil) + soil plastering with cowdung slurry for two weeks (basal application)
T_3	T_1 + soil application of cowdung and neem cake enriched with *T. viride* (KAU isolate) at one month after planting (MAP)
T_4	T_2 + soil application of cowdung and neem cake enriched with *T. viride* (KAU isolate) at one MAP
T_5	Soil application of cowdung and neem cake enriched with *T. viride* (KAU isolate) at one MAP alone
T_6	Soil application of *Pseudomonas fluorescens* (KAU isolate) (2% drench) at one MAP alone
T_7	Inoculated control (*S. rolfsii*)
T_8	Uninoculated control
T_9	Mancozeb M 45 @ 3g $L^{-}1$

3.5.2. Preparation of Inoculum of the Virulent Isolate (S_1) of *S. rolfsii*

The virulent isolate of *S. rolfsii* (Sr_1) was multiplied in sand oats medium (Chaurasia *et al*., 2013). Sand and oats were mixed in 9:1 ratio and moistened with sterile water to maintain sufficient moisture. The mixture was filled in poly propylene bags and autoclaved at 121^0C and 15 psi for 15-20 min for two consecutive days. Seven day-old five mm mycelial discs of *S. rolfsii* were inoculated at the rate of five mycelial discs per bag. The inoculated bags were incubated at room temperature for two weeks. The inoculum of *S. rolfsii* at two per cent concentration was inoculated into the grow bags at two weeks before transplanting of the vegetable cowpea seedlings. Thus, the

soil was artificially inoculated with *S. rolfsii* to undertake the *in vivo* management studies.

3.5.3. Treatment Application

Grow bags of dimension 40 cm x 24 cm x 24 cm were selected for the *in vivo* study. One fourth of the bag was filled with soil above which, the inoculum of the pathogen multiplied on sand oats medium was added at the rate of two per cent inoculum per grow bag. Two best effective biofumigants selected from the *in vitro* studies *viz*., macerated garlic bulb and garlic creeper leaves were added to the soil separately (maintained as T_1 and T_2) and sprinkled with tap water to maintain the moisture at field capacity. Another layer of soil was added again and the top portion of the grow bags and was plastered with cowdung slurry mixed with equal quantity of soil (1:1 ratio) in these treatments. In untreated inoculated control (T_7), the pathogen inoculum multiplied in sand oats medium at two per cent was inoculated into the soil without any biofumigant application. The uninoculated control (T_8) was maintained without the addition of any inoculum of *S. rolfsii* in soil or treatment application. Application of the fungicide *viz*., mancozeb (T_9) (0.30%) was maintained as the chemical control. The grow bags were left undisturbed for two weeks. After two weeks, the soil was turned and mixed well and two week old vegetable cowpea seedlings (var. Gitika) were transplanted at the rate of one per bag. The soil application of *T. viride* (500 g / plant) and *P. fluorescens* (2% drench) alone at one MAP represented the treatments *viz*., T_5 and T_6, whereas their application in treatments having garlic bulb and garlic creeper leaf extracts at one MAP represented T_3 and T_4. The crop production and protection practices were undertaken as and when required as per the package of practices of KAU. The vine length and the number of leaves in each treatment were also recorded. The various observations on the days for disease development, days for production of sclerotia, disease incidence and disease intensity were recorded in each treatment. The disease incidence (Mayee and Datar, 1986) and disease intensity (Lee *et al*., 2012) of basal stem rot and blight of vegetable cowpea incited by *S. rolfsii* were calculated using the formulae as follows.

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants affected} \times 100}{\text{Total number of plants}}$$

Grade	Description
0	No disease symptoms
1	Disease symptoms without visible outgrowth of fungus
2	Disease symptoms with visible outgrowth of fungus
3	Partial wilting of plants
4	Complete wilting and plant death

$$PDI = \frac{\text{Sum of grade of each plant x 100}}{\text{Number of plants assessed x Maximum grade}}$$

3.6. ESTIMATION OF SOIL FUNGAL POPULATION DYNAMICS

The effect of biofumigation on fungal population can be studied by estimating the fungal colonies in soil by serial dilution and plating technique (Wakesman, 1922). The fungal colony count as colony forming units (cfu / g of soil) was estimated before biofumigation and one month after it. For the estimation of fungal population dynamics before and after biofumigation, representative soil sample of ten gram was collected from grow bags of each treatment and added to 90 ml sterile, distilled water which represented the 10^{-1} dilution. One ml from this dilution was pipetted and transferred to 9 ml sterile water containing test tubes to form 10^{-2} dilution and the same procedure was followed to prepare upto 10^{-4} dilution. One ml from each of 10^{-3} and 10^{-4} dilutions were pipetted out into sterile Petri plates. Fifteen ml of Martin Rose Bengal Agar medium was poured over it and rotated in both clockwise and anticlockwise direction for the development of scattered fungal colonies. Subsequently, the plates were incubated at room temperature for three days and observations on the number of fungal colonies (cfu / g soil) developed in each treatment were enumerated.

3.7. STATISTICAL ANALYSIS

The data collected from all the *in vitro* and *in vivo* studies were analysed statistically using the analysis of variance (ANOVA). Arc sine transformation of the data was undertaken, wherever necessary. The treatments were compared based on the Duncans Multiple Range Test (DMRT) as developed by Steel and Torrie (1960).

Results

4. RESULTS

The present research work entitled “Biofumigation for the management of *Sclerotium rolfsii* in vegetable cowpea” was carried out at Integrated Farming System Research Station (IFSRS), Karamana and Department of Plant Pathology, College of Agriculture, Vellayani during 2018-2020 to assess the biofumigation potential of selected botanicals and oil cakes against *S. rolfsii* inciting basal stem rot and blight disease of vegetable cowpea. The data recorded from the various *in vitro* and *in vivo* experiments were analysed statistically. The results of the experiments conducted as per the study are detailed in this chapter.

4.1. ISOLATION OF S. ROLFSII FROM VEGETABLE COWPEA

4.1.1. Collection of Infected Plant Samples

Six isolates of *S. rolfsii* inciting basal stem rot and blight of vegetable cowpea were collected from different vegetable cultivating areas. The disease was observed to affect the crop at all stages of growth *viz*., seedling, vegetative, flowering, pod formation and harvesting stages. Basal stem rot and blight disease was manifested as wilting, yellowing of leaves, defoliation and stem shredding (Plate 1). The disease in all the infected vegetable cowpea plants appeared to have white coloured, fan or thread like mycelial growth at the collar region (Table 3).

4.1.2. Isolation and Purification of Pathogen Isolates

Among the six isolates of *S. rolfsii*, five isolates were obtained from basal stem rot and blight affected vegetable cowpea plant samples collected from the commonly cultivated varieties *viz*., Gitika, Vellayani Jyothika and NS 621 from the predominant vegetable cultivating areas of Pathanamthitta, Karamana and Vellayani. A new isolate was also obtained from the variety Kashi Kanchan collected from Indian Institute of Vegetable Research, Varanasi. These isolates were purified and maintained on PDA medium to conduct virulence rating in order to identify the most virulent isolate.

a. Wilting

b.Yellowing

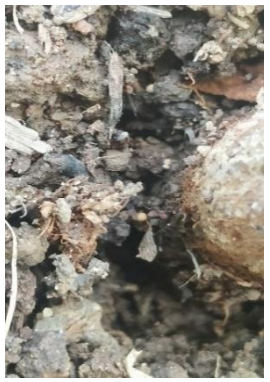
c. Defoliation

d. Mycelial growth

e. Presence of sclerotia

f. Complete drying

g. Shredding of infected tissue



h. Sclerotia at 15cm depth

Plate 1. Stages of basal stem rot and blight disease of vegetable cowpea caused by *S. rolfsii*

Table 3. Nature of symptoms of basal stem rot and blight disease caused by *S. rolfsii* in different varieties of vegetable cowpea collected from different locations

Isolates	Locations	Variety	Crop stages	Nature of symptoms
Sr_1	Pathanamthitta	Gitika	Flowering	White, fan like mycelial growth on collar region
Sr_2	Karamana	Vellayani Jyothika	Pod formation	Initiation of mycelial growth on collar region
Sr_3	Vellayani	Vellayani Jyothika	Seedling	Water soaked lesions with mycelial growth on collar region
Sr_4	Varanasi, UP	Kashi Kanchan	Vegetative	White, fan like mycelial growth on collar region
Sr_5	Karamana	Gitika	Flowering	Fan like mycelial growth on collar region
Sr_6	Karamana	NS 621	Towards harvest	Thread like mycelial growth on collar region

4.1.3. Virulence Rating of the Isolates

4.1.3.1. Symptomatology of Isolates in Vegetable Cowpea on Artificial Inoculation

Stem inoculation of vegetable cowpea seedlings was done separately using the six isolates of *S. rolfsii*. The isolate Sr_1 took the minimum days (two) for lesion initiation, whereas the maximum duration of five days was taken by the isolate Sr_6. The isolates Sr_2, Sr_3, Sr_4 and Sr_5 started to develop lesions on third day after inoculation (DAI). The maximum lesion size of 8.20 cm was produced by the isolate, Sr_1 compared to the other five isolates 9 DAI. Sr_1 was also found to produce the maximum number of sclerotia (18) on the artificially inoculated vegetable cowpea seedlings at 15 DAI (Table 4).

4.1.3.2. Mycelial and Sclerotial Characters of Isolates

The six isolates of *S. rolfsii* were grown separately on PDA medium and their mycelial and sclerotial characters were compared to identify the most virulent isolate among them. The mycelial growth of the isolates was recorded on 1, 2, 3, 4 and 5 DAI. The isolate *viz*., Sr_1 completed its mycelial growth within 3 day followed by Sr_2, Sr_3, Sr_4 and Sr_6 which took 4 day to complete their mycelial growth in PDA medium. The isolate Sr_5 took 5 day to complete its mycelial growth. The mycelia of the isolates appeared dull (Sr_2 and Sr_3) to pure white (Sr_1, Sr_4, Sr_5 and Sr_6). The colony growth of the isolates varied from thread like (Sr_1, Sr_3 and Sr_6) to dense (Sr_2 and Sr_4) and fluffy (Sr_5) (Table 5; Plate 2).

All the isolates exhibited the presence of sclerotia when grown in PDA medium. The sclerotial growth initiated within four days in the case of Sr_1, which took the minimum days for sclerotial initiation, whereas the maximum duration (seven days) was taken by the isolate Sr_5. The isolate, Sr_1 also produced the maximum number of sclerotia (332) whereas Sr_5 produced the least number of sclerotia (18) on PDA medium. The sclerotia appeared smooth round in all the isolates except in Sr_6, where they appeared as irregular round in shape. The sclerotial colour varied from dark brown (Sr_1 and Sr_2), reddish brown (Sr_3 and Sr_4), white (Sr_5) to golden brown (Sr_6). The arrangement of the sclerotia

Table 4. Symptom development in vegetable cowpea by different isolates of *S. rolfsii* on artificial inoculation

Isolates	Days taken for lesion initiation	Lesion size (cm)*	Number of sclerotia*
Sr_1	2	8.20 ± 1.155^{a}	18.00 ± 1.155^{a}
Sr_2	3	7.40 ± 0.058^{ab}	9.00 ± 2.309^{b}
Sr_3	3	6.20 ± 0.866^{abc}	7.00 ± 0.577^{bc}
Sr_4	3	5.90 ± 0.751^{bc}	6.00 ± 1.732^{bc}
Sr_5	3	4.20 ± 0.115^{cd}	5.00 ± 0.000^{bc}
Sr_6	5	3.60 ± 0.751^{d}	4.00 ± 1.155^{c}
CD (0.05)	-	2.285	4.282
SEm±	-	0.733	1.374

*****Mean ± SD of three replications

Values followed by similar superscripts are not significantly different at 5% level

Table 5. Mycelial growth and characteristics of different isolates of *S. rolfsii* on PDA medium

Isolates	Mycelial growth (Diameter in cm)*				Mycelial characteristics	
	1 day	2 day	3 day	4 day	Colour	Colony pattern
Sr_1	2.20±0.173^{a}	5.90±0.058^{a}	9.00±0.000^{a}	9.00±0.000^{a}	Pure white	Thread like mycelial growth
Sr_2	2.00±0.115^{ab}	5.20±0.115^{b}	7.40±0.115^{bc}	9.00±0.000^{a}	Dull white	Dense mycelial growth
Sr_3	1.70±0.115^{bc}	4.90±0.115^{b}	7.50±0.000^{b}	9.00±0.000^{a}	Dull white	Thread like mycelial growth
Sr_4	1.50±0.058^{cd}	4.40±0.173^{c}	7.40±0.058^{bc}	9.00±0.000^{a}	Pure white	Dense mycelial growth
Sr_5	1.30±0.115^{d}	3.50±0.058^{d}	5.70±0.115^{d}	8.60±0.115^{b}	Pure white	Fluffy mycelial growth
Sr_6	1.70±0.058^{bc}	4.20±0.115^{c}	7.20±0.058^{c}	9.00±0.000^{a}	Pure white	Thread like mycelial growth
CD (0.05)	0.352	0.352	0.232	0.147		
SEm±	0.113	0.113	0.075	0.047		

*Mean ± SD of three replications

Values followed by similar superscripts are not significantly different at 5% level

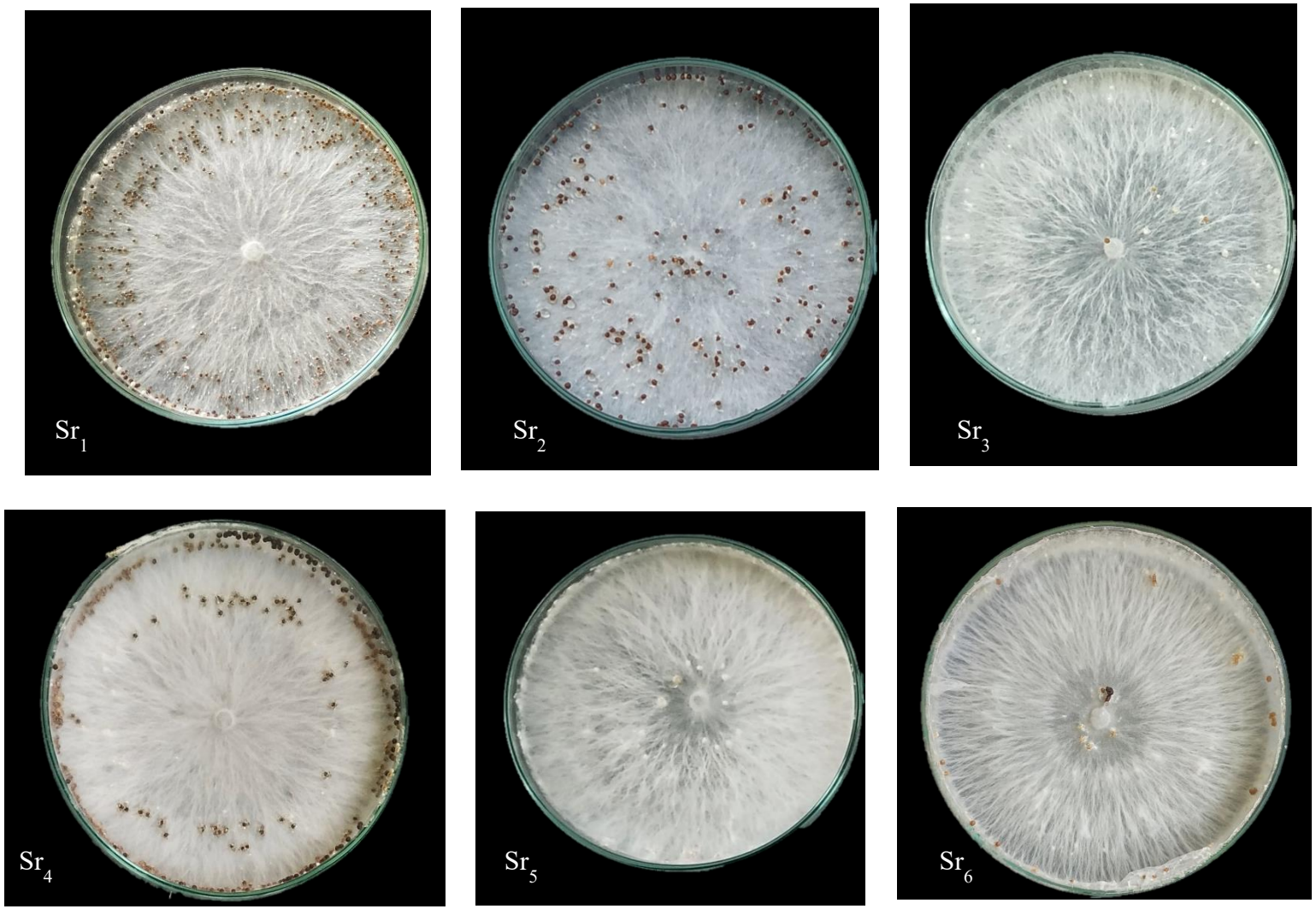


Plate 2. Mycelia and sclerotia of different isolates of *S. rolfsii* collected from different locations on PDA medium at 15 days of growth

appeared as dense towards rim (Sr_1 and Sr_4), dense at centre (Sr_5), scattered (Sr_2) as well as scattered and sparse (Sr_3 and S_6). The degree of formation of sclerotia was graded as excellent in Sr_1, fair in Sr_2 and poor in the remaining isolates (Table 6; Plate 2).

4.1.3.3. Selection of the most virulent isolate

The most virulent isolate from the six isolates of *S. rolfsii* was identified based on symptomatology on plants and growth characters on PDA medium. The study revealed that the minimum days for lesion formation was observed in the seedlings which were artificially inoculated with Sr_1 isolate. This isolate produced lesions with the maximum size (8.20 cm) and recorded the maximum number of sclerotia (18) (Table 4; Plate 3).

It was revealed that the isolate Sr_1 took three days for completing the mycelial growth, which was the minimum among all the isolates (Table 5). The isolate also took only four days for the initiation of sclerotia with excellent degree of sclerotial formation with the maximum number of sclerotia (332.00) (Table 6). Thus, from both the studies, the isolate *viz*., Sr_1 was identified as the most virulent among the six isolates of *S. rolfsii*.

4.1.4. Pathogenicity Studies

4.1.4.1. Soil Inoculation

When soil inoculation of vegetable cowpea seedlings (var. Gitika) was undertaken using the virulent isolate (Sr_1) of *S. rolfsii*, water soaked lesions were observed initially at the collar region on third DAI. Gradually, the lesions increased in size and resulted in collar rot. There was yellowing of the leaves which later got defoliated. Finally, the seedlings were observed to completely wilt on six DAI (Table 7; Plate 4).

Table 6. Characteristics of sclerotia produced by different isolates of *S. rolfsii* on PDA medium

Isolates	Days for sclerotia initiation	No. of sclerotia on 15 DAI*	Shape	Colour	Arrangement in Petri plate	Degree of formation	Symbol
Sr_1	4	332.00±11.547[a]	Smooth round	White to dark brown	Dense towards rim	Excellent	4+
Sr_2	5	106.00±2.309[b]	Smooth round	Cream to dark brown	Scattered	Fair	3+
Sr_3	5	26.00±4.619[d]	Smooth round	White to reddish brown	Scattered and sparse	Poor	2+
Sr_4	6	79.00±2.887[c]	Smooth round	Reddish brown	Dense towards rim	Poor	2+
Sr_5	7	18.00±1.732[d]	Smooth round	White	Dense at centre	Poor	2+
Sr_6	6	30.00±2.309[d]	Irregular round	White to golden brown	Scattered and sparse	Poor	2+
CD (0.05)		16.905					
SEm±		5.426					

DAI - days after inoculation, *Mean ± SD of three replications

Values followed by similar superscripts are not significantly different at 5% level

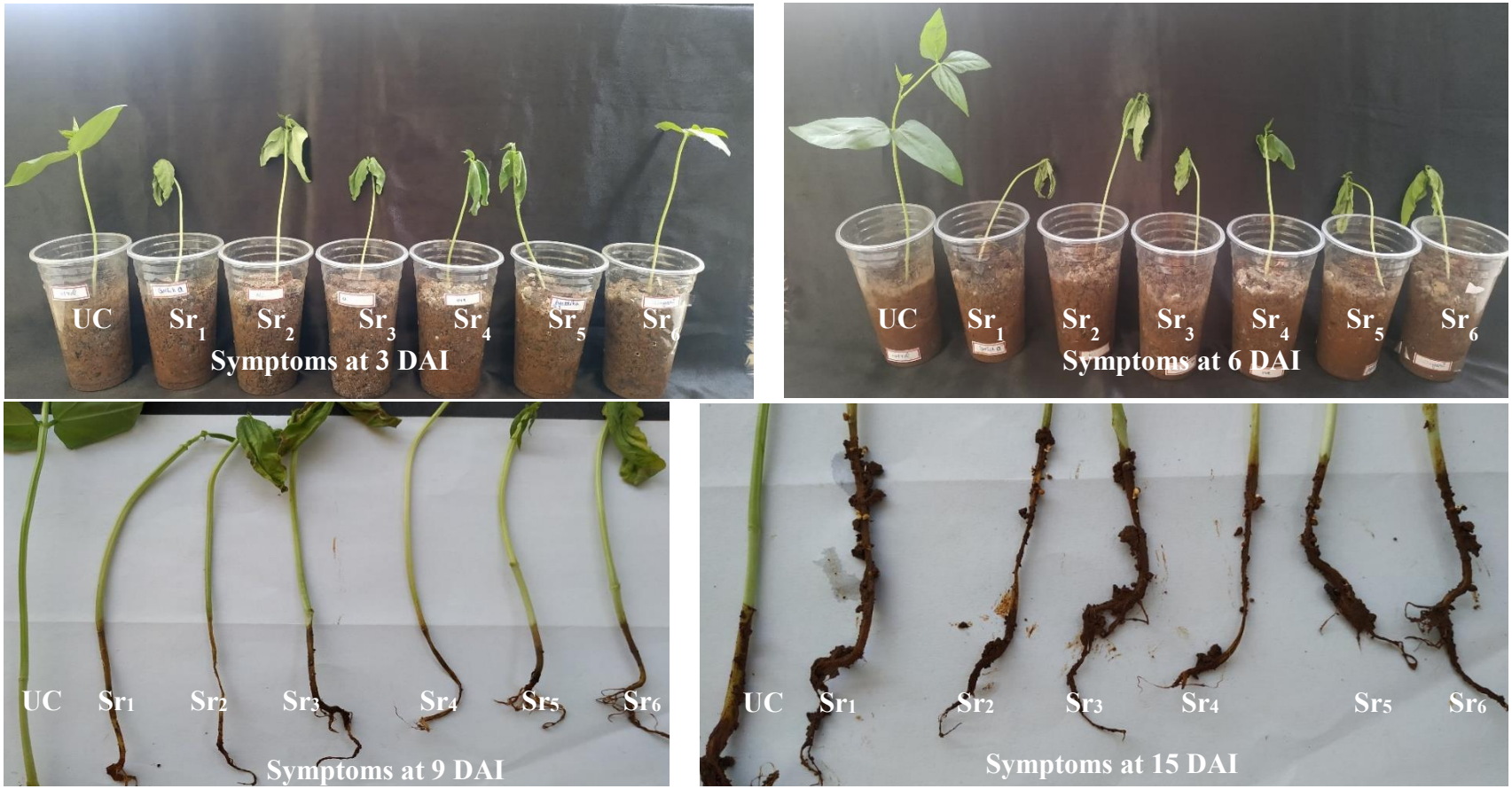
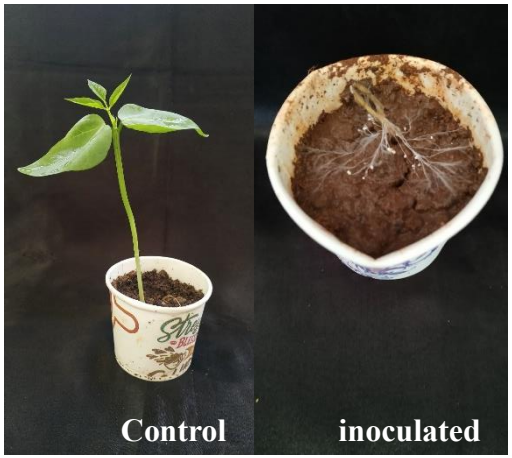


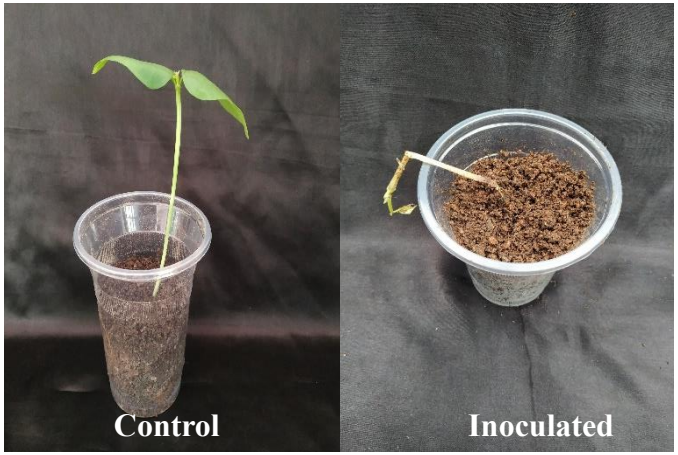
Plate 3. Testing of virulence of different isolates of *S. rolfsii* on vegetable cowpea seedlings var. Gitika at 3, 6, 9 and 15 days after inoculation

Table 7. Symptom development in vegetable cowpea var. Gitika by the most virulent isolate of *S. rolfsii* on different methods of artificial inoculation

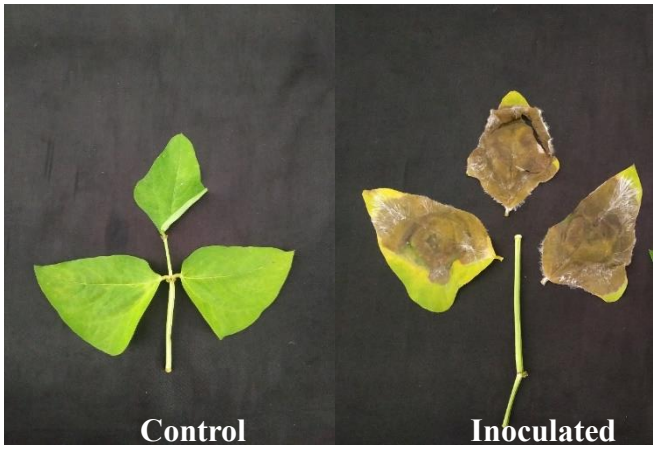
Method of inoculation	Nature of symptom development	Days for symptom initiation
Stem inoculation	Water soaked lesion at collar region, gradually resulting in collar rot	2
Soil inoculation	Water soaked lesion at collar region, gradually resulting in collar rot	3
Leaf inoculation	Blighted appearance of leaves, detachment from petiole and complete drying	1



a. Stem inoculation



b. Soil inoculation



c. Leaf inoculation

Plate 4. Symptoms of basal stem rot and blight disease of vegetable cowpea var. Gitika caused by the most virulent isolate (Sr_1) of *S. rolfsii*

4.1.4.2. Stem Inoculation

In stem inoculation method, seven day-old five mm mycelial discs of the virulent isolate (Sr_1) of the fungus were placed at the collar region of seedlings. Water soaked lesions were observed at the inoculated region on two DAI, which gradually enlarged in size. The infected seedlings toppled down at the collar region and were completely covered with white, thread like mycelial growth of the fungus along with mustard shaped, white coloured sclerotia. The complete rotting and death of the seedlings were recorded on six DAI (Table 7; Plate 4).

4.1.4.3. Leaf Inoculation

Seven day-old five mm mycelial discs of the fungus were placed on the under surface of detached leaves of the crop. Yellowing and blighting of the leaves were observed on one DAI, first at the site of inoculation, which gradually increased and covered the entire leaf within three days. White mycelial growth of the fungus was clearly evident on the blighted portions of the leaves from three DAI. The infected trifoliate leaves finally got detached individually from the petiole and appeared as blighted (Table 7; Plate 4).

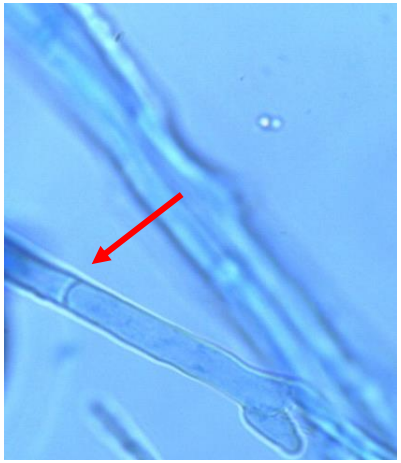
4.2. CHARACTERIZATION OF S. ROLFSII

4.2.1. Morphological Characters

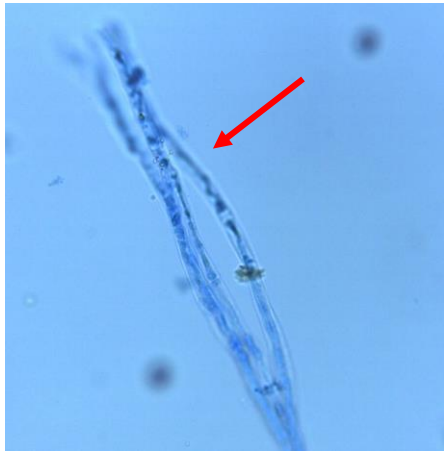
The virulent isolate, Sr_1 was used for studying the various morphological characters of *S. rolfsii*. The fungus had hyaline and septate mycelium with granular cytoplasm. Characteristic clamp connections were also observed under microscope (Plate 5).

4.2.2. Cultural Characters

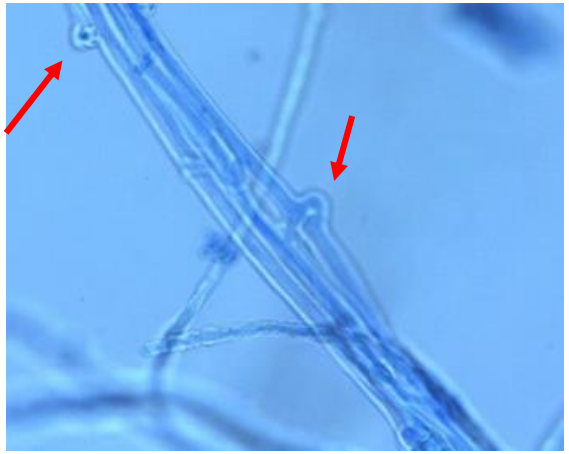
The virulent isolate (Sr_1) completed its mycelial growth on PDA medium within three DAI. The mycelia appeared to be pure white in colour with conspicuous thread like growth on PDA medium. The initiation of sclerotia started on four DAI which appeared



Mycelial septation at 1000X



Granular cytoplasm at 1000X



Clamp connection at 1000X

Plate 5. Morphological characters of *S. rolfsii* causing basal stem rot and blight disease in vegetable cowpea

as smooth, round and dark brown in colour. Their arrangement was dense towards rim with excellent degree of formation (Table 5 and 6).

4.2.3. Identification of the Fungus

The identification of the fungus was done based on its morphological and cultural characters.

4.2.3.1. Morphological and Cultural Characters

The presence of hyaline and septate mycelium, granular cytoplasm, characteristic clamp connections as well as the presence of moniloid cells in sclerotia confirmed the pathogen to be *S. rolfsii* (Plate 5). Conspicuous thread like, white, mycelial growth with the presence of mustard like, dark brown sclerotia (Plate 2) also confirmed the pathogen to be *Sclerotium rolfsii* Sacc. (teleomorph *Athelia rolfsii* [Curzi] C. C. Tu. & Kimbr.) (division basidiomycota and family Atheliaceae) (http://www.indexfungorum.org/names/Names.asp).

4.2.3.2. Molecular Identification

Molecular identification based on the ITS region of the rDNA of *S. rolfsii* revealed 99.68 per cent identity with four strains of *S. rolfsii* (teleomorph: *Athelia rolfsii*) having the accession numbers *viz*., KT222900, KY640623, KU514414 and AB042626 respectively (Table 8). Thus, the fungus was identified and confirmed to be *S. rolfsii* by morphological, cultural and molecular characterization. The sequence of the fungus was deposited in National Centre for Biotechnology Information (NCBI) and was assigned with the accession number MT560347.

4.2.4. Standardization of Optimum pH for the Growth of *S. rolfsii*

Among the different levels of pH tested, the fungus completed its mycelial growth (9 cm) within three DAI in PDA medium of pH 6.0. The maximum number of sclerotia (245) was recorded in the medium of pH 7 at 15 DAI. The study revealed that

Table 8. Molecular identification of *S. rolfsii* isolated from vegetable cowpea based on ITS region and BLAST at NCBI

Gen Bank Accession No.	Description	Maximum score	Query coverage	E value	Identity (%)
KT222900.1	*Athelia rolfsii* * strain 13M-0067	1158	96%	0.0	99.68
KY640623.1	*Athelia rolfsii* isolate SrCAMKS12	1155	97%	0.0	99.68
KU514414.1	*Athelia rolfsii* isolate SrKK19_1090	1136	96%	0.0	99.68
AB042626.1	*Athelia rolfsii*	1136	96%	0.0	99.68

**Athelia rolfsii* is the teleomorph of *S. rolfsii*

the mycelial growth as well as the production of sclerotia was comparatively less in the alkaline pH range (7.5 to 8.5) (Table 9; Plate 6).

4.2.5. Standardization of Optimum Moisture for the Growth of *S. rolfsii*

The study revealed that under high moisture percentage (60 to 80%), more number of days (10 to 12 days) was taken by the fungus to develop the basal stem rot and blight disease symptoms in vegetable cowpea seedlings. The disease development and symptom expression were favoured in the moisture range from 35 to 50 per cent at which the disease occurred within a short period of 5 to 8 days (Table 10; Plate 7).

4.2.6. Standardization of Inoculum Level of the Fungus for Disease Development

Among the different inoculum levels **(**1, 2, 3, 5, 7, 9 and 11%) of *S. rolfsii* inoculated into the soil for symptom expression, the maximum number of days (6) was taken for the disease to exhibit in the seedlings inoculated with one per cent concentration of the inoculum. Three days were taken for symptom expression in the seedlings (Plate 8 and 9) at all other levels of the inoculum (2 to 11%), the least being at 2 per cent, this inoculum level (2%) was selected for further studies (Table 11).

4.2.7. Screening of Fungicides for the Inhibition of Growth of *S. rolfsii*

Among the different fungicides tested for their potential in inhibiting the growth of *S. rolfsii,* mancozeb alone and in combination with carbendazim completely inhibited the mycelial growth of the fungus, whereas the other fungicides *viz*., carbendazim, copper oxychloride and wettable sulphur had no inhibitory effect at all on the fungus (Table 12; Plate 10).

4.3. *IN VITRO* MANAGEMENT STUDIES

The selected plant extracts (mustard, cabbage, garlic creeper, castor, cauliflower, a combination of onion and garlic bulbs, garlic bulb and onion bulb individually) and oil cakes (castor, mustard and pongamia cakes) were assessed for their biofumigation and antifungal potential in inhibiting the mycelial growth as well as the mycelial

Table 9. Effect of pH on mycelial growth and sclerotial production in *S. rolfsii* causing basal stem rot and blight of vegetable cowpea

pH	Mycelial growth (Diameter in cm)*			Nature of mycelial growth	No. of sclerotia on 15th day of growth	Colour of sclerotia on 15th day
	1 day	2 day	3 day			
5.0	1.50±0.058^{d}	3.90±0.635^{bc}	8.00±0.577^{cd}	Fluffy growth	105.67±2.963^{b}	Reddish brown
5.5	2.00±0.115^{c}	4.20±0.115^{b}	8.80±0.115^{ab}	Sparse growth	80.67±2.186^{c}	Brilliant brown
6.0	2.50±0.173^{b}	5.10±0.058^{a}	9.00±0.058^{a}	Fluffy growth	60.33±2.404^{d}	Reddish brown
6.5	2.90±0.058^{a}	4.50±0.058^{ab}	8.90±0.058^{ab}	Suppressed growth	101.67±2.728^{b}	Brilliant brown
7.0	1.50±0.058^{d}	3.20±0.115^{cd}	7.60±0.058^{d}	Fluffy growth	245.67±8.090^{a}	Light cream
7.5	2.00±0.115^{c}	4.30±0.058^{b}	8.10±0.115c^{d}	Fluffy growth	81.67±2.728^{c}	Brilliant brown
8.0	1.10±0.058^{e}	2.90±0.115^{d}	7.50±0.058^{d}	Sparse fluffy growth	85.67±2.963^{c}	Reddish brown
8.5	1.70±0.115c^{d}	4.00±0.115^{b}	8.30±0.058^{bc}	Fluffy growth	74.33±2.963^{c}	Honey colour
CD (0.05)	0.309	0.730	0.656		11.575	
SEm±	0.102	0.242	0.217		3.828	

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

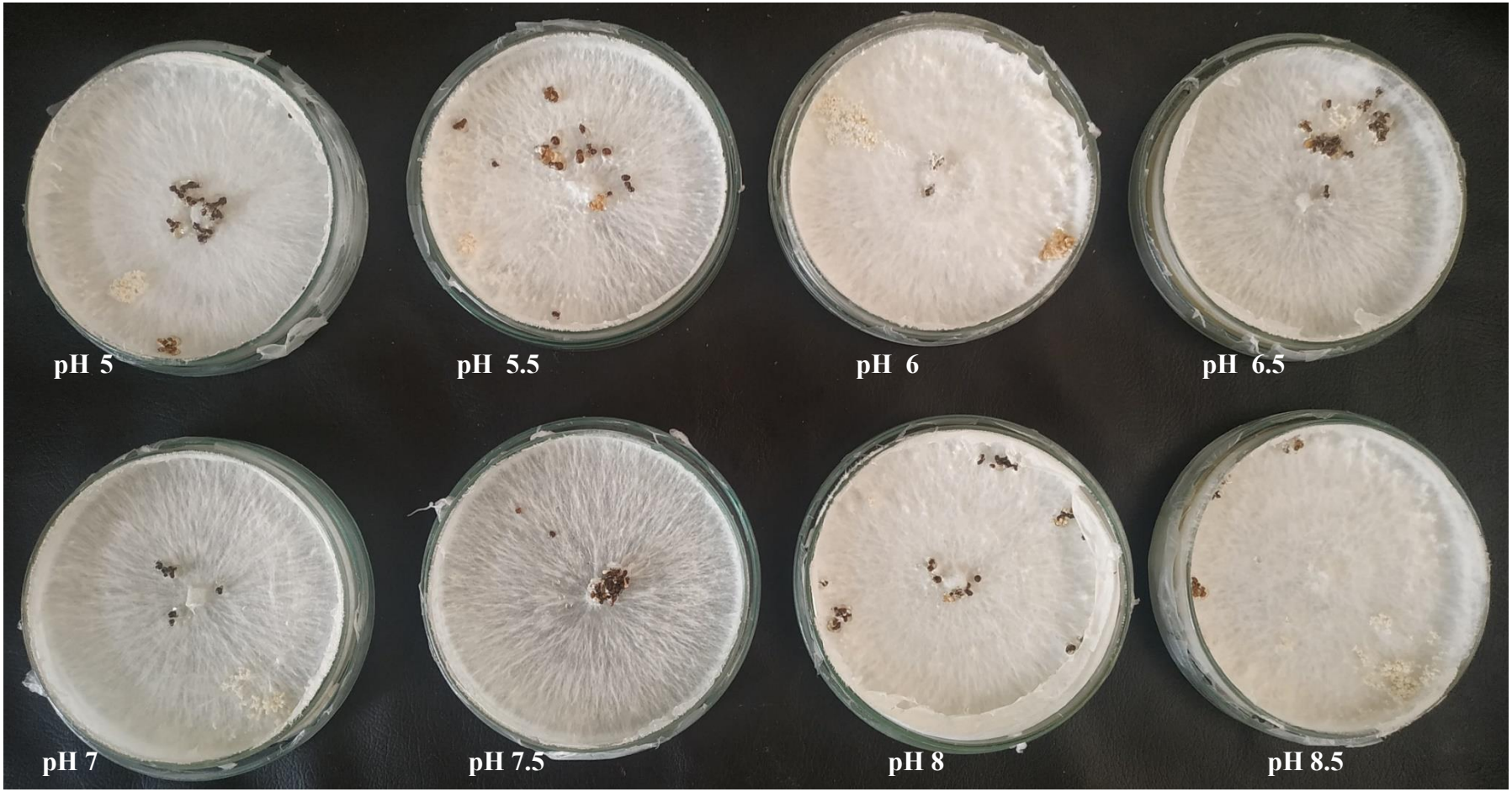


Plate 6. Effect of different levels of pH on mycelial growth and sclerotial production of *S. rolfsii* on PDA medium at 15 days of growth

Table 10. Effect of soil moisture levels on symptom initiation by *S. rolfsii* in vegetable cowpea

Sl. No.	Soil moisture percentage (%)	Days for symptom initiation *
1	35	4.67 ± 0.33^{e}
2	45	6.33 ± 0.33^{d}
3	50	7.67 ± 0.33^{c}
4	60	11.67 ± 0.33^{a}
5	70	10.67 ± 0.33^{ab}
6	80	10.33 ± 0.33^{b}
CD (0.05)		1.038
SEm±		0.333

*Mean ± SD of 3 replications

Values followed by similar superscripts are not significantly different at 5% level

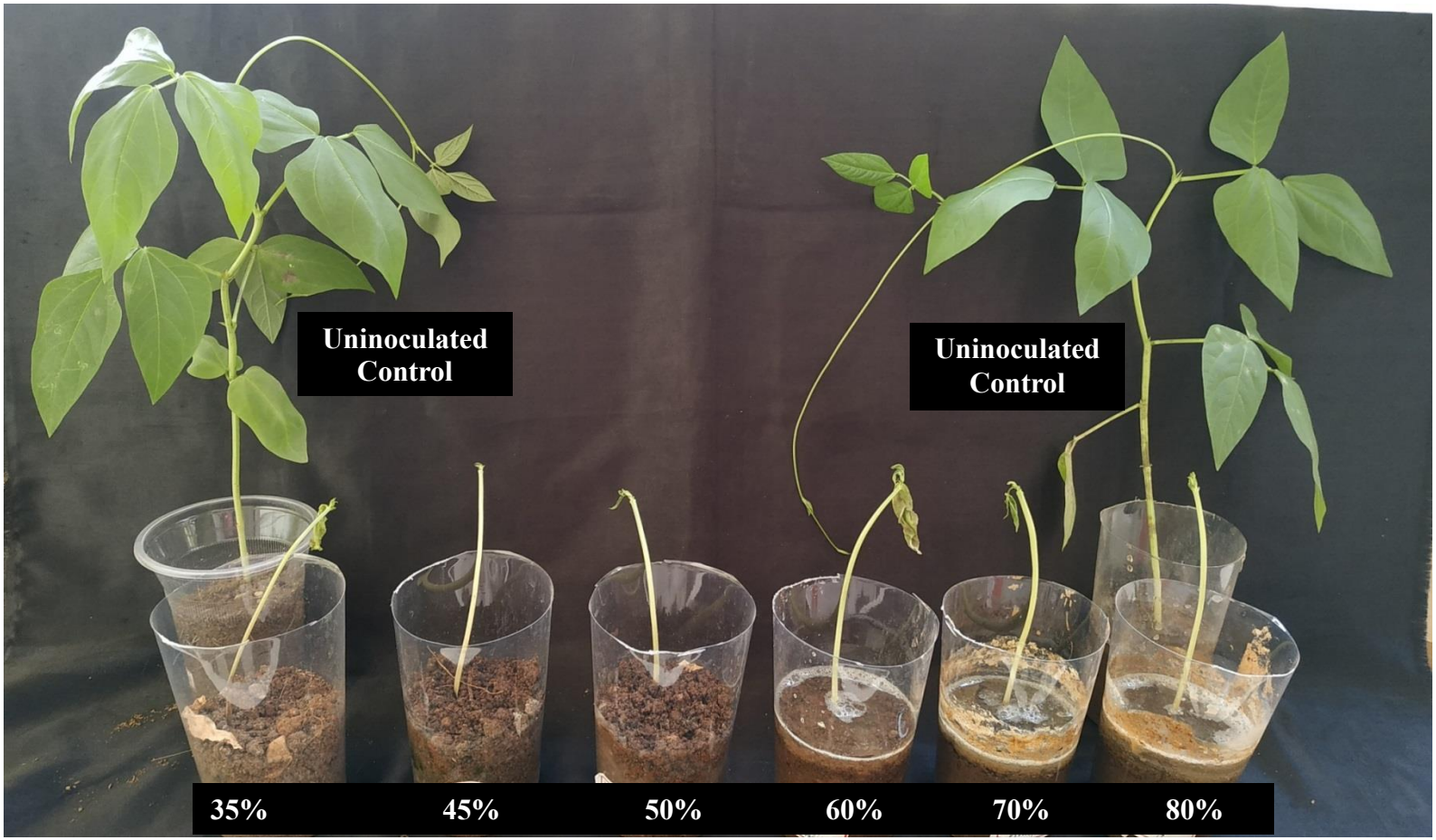
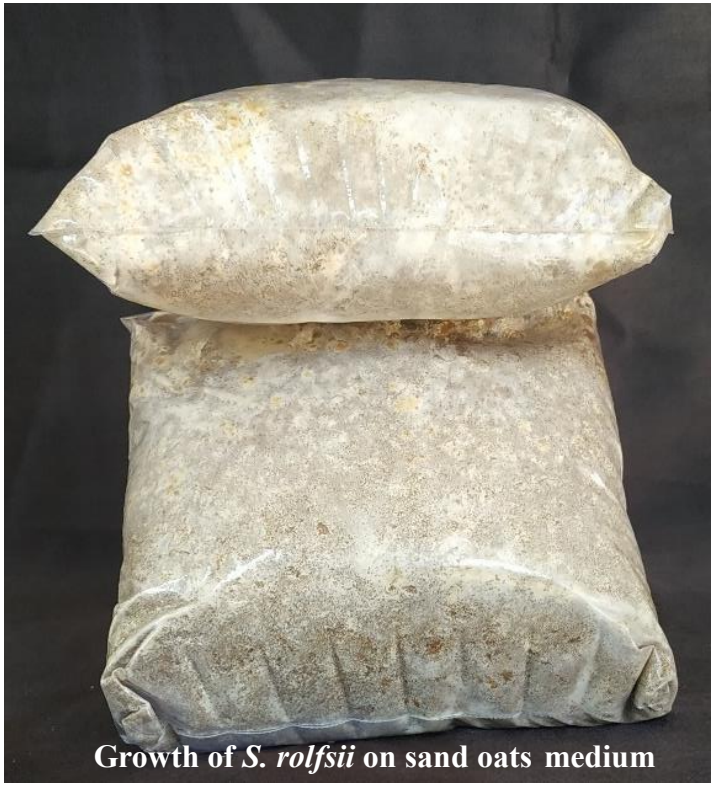


Plate 7. Effect of different levels of soil moisture on the incidence of basal stem rot and blight disease caused by *S. rolfsii* in vegetable cowpea var. Gitika at 16 days after inoculation

Sand oats medium for the multiplication of *S. rolfsii*



Growth of *S. rolfsii* on sand oats medium

Plate 8. Multiplication of *S. rolfsii* on sand oats medium for artificial inoculation in soil

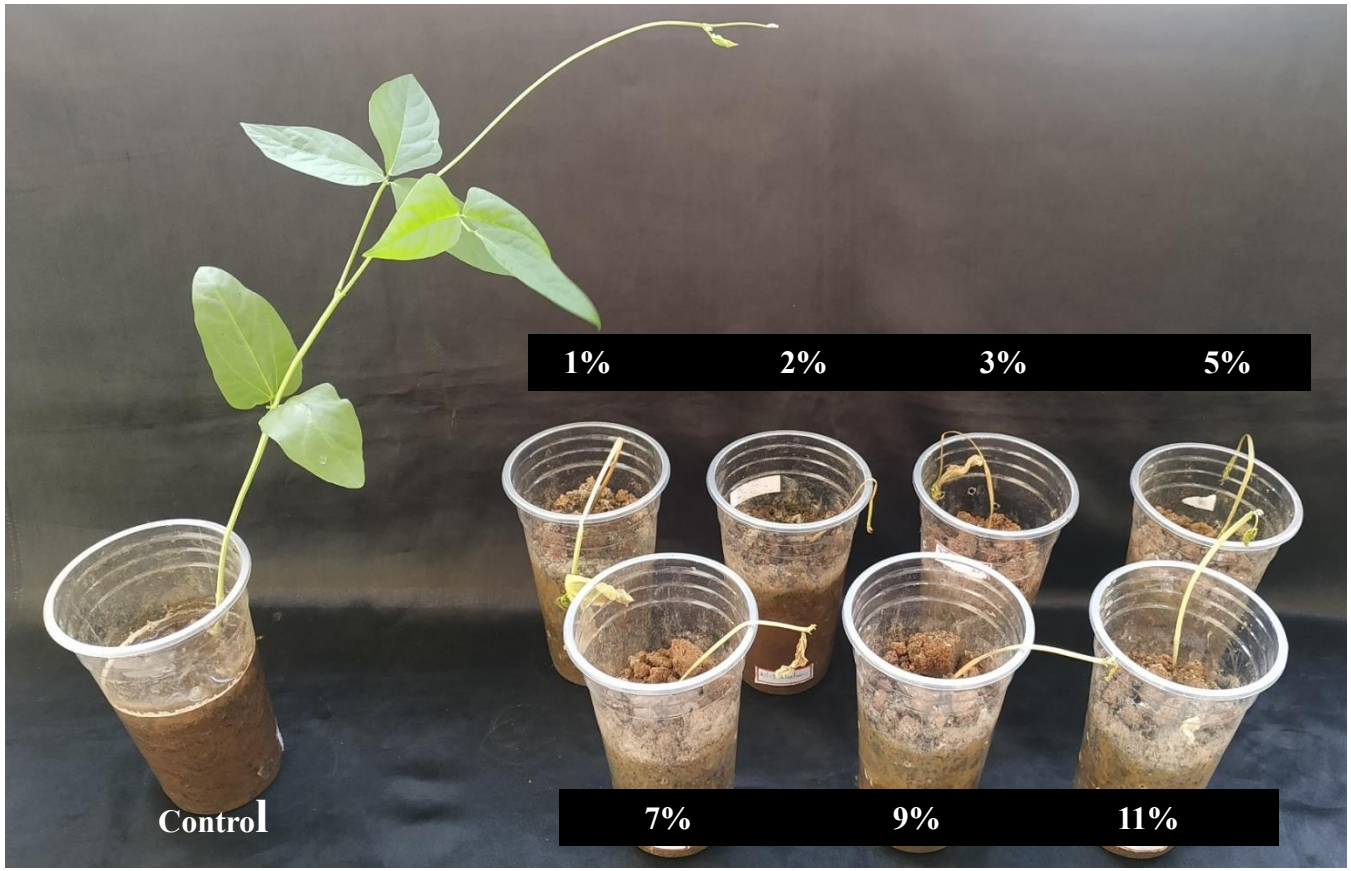


Plate 9. Effect of different levels of inoculum of *S. rolfsii* on the incidence of basal stem rot and blight disease in vegetable cowpea var. Gitika at 15 days after transplanting

Table 11. Effect of different levels of inoculum of *S. rolfsii* on symptom development in vegetable cowpea

Sl. No.	Inoculum level of *S. rolfsii* (%) (w/w)	Days for symptom development
1	1	6
2	2	3
3	3	3
4	5	3
5	7	3
6	9	3
7	11	3

Table 12. Evaluation of fungicides by poisoned food technique on mycelial growth and its inhibition of *S. rolfsii*

Treatments	Mycelial growth* (diameter in cm)	Percentage inhibition of mycelial growth
Wettable sulphur (0.25%)	9.00±0.000[a]	0.00 (0.00±0.00)[c]
Carbendazim (0.20%)	9.00±0.000[a]	0.00 (0.00±0.00)[c]
Copper oxychloride (0.30%)	3.37±0.033[b]	62.59 (52.27±0.22)[b]
Mancozeb + Carbendazim (0.20%)	0.00±0.000[c]	100.00 (90.00±0.00)[a]
Mancozeb (0.03%)	0.00±0.000[c]	100.00 (90.00±0.00)[a]
CD (0.05)	0.048	0.313
SEm±	0.015	0.098

*Mean ± SD of 3 replications

Values followed by similar superscripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

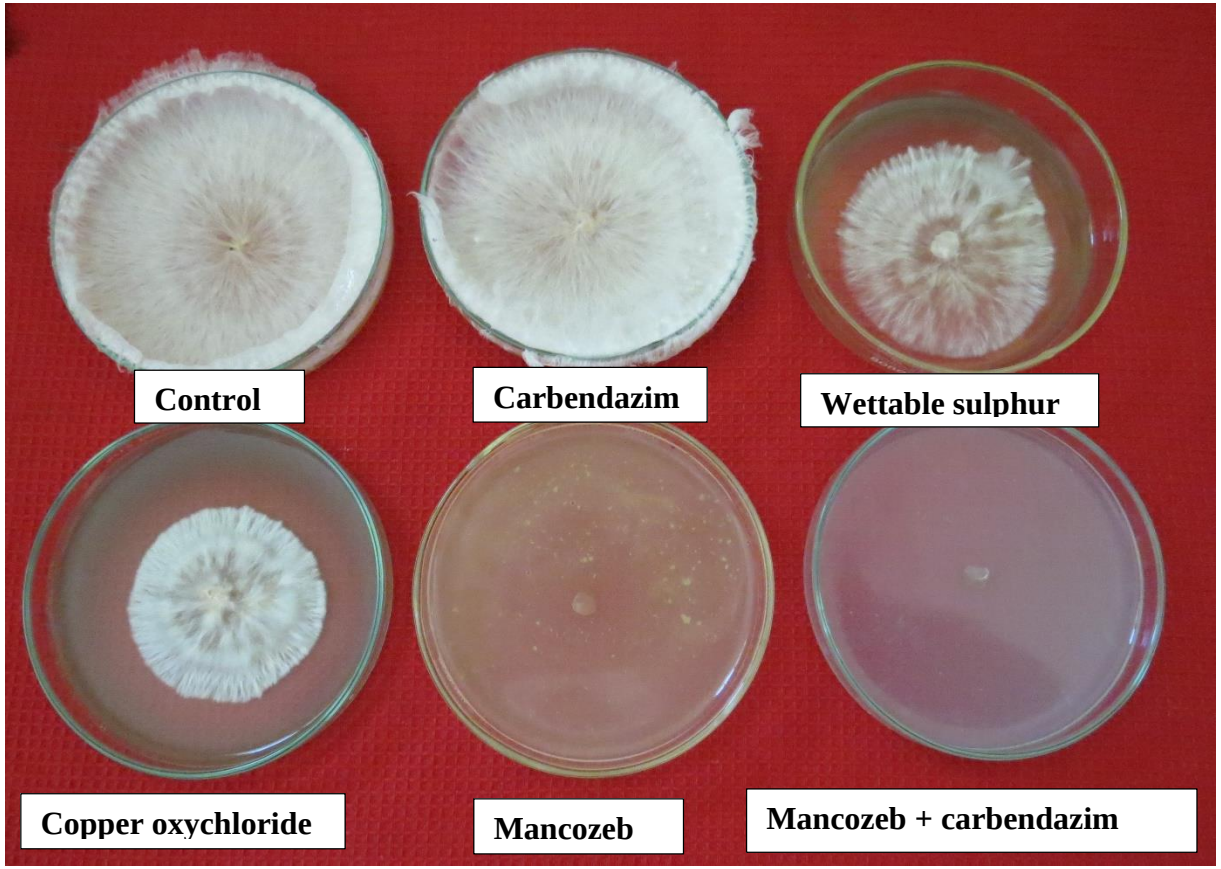


Plate 10. *In vitro* screening of fungicides for inhibition of mycelial growth and formation of sclerotia of *S. rolfsii*

regeneration from sclerotia of *S. rolfsii* under *in vitro* conditions. Since the survival of the fungus in soil is mainly dependent on sclerotial germination, confined container technique was also performed to reconfirm the potential of the treatments in inhibiting the mycelial regeneration from sclerotia using plastic containers (Plate 11).

4.3.1. Assessing the Antifungal Potential of Plant Extracts and Oil Cakes against Mycelia and Sclerotia of *S. rolfsii*

4.3.1.1. Plant Extracts

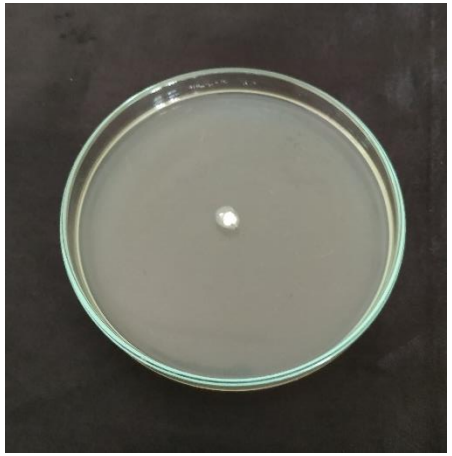
The antifungal potential of the plant extracts at different concentrations (1, 5, 10, 15, 20 and 25%) was tested against the mycelia of *S. rolfsii*. Garlic extract at one per cent followed by garlic creeper at five per cent resulted in complete inhibition (Plate 12) of the mycelial growth of the fungus (Table 13 and 14). When the formation of sclerotia from mycelia was analysed, garlic creeper, the combination of onion and garlic as well as garlic alone, even at one per cent could completely inhibit the formation of sclerotia (Table 15).

When the plant extracts were tested for their antifungal potential to inhibit the sclerotia of the fungus, garlic at one per cent alone resulted in complete inhibition of the mycelial regeneration from sclerotia followed by garlic creeper at ten per cent. At five per cent, garlic creeper resulted in 97 per cent reduction of the mycelial regeneration from sclerotia (Plate13). In this study also, when the production of sclerotia from mycelia was analysed, garlic creeper, the combination of onion and garlic as well as garlic alone even at one per cent completely inhibited the sclerotial formation (Table16, 17 and 18). Thus, the results of this study revealed that garlic (1%) followed by garlic creeper (5 and 10%) were the best effective antifungal extracts for inhibiting the mycelial growth as well as the sclerotial formation of the fungus.

4.3.1.2. Oil Cakes

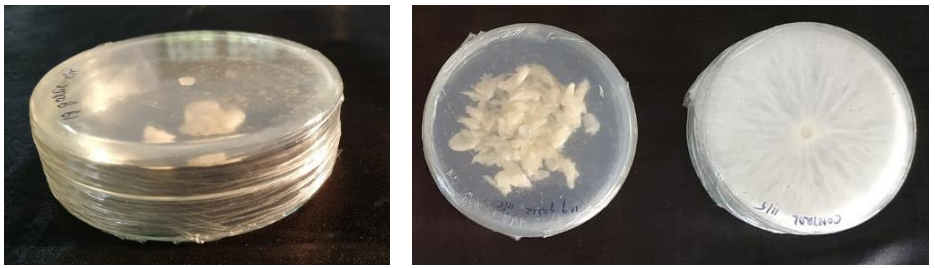
The oil cakes at different concentrations (1, 5, 10, 15, 20 and 25%) were tested for their antifungal potential against the fungus. Mustard cake at ten per cent concentration resulted in complete mycelial inhibition, but sclerotial production from

1. Antifungal potential



Poisoned food technique

2. Bio-fumigation potential



a. Paired plate technique

b. Confined container technique

Plate 11. Methodology used to assess antifungal potential of plants extracts and oil cakes by poisoned food technique; and biofumigation potential by paired plate and confined container techniques

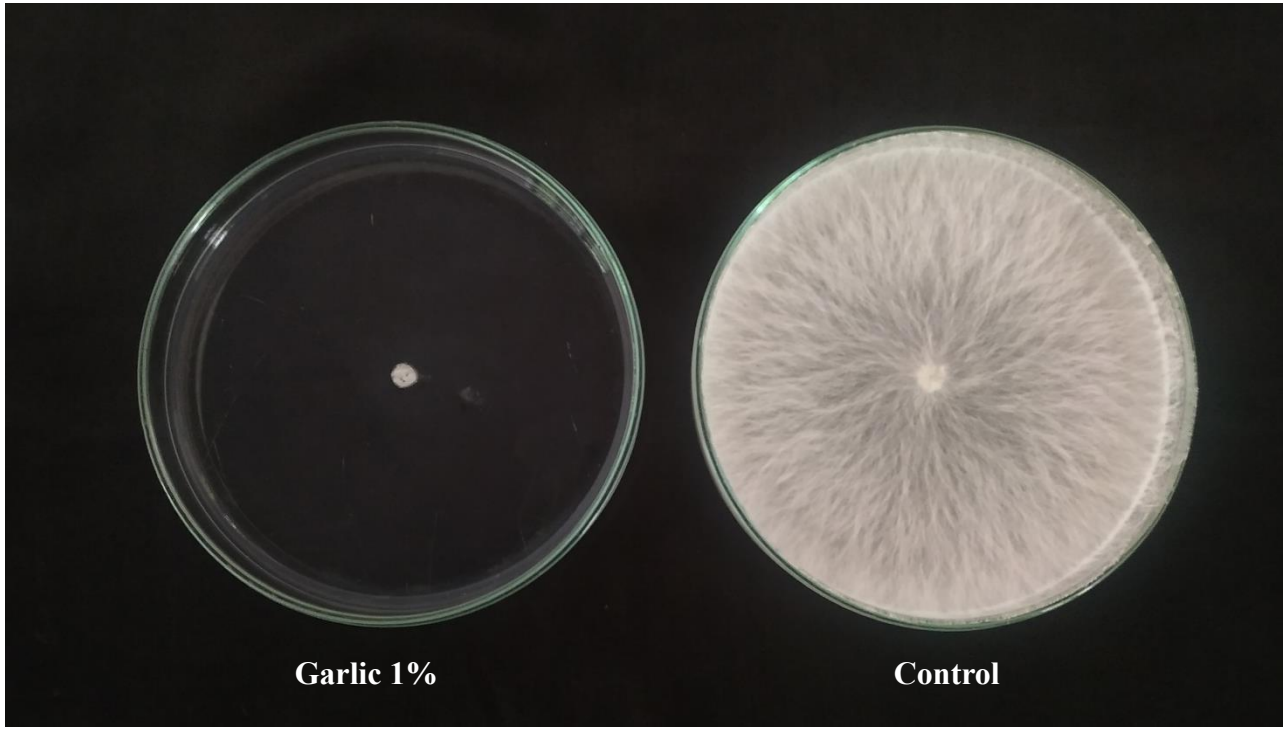


Plate 12. Complete inhibition of mycelial growth from mycelial disc of *S. rolfsii* by 1% garlic extract at 4th day of growth

Table 13. Effect of antifungal potential of plant extracts by poisoned food technique on mycelial growth of *S. rolfsii*

Sl. No.	Treatments	Quantity of selected plant extract for antifungal potential against *S. rolfsii* (%)*					
		1	5	10	15	20	25
		MG	MG	MG	MG	MG	MG
1	Mustard	9.00±0.00^{a}	3.83±0.44^{b}	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{c}
2	Cabbage	9.00±0.00^{a}	9.00±0.00^{a}	8.06±0.06^{c}	7.06±0.12^{d}	2.13±0.08^{c}	0.00±0.00^{c}
3	Garlic creeper	3.03±0.03^{b}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{c}
4	Castor	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	7.46±0.03^{c}	0.00±0.00^{d}	0.00±0.00^{c}
5	Cauliflower	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	8.20±0.05^{b}	8.00±0.00^{b}	6.36±0.08^{b}
6	Onion and garlic	0.63±0.06^{c}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{c}
7	Garlic	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{c}
8	Onion	9.00±0.00^{a}	9.00±0.00^{a}	8.26±0.13^{b}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{c}
9	Control	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}
	CD (0.05)	0.074	0.440	0.149	0.137	0.088	0.088
	SEm±	0.025	0.147	0.050	0.046	0.029	0.029

MG – Diameter of mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, Values followed by similar superscripts are not significantly different at 5% level

Table 14. Effect of antifungal potential of plant extracts by poisoned food technique on mycelial inhibition of *S. rolfsii*

Sl. No.	Treatments	Quantity of selected plant extract for antifungal potential against *S. rolfsii* (%)*					
		1	5	10	15	20	25
		PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
1	Mustard	0.00 (0.00±0.00)^{d}	57.40 (49.29±2.86)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
2	Cabbage	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	10.36 (18.75±0.71)^{b}	21.47 (27.58±0.94)^{b}	76.29 (60.85±0.66)^{b}	100.00 (90.00±0.00)^{a}
3	Garlic creeper	66.29 (54.49±0.22)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
4	Castor	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)c	17.03 (24.36±0.28)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
5	Cauliflower	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{c}	8.83 (17.31±0.65)^{d}	11.11 (19.46±0.00)^{c}	29.25 (32.72±0.62)^{b}
6	Onion and garlic	92.96 (74.63±0.85)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
7	Garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
8	Onion	0.00 (0.00±0.00)^{d}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
9	Control	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}
	CD (0.05)	0.879	2.858	1.661	1.175	0.656	0.616
	SEm±	0.294	0.954	0.555	0.393	0.219	0.206

PI – Percentage inhibition, Values in parenthesis are arcsine transformed values

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Table 15. Effect of antifungal potential of plant extracts by poisoned food technique on inhibition of formation of sclerotia of *S. rolfsii*

Treatments	Quantity of selected plant extract for antifungal potential against *S. rolfsii* (%)*											
	1		5		10		15		20		25	
	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI
Mustard	15	23 (69.33)	100 per cent inhibition									
Cabbage	6	20 (73.33)	5	26 (65.33)	100% inhibition							
Garlic creeper	100% inhibition											
Castor	6	30 (60)	7	20 (73.33)	7	15 (80)						
Cauliflower	6	28 (62.66)	6	45 (40)	6	10 (86.6)						
Onion and garlic	100% inhibition											
Garlic												
Onion	10	32 (57.33)	10	43 (42.66)	100% inhibition							
Control	5	75		75	-	75		75		75	-	75

DSF - Days for sclerotia formation, SN – Sclerotia number, PI – Percentage inhibition of formation of sclerotia; values in parenthesis are percentage inhibition values

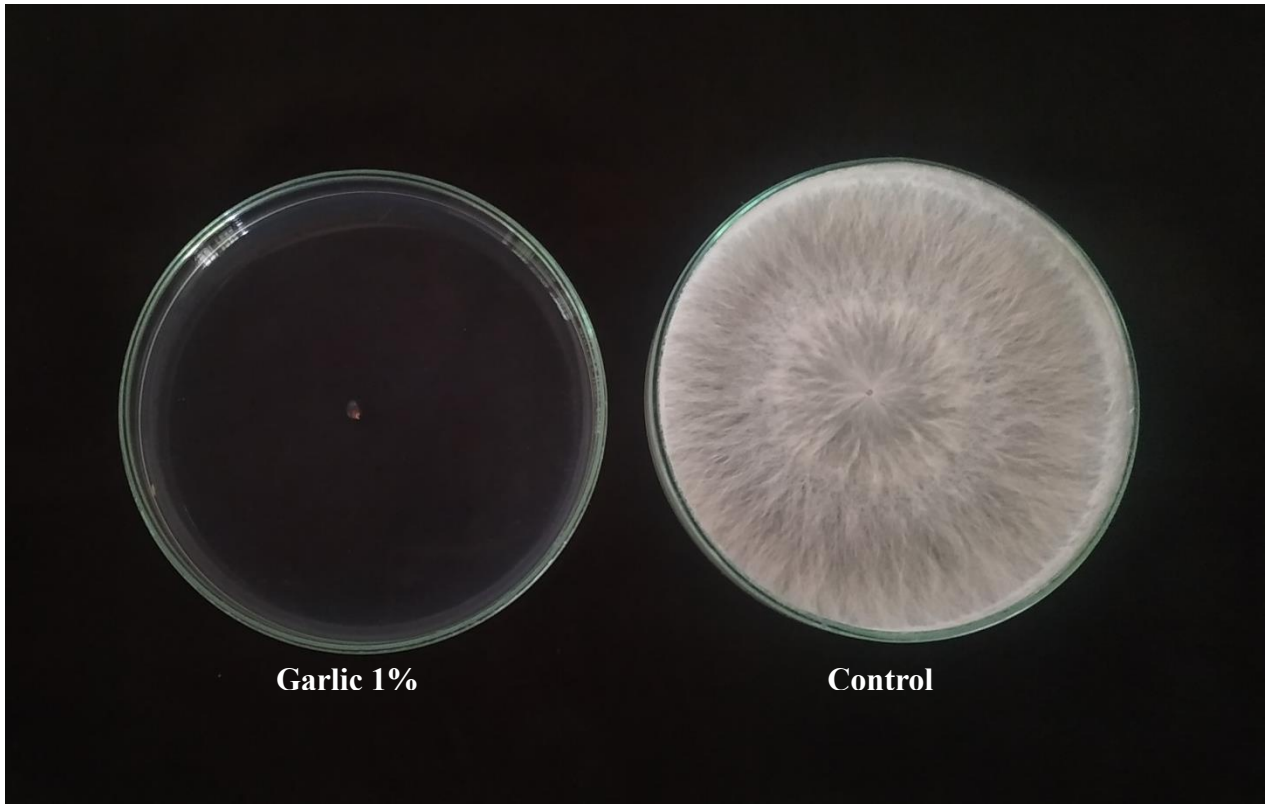


Plate 13. Complete inhibition of mycelial regeneration from sclerotia of *S. rolfsii* by 1% garlic bulb extract at 4th day of growth

Table 16. Effect of antifungal potential of plant extracts by poisoned food technique on mycelial regeneration of *S. rolfsii* from sclerotia

Sl. No.	Treatments	Quantity of selected plant extract for antifungal potential against *S. rolfsii* (%)*					
		1	5	10	15	20	25
		MG	MG	MG	MG	MG	MG
1	Mustard	9.00±0.000^{a}	9.00±0.000^{a}	6.03±0.133^{b}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{e}
2	Cabbage	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	8.33±0.167^{b}	5.07±0.186^{b}	0.93±0.033^{d}
3	Garlic creeper	9.00±0.000^{a}	0.27±0.033^{c}	0.00±0.000^{c}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{e}
4	Castor	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	3.17±0.167^{c}	1.23±0.033^{c}
5	Cauliflower	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	8.97±0.033^{a}	5.13±0.133^{b}	4.10±0.058^{b}
6	Onion and garlic	0.87±0.033^{b}	0.00±0.000^{d}	0.00±0.000^{c}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{e}
7	Garlic	0.00±0.000^{c}	0.00±0.000^{d}	0.00±0.000^{c}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{e}
8	Onion	9.00±0.000^{a}	7.13±0.120^{b}	5.77±0.384^{b}	3.60±0.058^{c}	0.00±0.000^{d}	0.00±0.000^{e}
9	Control	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}
	CD (0.05)	0.033	0.124	0.406	0.179	0.282	0.074
	SEm±	0.011	0.042	0.136	0.060	0.094	0.025

MG – Mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Table 17. Effect of antifungal potential of plant extracts by poisoned food technique on inhibition of mycelial regeneration

Sl. No.	Treatments	Quantity of selected plant extract for antifungal potential against *S. rolfsii* (%)*					
		1	5	10	15	20	25
		PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
1	Mustard	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{d}	32.96 (35.01±0.91)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
2	Cabbage	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{c}	7.40 (15.57±1.95)^{b}	43.7 (41.36±1.19)^{c}	89.62 (71.19±0.35)^{b}
3	Garlic creeper	0.00 (0.00±0.00)^{c}	97.03 (80.09±0.65)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
4	Castor	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{c}	64.81 (53.61±1.10)^{b}	86.29 (68.24±0.31)^{c}
5	Cauliflower	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{c}	0.37 (2.02±2.02)^{c}	42.96 (40.93±0.86)^{c}	54.44 (47.53±0.37)^{d}
6	Onion and garlic	90.37 (71.90±0.36)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
7	Garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
8	Onion	0.00 (0.00±0.00)^{c}	35.92 (27.04±0.95)^{c}	35.92 (36.73±2.58)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100. (90.00±0.00)^{a}
9	Control	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{e}
	CD (0.05)	0.362	1.148	2.734	2.797	1.831	0.591
	SEm±	0.121	0.383	0.913	0.934	0.611	0.197

PI – Percentage inhibition, *Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level
Values in parenthesis are arcsine transformed values

Table 18. Effect of antifungal potential of plant extracts by poisoned food technique on formation of *s*clerotia from regenerated mycelia of *S. rolfsii*

Treatments	Quantity of selected plant extract for antifungal potential against *S. rolfsii* (%)*											
	1		5		10		15		20		25	
	DSF	SN &PI	DSF	SN &PI	DSF	SN &PI	DSF	SN &PI	DSF	SN &PI	DSF	SN &PI
Mustard	10	32 (60.97)	5	20 (75.60)	100% inhibition							
Cabbage	7	30 (63.41)	12	52 (36.58)	9	18 (78.04)	100% inhibition					
Garlic creeper	100% inhibition											
Castor	6	42 (48.78)	9	29 (64.63)	9	12 (89.02)	100% inhibition					
Cauliflower	6	37	6	25	7	18						
Onion and garlic	100% inhibition											
Garlic												
Onion	8	16 (80.48)	100% inhibition									
Control	5	82	5	82	5	-	5	82	5	82	5	82

DSF – Days for sclerotia formation, SN – Sclerotia number, PI – Percentage inhibition

Values in parenthesis are percentage inhibition values

this mycelia was inhibited even at one per cent (Table 19, 20 and 21).

The oil cakes at different concentrations (1, 5, 10, 15, 20 and 25%) were tested for their antifungal potential against the sclerotia of the fungus. The mycelial regeneration from sclerotia was completely inhibited only at 15 per cent concentration of mustard cake, but formation of sclerotia from the regenerated mycelium was completely inhibited even at one per cent concentration (Table 22, 23 and 24).

4.3.2. Biofumigation Potential of Plant Extracts and Oil Cakes against Mycelia and Sclerotia

4.3.2.1. Plant Extracts

The biofumigation potential of the plant extracts at different concentrations (1, 5, 10, 15, 20 and 25g) was tested against *S. rolfsii*. Among all the extracts tested at different concentrations, garlic extract (1g) and the combination of garlic and onion extracts (1g) resulted in complete inhibition of the mycelial growth (Plate 14 and 15). However, onion extract (1g) when tested individually resulted only in 52.55 per cent inhibition of the mycelial growth. Hence, it can be concluded that the effect of the combination of garlic and onion extract (1g) is due to the biofumigation potential of garlic bulbs alone (1g). The next best effective treatments were garlic creeper (5g) and onion (5g) which also resulted in the complete mycelial inhibition. However, onion at 15g revealed a reduction in inhibiting the mycelial growth and hence, cannot be considered as an effective biofumigant against *S. rolfsii.* The inhibition of sclerotial formation from mycelia was also analysed and it was found that mustard, garlic creeper, the combination of onion and garlic as well as garlic alone at one per cent completely inhibited the sclerotial formation (Table 25, 26 and 27).

The biofumigation potential of the plant extracts at different concentrations (1, 5, 10, 15, 20 and 25g) was tested against the mycelial regeneration from sclerotia. Among all the extracts tested at different concentrations, garlic extract (1g), onion (1g) and the combination of garlic and onion extracts (1g) (Plates 16 and 17) resulted in complete inhibition of sclerotia followed by garlic creeper (5g). The combination of onion and

Table 19. Effect of antifungal potential of oil cakes by poisoned food technique on mycelial growth of *S. rolfsii*

Sl. No.	Treatments	Quantity of selected oil cake extract for antifungal potential against *S. rolfsii* (%)*					
		1	5	10	15	20	25
		MG	MG	MG	MG	MG	MG
1	Castor cake	9.00 ± 0.000^{a}	9.00 ± 0.000^{a}	0.37 ± 0.120^{c}	0.00 ± 0.000^{c}	0.07 ± 0.07^{b}	0.00 ± 0.000^{b}
2	Mustard cake	4.10 ± 0.153^{b}	2.03 ± 0.033^{b}	0.00 ± 0.000^{d}	0.00 ± 0.000^{c}	0.00 ± 0.00^{b}	0.00 ± 0.000^{b}
3	Pongamia cake	9.00 ± 0.000^{a}	9.00 ± 0.000^{a}	5.30 ± 0.115^{b}	4.63 ± 0.033^{b}	0.00 ± 0.00^{b}	0.03 ± 0.033^{b}
4	Control	9.00 ± 0.000^{a}	9.00 ± 0.000^{a}	9.00 ± 0.000^{a}	9.00 ± 0.000^{a}	9.00 ± 0.00^{a}	9.00 ± 0.000^{a}
	CD (0.05)	0.253	0.055	0.276	0.055	0.110	0.055
	SEm±	0.076	0.017	0.083	0.017	0.033	0.017

MG - Diameter of mycelial growth in cm on 4^{th} day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Table 20. Effect of antifungal potential of oil cakes by poisoned food technique on mycelial inhibition of *S. rolfsii*

Sl. No	Treatments	Quantity of selected oil cake extract for antifungal potential against *S. rolfsii* (%)*					
		1	5	10	15	20	25
		PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
1	Castor cake	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	95.92 (78.61±1.89)^{b}	100.00 (90.00±0.00)^{a}	99.25 (87.13±2.87)^{a}	100.00 (90.00±0.00)^{a}
2	Mustard cake	54.51 (47.57±0.96)^{a}	77.4 (61.59±0.25)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
3	Pongamia cake	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	41.10 (39.86±0.75)^{c}	48.51 (44.13±0.21)^{b}	100.00 (90.00±0.00)^{a}	99.62 (87.96±2.04)^{a}
4	Control	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}
	CD (0.05)	1.596	0.418	3.361	0.351	4.758	3.372
	SEm±	0.482	0.126	1.015	0.106	1.437	1.018

PI – Percentage inhibition

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

Table 21. Effect of antifungal potential of oil cakes on *in vitro* inhibition of formation of sclerotia of *S. rolfsii* by poisoned food technique

Sl. No.	Treatments	Quantity of selected oil cake extract for antifungal potential against *S. rolfsii* (%)*											
		1		5		10		15		20		25	
		DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI
1	Castor cake	9	23 (69.33)	100% inhibition									
2	Mustard cake	100% inhibition											
3	Pongamia cake	7	42 (44)	11	15 (80)	100% inhibition							
4	Control	5	75	5	75	5	75	5	75	5	75	5	75

DSF – Days for sclerotia formation, SN – Sclerotia number, PI – Percentage inhibition
Values in parenthesis are percentage inhibition values

Table 22. Effect of antifungal potential of oil cakes by poisoned food technique on mycelial regeneration from sclerotia of *S. rolfsii*

Sl. No.	Treatments	Quantity of selected oil cake extract for antifungal potential against *S. rolfsii* (%)*					
		1	5	10	15	20	25
		MG	MG	MG	MG	MG	MG
1	Castor cake	9.00±0.000[a]	9.0±0.000[a]	3.10±0.153[c]	2.03±0.120[c]	0.00±0.00[b]	0.03±0.03[b]
2	Mustard cake	5.83±0.120[b]	5.3±0.033[b]	2.47±0.120[d]	0.00±0.000[d]	0.00±0.00[b]	0.00±0.00[b]
3	Pongamia cake	9.00±0.000[a]	9.0±0.000[a]	6.10±0.115[b]	3.33±0.203[b]	0.03±0.03[b]	0.00±0.00[b]
4	Control	9.00±0.000[a]	9.0±0.000[a]	9.00±0.000[a]	9.00±0.000[a]	9.00±0.00[a]	9.00±0.00[a]
	CD (0.05)	0.199	0.055	0.374	0.390	0.055	0.055
	SEm±	0.060	0.017	0.113	0.118	0.017	0.017

MG – Diameter of mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Table 23. Effect of antifungal potential of oil cakes by poisoned food technique on inhibition of mycelial regeneration from sclerotia of *S. rolfsii*

Sl. No.	Treatments	Quantity of selected oil cake extract for antifungal potential against *S. rolfsii* (%)*					
		1	5	10	15	20	25
		PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
1	Castor cake	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	65.5 (54.06±1.03)^{b}	77.04 (61.61±0.92)^{b}	100.00 (90.00±0.00)^{a}	99.62 (87.96±2.04)^{a}
2	Mustard cake	35.18 (36.36±0.80)^{a}	41.48 (40.08±0.22)^{a}	72.5 (58.40±0.84)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
3	Pongamia cake	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	32.06 (34.55±0.78)^{c}	62.95 (52.51±1.34)^{c}	99.62 (87.96±2.04)^{a}	100.00 (90.00±0.00)^{a}
4	Control	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}
	CD (0.05)	0.199	1.321	0.055	0.356	0.374	2.553
	SEm±	0.060	0.399	0.017	0.107	0.113	0.771

PI – Percentage inhibition of mycelial regeneration from sclerotia

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

Table 24. Effect of antifungal potential of oil cakes by poisoned food technique on inhibition of sclerotia formation from regenerated mycelium of *S. rolfsii*

		Quantity of selected oil cake extract for antifungal potential against *S. rolfsii* (%)*											
		1		**5**		**10**		**15**		**20**		**25**	
		DSF	**SN & PI**	**DSF**	**SN & PI**	**DSF**	**SN & PI**	**DSF**	**SN & PI**	**DSF**	**SN & PI**	**DSF**	**SN & PI**
1	Castor cake	7	20 (70.60)	12	32 (60.97)	100% inhibition							
2	Mustard cake	100% inhibition											
3	Pongamia cake	6	40 (51.21)	8	35 (57.31)	100% inhibition							
4	Control	5	82	5	82	5	82	5	82	5	82	5	82

DSF – Days for sclerotia formation, SN – Sclerotia number, PI – Percentage inhibition of mycelial regeneration from sclerotia
Values in parenthesis are percentage inhibition values

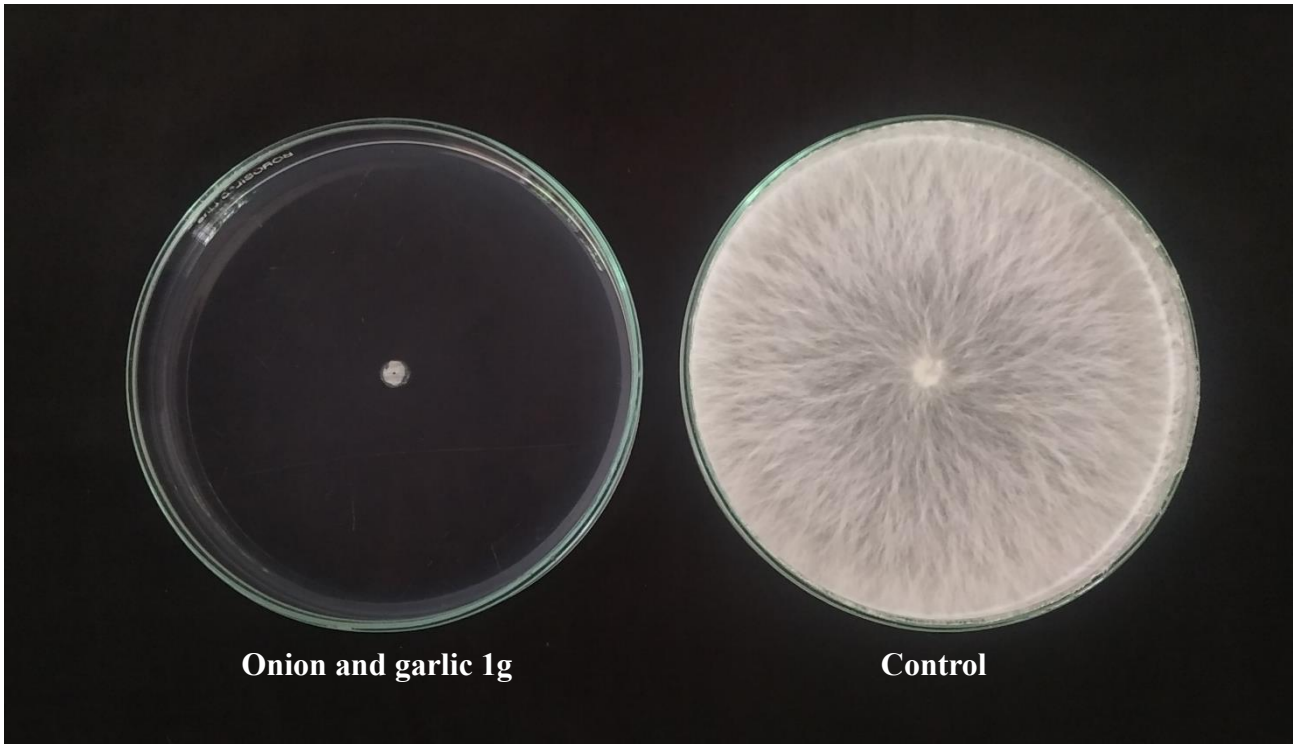


Plate 14. Complete inhibition of mycelial growth from mycelial bit of *S. rolfsii* by 1g onion and garlic at 4th day of growth

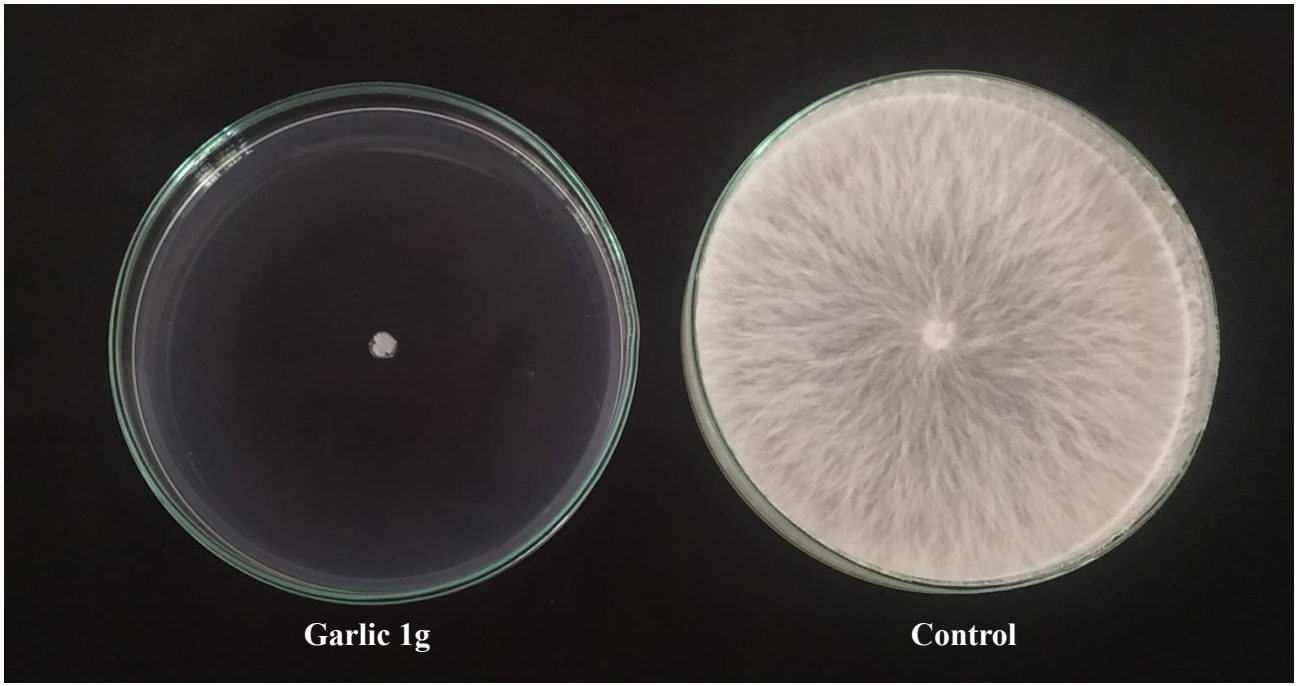


Plate 15. Complete inhibition of mycelial growth from mycelial bit of *S. rolfsii* by 1g garlic at 4th day of growth

Table 25. Effect of biofumigation potential of plant extracts by paired plate technique on mycelial growth of *S. rolfsii*

Sl. No.	Treatments	Quantity of macerated tissue of selected plants for biofumigation potential against *S. rolfsii* (g)*					
		1	5	10	15	20	25
		MG	MG	MG	MG	MG	MG
1	Mustard	9.00±0.000^{a}	9.00±0.000^{a}	4.17±0.088^{c}	0.00±0.000^{d}	0.00±0.000^{d}	0.57±0.088^{bc}
2	Cabbage	9.00±0.000^{a}	5.47±0.033^{c}	2.43±0.088^{d}	0.70±0.058^{b}	0.87±0.033^{b}	0.47±0.088^{c}
3	Garlic creeper	3.03±0.033^{c}	0.00±0.000^{d}	0.00±0.000^{e}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{d}
4	Castor	9.00±0.000^{a}	8.13±0.088^{b}	0.00±0.000^{e}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{d}
5	Cauliflower	9.00±0.000^{a}	9.00±0.000^{a}	6.10±0.100^{b}	0.00±0.000^{d}	0.57±0.033^{c}	0.67±0.088^{b}
6	Onion and garlic	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{e}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{d}
7	Garlic	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{e}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{d}
8	Onion	4.27±0.120^{b}	0.00±0.000^{d}	0.00±0.000^{e}	0.43±0.088^{c}	0.00±0.000^{d}	0.00±0.000^{d}
9	Control	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000
	CD (0.05)	0.124	0.094	0.160	0.105	0.047	0.152
	SEm±	0.042	0.031	0.053	0.035	0.016	0.051

MG – Mycelial growth on 4th day, * Mean ± SD of 3 replications, Values followed by similar superscripts are not significantly different at 5% level

Table 26. Effect of biofumigation potential of plant extracts by paired plate technique on mycelial growth inhibition of *S. rolfsii*

Sl. No.	Treatments	Quantity of macerated tissue of selected plants for biofumigation potential against *S. rolfsii* (g)*					
		1	5	10	15	20	25
		PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
1	Mustard	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	53.70 (47.10±0.56)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	93.70 (75.52±1.19)^{b}
2	Cabbage	0.00 (0.00±0.00)^{d}	39.25 (38.78±0.22)^{b}	72.96 (58.65±0.62)^{b}	92.22 (73.80±0.69)^{c}	90.37 (71.90±0.36)c	94.81 (76.92±1.31)^{b}
3	Garlic creeper	66.29 (54.49±0.22)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
4	Castor	0.00 (0.00±0.00)^{d}	9.62 (18.02±0.97)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
5	Cauliflower	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	32.22 (34.57±0.68)^{d}	100.00 (90.00±0.00)$_{a}$	93.70 (75.45±0.44)b	93.33 (75.04±0.74)^{b}
6	Onion and garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)$_{a}$	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
7	Garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
8	Onion	52.59 (35.78±11.43)^{c}	95.18 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	95.18 (77.41±1.29)^{b}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
9	Control	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{e}	0.00 (0.000±0.00)^{d}	0.00 (0.00±0.00)d	0.00 (0.00±0.00)^{c}
	CD (0.05)	11.413	0.992	1.084	1.462	0.571	1.916
	SEm±	3.812	0.331	0.362	0.488	0.191	0.640

PI – Percentage inhibition, values followed by similar superscripts are not significantly different at 5%, values in parenthesis are arcsine transformed values

Table 27. Effect of biofumigation potential of plant extracts by paired plate technique on inhibition of formation of sclerotia of *S. rolfsii*

Sl. No.	Treatments	Quantity of macerated tissue of selected plants for biofumigation potential against *S. rolfsii* (g)											
		1		5		10		15		20		25	
		DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI
1	Mustard	100% inhibition											
2	Cabbage	7	30 (62.02)	100% inhibition									
3	Garlic creeper	100% inhibition											
4	Castor	9	27 (65.82)	100% inhibition									
5	Cauliflower	6	35 (55.69)	6	39 (50.63)	100% inhibition							
6	Onion and garlic	100% inhibition											
7	Garlic												
8	Onion	8	16 (79.74)	-	-	-		-	-	-	-	-	-
9	Control	5	79	5	79	5	79	5	79	5	79	5	79

DSF – Days for sclerotial formation, SN – Sclerotial number, PI – Percentage inhibition; Values in parenthesis are percentage inhibition values

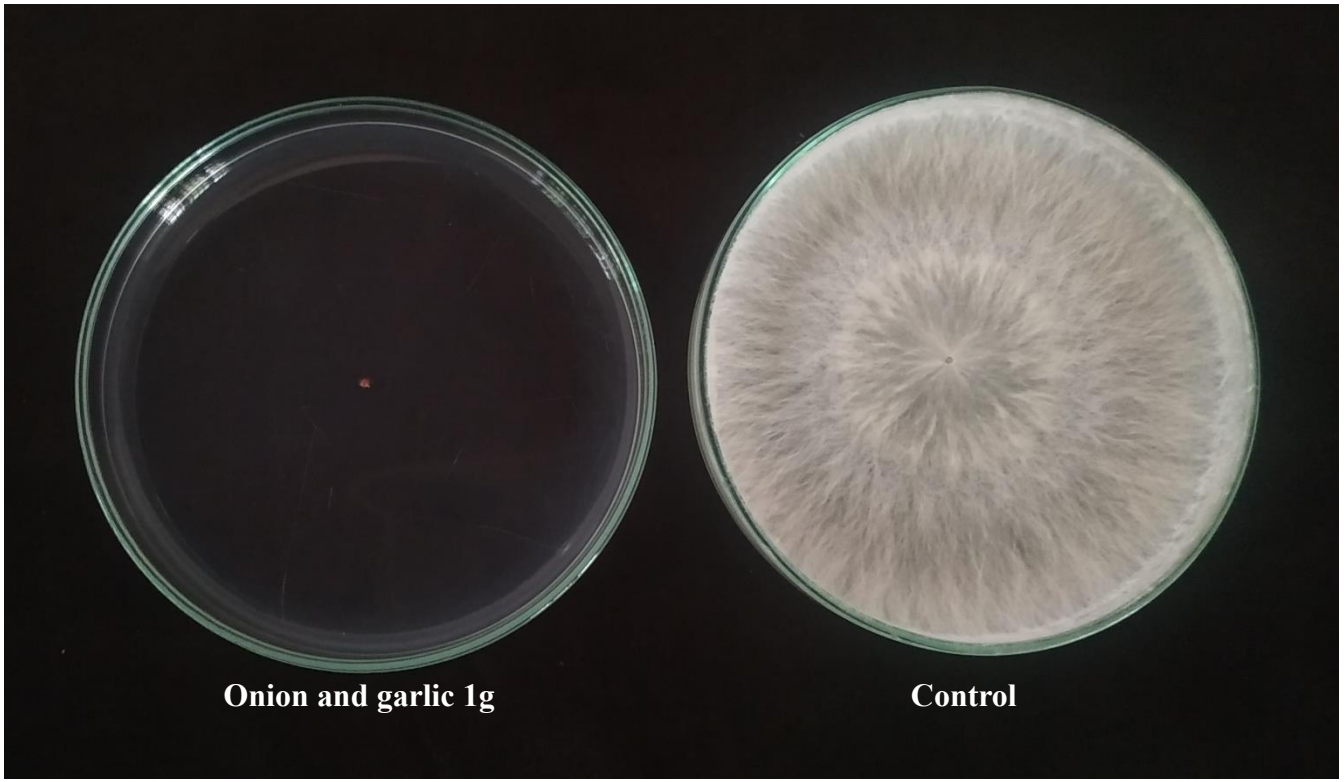


Plate 16. Complete inhibition of mycelial regeneration from sclerotia of *S. rolfsii* by 1g onion and garlic at 4th day of growth

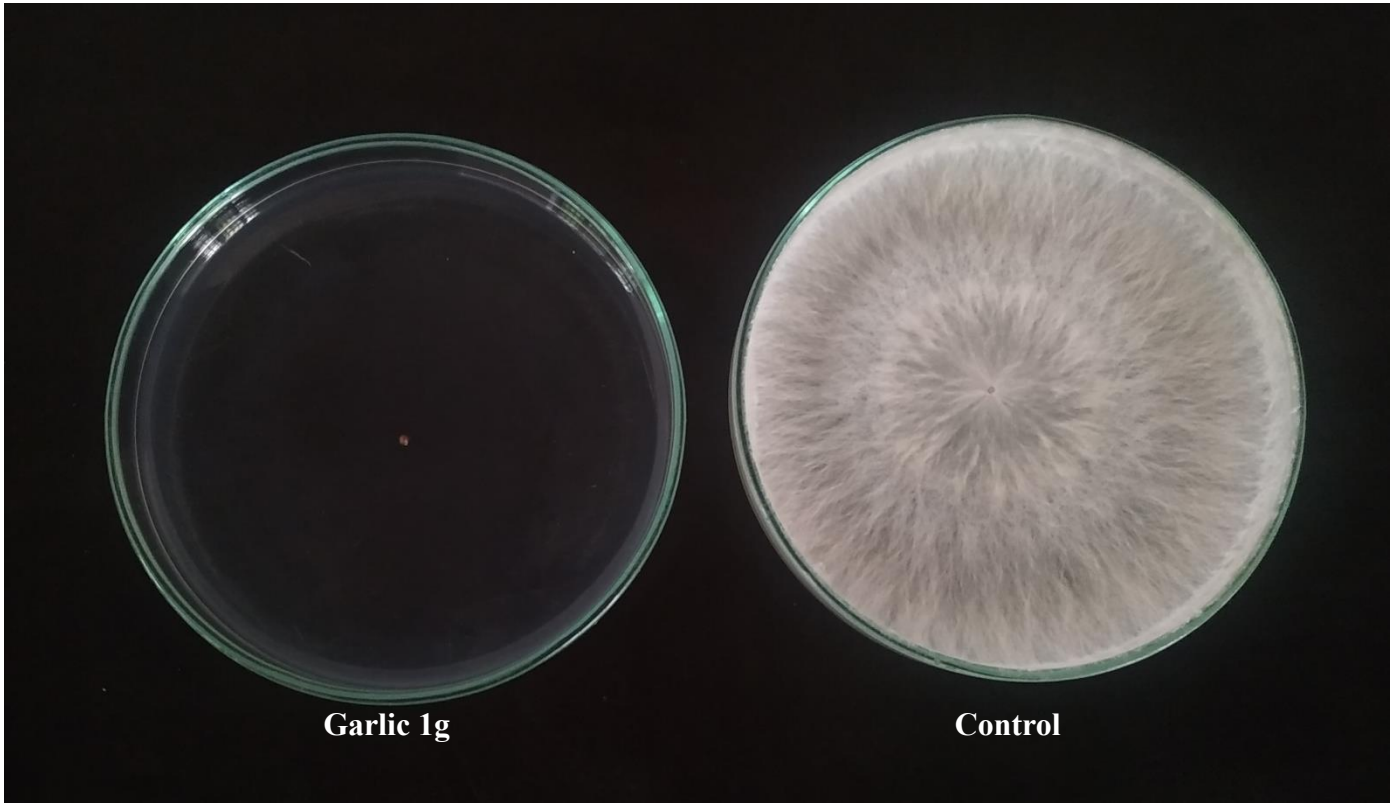


Plate 17. Complete inhibition of mycelial regeneration from sclerotia of *S. rolfsii* by 1g garlic at 4th day of growth

garlic as well as garlic alone at one per cent completely inhibited sclerotial formation (Table 28, 29 and 30). Thus, from this study, garlic (1g) followed by garlic creeper (5g) were selected as the best effective biofumigants for inhibiting the mycelia as well as sclerotia of the fungus.

4.3.2.2. Oil Cakes

The biofumigation potential of the oil cakes at different concentrations (1, 5, 10, 15, 20 and 25g) was tested against both the mycelia and sclerotia of the fungus. Among them, mustard cake at 5g concentration resulted in complete inhibition of the mycelial growth. It also inhibited the production of sclerotia even at 1g concentration (Table 31, 32 and 33). The mycelial regeneration from sclerotia was completely inhibited only at 10 g concentration of mustard cake, but the sclerotial formation was completely inhibited at 1g concentration of this oil cake (Table 34, 35 and 36).

4.3.3. Assessing the Inhibition of Mycelial Regeneration from Sclerotia Using Confined Container Technique

4.3.3.1. Plant Extracts

The biofumigation potential of plant extracts at one per cent was tested by confined container technique to study their effect on the inhibition of sclerotia of *S. rolfsii*. Garlic extract at one per cent resulted in complete inhibition of the regeneration of mycelia from sclerotia (Plate 18, 19 and 20) up to three week duration (Table 37).

4.3.3.2. Oil Cakes

Among the oil cakes tested at five per cent concentration, mustard cake resulted in complete inhibition of the mycelial growth up to one week after which, the inhibition of the mycelial regeneration had decreased (Table 38).

4.3.4. Selection of the Best Treatments

Based on the above experiments, garlic at one per cent and garlic creeper at five per cent were selected as the two best treatments based on their biofumigation and

Table 28. Effect of biofumigation potential of plant extracts by paired plate technique on mycelial regeneration of *S. rolfsii* from sclerotia

Sl. No.	Treatments	Quantity of macerated tissue of selected plants for biofumigation potential against *S. rolfsii* (g)*					
		1	5	10	15	20	25
		MG	MG	MG	MG	MG	MG
1	Mustard	9.00±0.000^{a}	9.00±0.000^{a}	6.10±0.100^{b}	0.00±0.000^{c}	0.00±0.000^{d}	0.73±0.120^{b}
2	Cabbage	9.00±0.000^{a}	6.50±0.200^{c}	5.00±0.153^{c}	0.00±0.000^{c}	0.40±0.115^{c}	0.33±0.088^{c}
3	Garlic creeper	4.13±0.033^{c}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{c}	0.00±0.000^{d}	0.00±0.000^{d}
4	Castor	9.00±0.000^{a}	8.63±0.088^{b}	0.00±0.000^{d}	0.00±0.000^{c}	0.87±0.033^{b}	0.00±0.000^{d}
5	Cauliflower	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	5.17±0.088^{b}	0.00±0.000^{d}	0.27±0.033^{c}
6	Onion and garlic	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{c}	0.00±0.000^{d}	0.00±0.000^{d}
7	Garlic	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{c}	0.00±0.000^{d}	0.00±0.000^{d}
8	Onion	5.37±0.233^{b}	0.00±0.000^{d}	0.00±0.000^{d}	0.00±0.000^{c}	0.00±0.000^{d}	0.00±0.000^{d}
9	Control	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}
	CD (0.05)	0.235	0.218	0.182	0.088	0.120	0.152
	SEm±	0.079	0.073	0.061	0.029	0.040	0.051

MG – Diameter of mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Table 29. Effect of biofumigation potential of plant extracts by paired plate technique on inhibition of mycelial regeneration from *sclerotia*

Sl. No.	Treatments	Quantity of macerated tissue of selected plants for biofumigation potential against *S. rolfsii* (g)*					
		1	5	10	15	20	25
		PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
1	Mustard	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	32.22 (34.57±0.68)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	91.85 (73.50±1.45)^{c}
2	Cabbage	0.00 (0.00±0.00)^{d}	27.77 (31.76±1.44)^{b}	44.44 (41.79±0.98)^{b}	100.00 (90.00±0.00)^{a}	96.21 (78.06±1.85)^{b}	96.29 (79.05±1.47)^{b}
3	Garlic creeper	54.07 (47.32±0.21)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
4	Castor	0.00 (0.00±0.00)^{d}	4.07 (11.45±1.50)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	90.37 (71.90±0.36)^{c}	100.00 (90.00±0.00)^{a}
5	Cauliflower	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	42.59 (40.72±0.57)^{b}	100.00 (90.00±0.00)^{a}	97.03 (80.09±0.65)^{b}
6	Onion and garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
7	Garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
8	Onion	40.36 (39.41±1.51)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
9	Control	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}
	CD (0.05)	1.520	2.078	1.192	0.565	1.877	2.161
	SEm±	0.508	0.694	0.398	0.189	0.627	0.722

MG – Diametre of mycelial growth in cm on 4th day; *Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level, values in parenthesis are arcsine transformed values

Table 30. Effect of biofumigation potential of plant extracts by paired plate technique on inhibition of sclerotia formation of *S. rolfsii* sclerotia

Sl. No.	Treatments	Quantity of macerated tissue of selected plants for biofumigation potential against *S. rolfsii* (g)											
		1		5		10		15		20		25	
		DSF	SN &PI	DSF	SN &PI	DSF	SN &PI	DSF	SN &PI	DSF	SN &PI	DSF	SN &PI
1	Mustard	9	36 (42.85)	100% Inhibition									
2	Cabbage	9	33 (47.61)										
3	Garlic creeper	4	29 (53.96)										
4	Castor	6	34 (46.03)										
5	Cauliflower	6	42 (33.33)	11	31 (50.79)	9	16 (74.60)	100% Inhibition					
6	Onion and garlic	100% Inhibition											
7	Garlic												
8	Onion	8	16	-	-	-	-	-	-	-	-	-	-
9	Control	5 I	63	5	63	5	63	5	63	5	63	5	63

DSF – Days for sclerotia formation, SN – Sclerotia number, PI – Percentage inhibition ;Values in parenthesis are percentage inhibition values

Table 31. Effect of biofumigation potential of oil cakes by paired plate technique on mycelial growth of *S. rolfsii*

Sl. No.	Treatments	Quantity of macerated tissue of oil cakes for biofumigation potential against *S. rolfsii* (g)*					
		1	5	10	15	20	25
		MG	MG	MG	MG	MG	MG
1	Castor cake	9.00±0.00^{a}	8.97±0.03^{a}	1.10±0.11^{c}	0.03±0.03^{b}	0.00±0.00^{b}	0.03±0.03^{b}
2	Mustard cake	5.13±0.23^{b}	0.00±0.00^{b}	0.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
3	Pongamia cake	9.00±0.00^{a}	8.93±0.06^{a}	7.10±0.11^{b}	0.00±0.00^{b}	0.03±0.03^{b}	0.03±0.03^{b}
4	Control	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}
	CD (0.05)	0.386	0.123	0.270	0.055	0.055	0.078
	SEm±	0.117	0.037	0.082	0.017	0.017	0.024

MG – Diametre of mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Table 32. Effect of biofumigation potential of oil cakes by paired plate technique on mycelial inhibition of *S. rolfsii*

Sl. No.	Treatments	Quantity of macerated tissue of oil cakes for biofumigation potential against *S. rolfsii* (g)*					
		1	5	10	15	20	25
		PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
1	Castor cake	0.00 (0.00±0.00)^{b}	0.37 (2.02±2.02)^{b}	87.77 (69.56±1.13)^{b}	99.63 (87.96±2.04)^{a}	100 (90.00±0.00)^{a}	99.63 (87.96±2.04)^{a}
2	Mustard cake	42.95 (40.92±1.50^{a}	100 (90.00±0.00)^{a}	100 (90.00±0.00)^{a}	100 (90.00±0.00)^{a}	100 (90.00±0.00)^{a}	100 (90.00±0.00)^{a}
3	Pongamia cake	0.00 (0.00±0.00)^{b}	0.74 (2.86±2.86)^{b}	21.11 (27.32±0.90)^{c}	100 (90.00±0.00)^{a}	99.63 (87.96±2.04)^{a}	99.63 (87.96±2.04)^{a}
4	Control	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}
	CD (0.05)	2.483	5.787	2.392	3.372	3.372	4.768
	SEm±	0.750	1.747	0.722	1.018	1.018	1.440

MG – Diametre of mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

Table 33. Effect of biofumigation potential of oil cakes by paired plate technique on inhibition of sclerotia formation of *S. rolfsii*

Sl. No.	Treatments	Quantity of macerated tissue of oil cakes for biofumigation potential against *S. rolfsii* (g)											
		1		5		10		15		20		25	
		DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI
1	Castor cake	5	37 (53.16)	100% Inhibition									
2	Mustard cake	100% Inhibition											
3	Pongamia cake	7	26 (67.08)	100% Inhibition									
4	Control	5	79	5	79	5	79	5	79	5	79	5	79

DSF – Days for sclerotial formation, SN – Sclerotia number, PI – Percentage inhibition; values in parenthesis are percentage inhibition values

Table 34. Effect of biofumigation potential of oil cakes by paired plate technique on mycelial regeneration of *S. rolfsii* from *s*clerotia

Sl. No.	Treatments	Quantity of macerated tissue of oil cakes for biofumigation potential against *S. rolfsii* (g)*					
		1	5	10	15	20	25
		MG	MG	MG	MG	MG	MG
1	Castor cake	9.00±0.000^{a}	9.00±0.000^{a}	2.10±0.200^{b}	0.03±0.033^{b}	0.07±0.067^{b}	0.03±0.033^{b}
2	Mustard cake	6.63±0.176^{b}	2.10±0.100^{b}	0.00±0.000^{c}	0.00±0.000^{b}	0.00±0.000^{b}	0.00±0.000^{b}
3	Pongamia cake	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	0.07±0.067^{b}	0.03±0.033^{b}	0.10±0.100^{b}
4	Control	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}
	CD (0.05)	0.292	0.166	0.331	0.123	0.123	0.175
	SEm±	0.088	0.050	0.100	0.037	0.037	0.053

MG – Diametre of mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Table 35. Effect of biofumigation potential of oil cakes by paired plate technique on inhibition mycelial regeneration from *s*clerotia of *S. rolfsii*

Sl. No.	Treatments	Quantity of macerated tissue of oil cakes for biofumigation potential against *S. rolfsii* (g)*					
		1	5	10	15	20	25
		PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
1	Castor cake	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	76.66 (61.14±1.54)^{b}	99.62 (87.96±2.04)^{a}	99.25 (87.13±2.87)^{a}	99.6 (87.96±2.04)^{a}
2	Mustard cake	26.18 (30.81±1.27)^{a}	76.66 (61.10±0.75)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
3	Pongamia cake	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{c}	99.25 (87.13±2.87)^{a}	99.62 (87.96±2.04)^{a}	98.88 (86.48±3.52)^{b}
4	Control	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{b}	0.00 (0.00±0.00)^{c}
	CD (0.05)	2.102	1.235	2.542	5.832	5.832	6.735
	SEm±	0.635	0.373	0.768	1.761	1.761	2.034

MG – Diametre of mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

Table 36. Effect of biofumigation potential of oil cakes by paired plate technique on inhibition of *s*clerotia formation from *S. rolfsii*

Sl. No.	Treatments	Quantity of macerated tissue of oil cakes for biofumigation potential against *S. rolfsii* (g)											
		1		5		10		15		20		25	
		DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI	DSF	SN & PI
1	Castor cake	7	42 (33.33)	7	19 (69.84)	100% Inhibition							
2	Mustard cake	100% Inhibition											
3	Pongamia cake	7	38 (39.68)	10	29 (53.96)	9	31 (50.7)	100% Inhibition					
4	Control	5	63	5	63	5	63	5	63	5	63	5	63

DSF – Days for sclerotia formation, SN – Sclerotia number, PI – Percentage inhibition
Values in parenthesis are percentage inhibition values

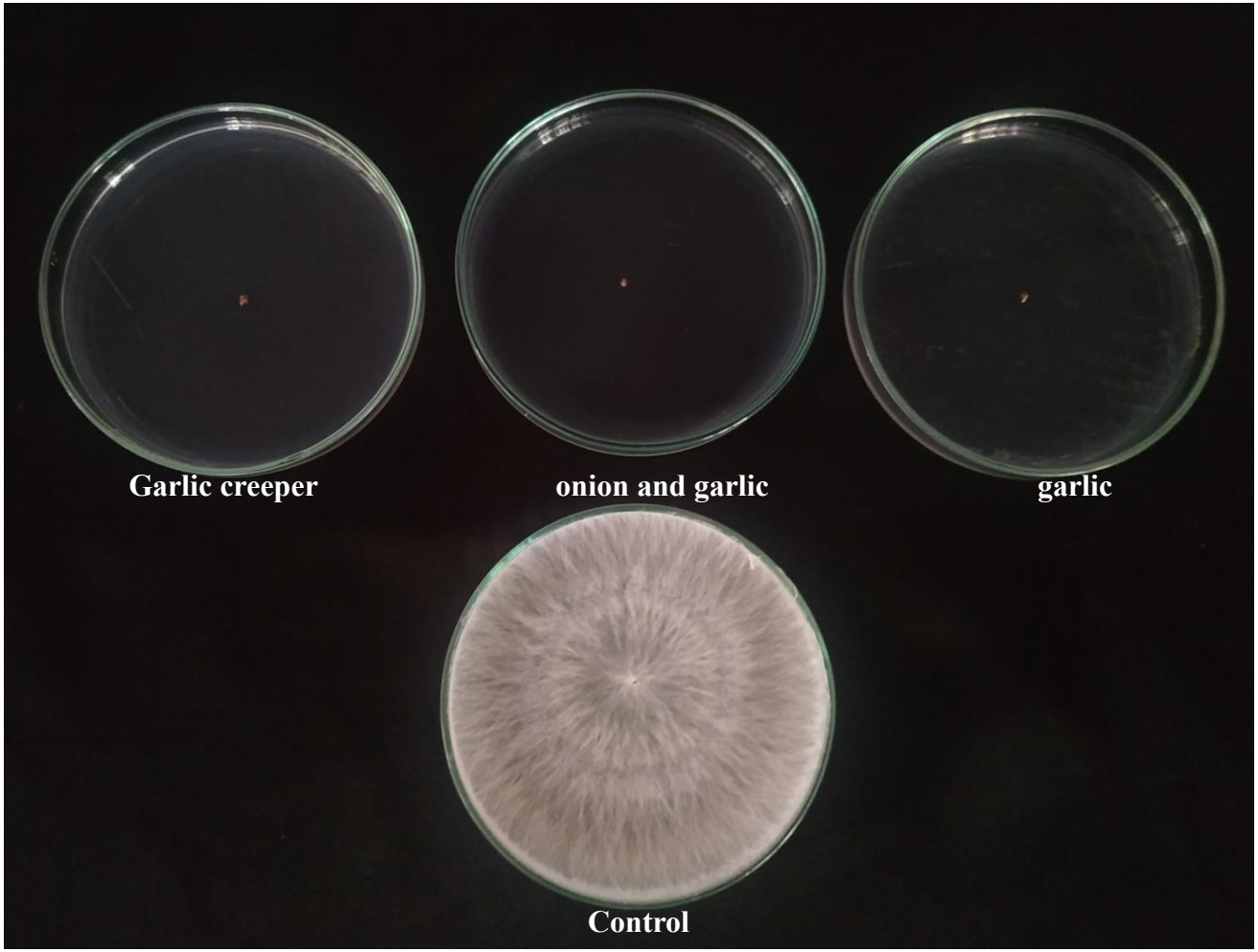


Plate 18. Complete inhibition of mycelial regeneration from sclerotia of *S. rolfsii* at 1 week after biofumigation

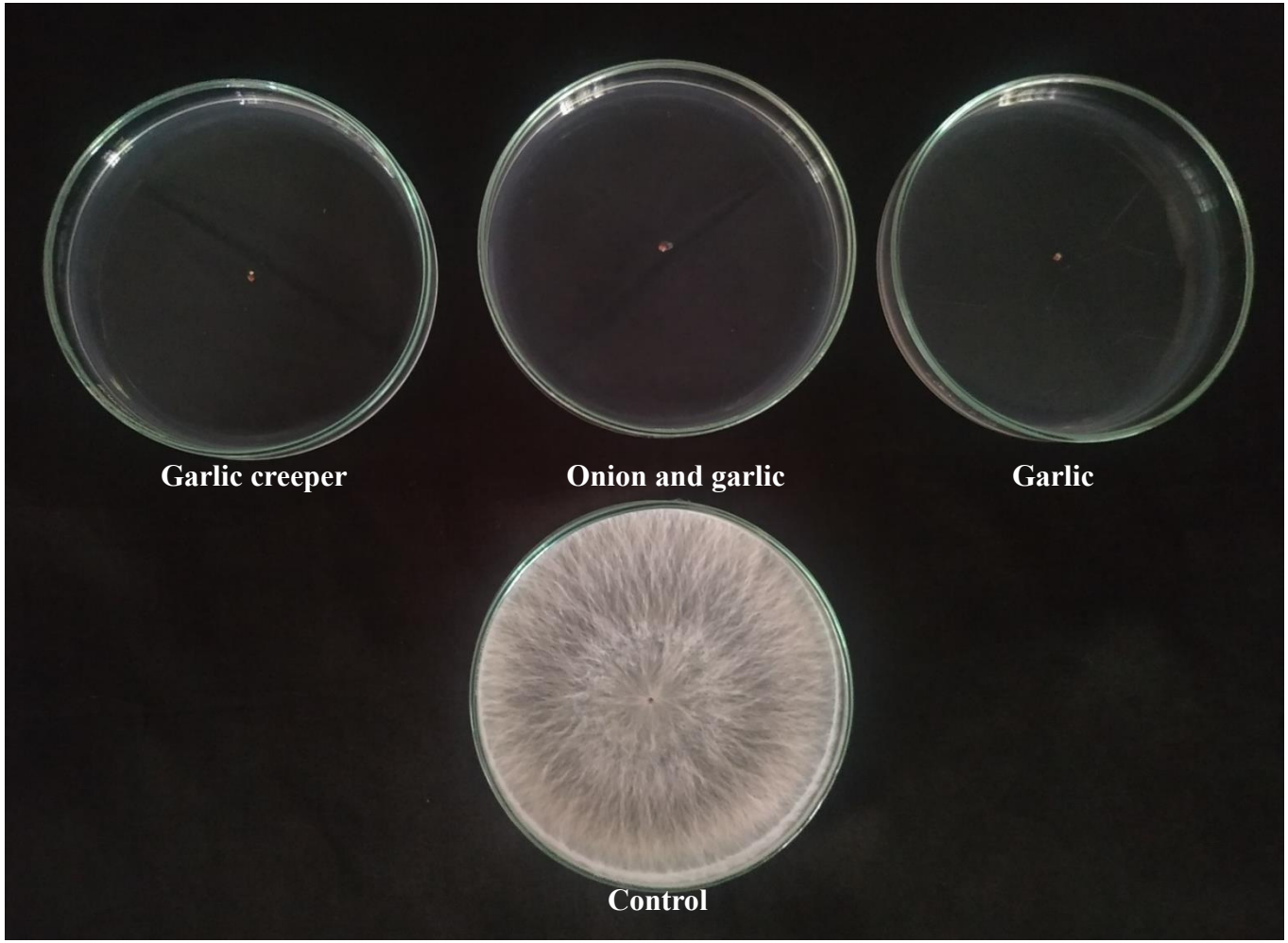


Plate 19. Complete inhibition of mycelial regeneration from sclerotia of *S. rolfsii* at 2 weeks after biofumigation

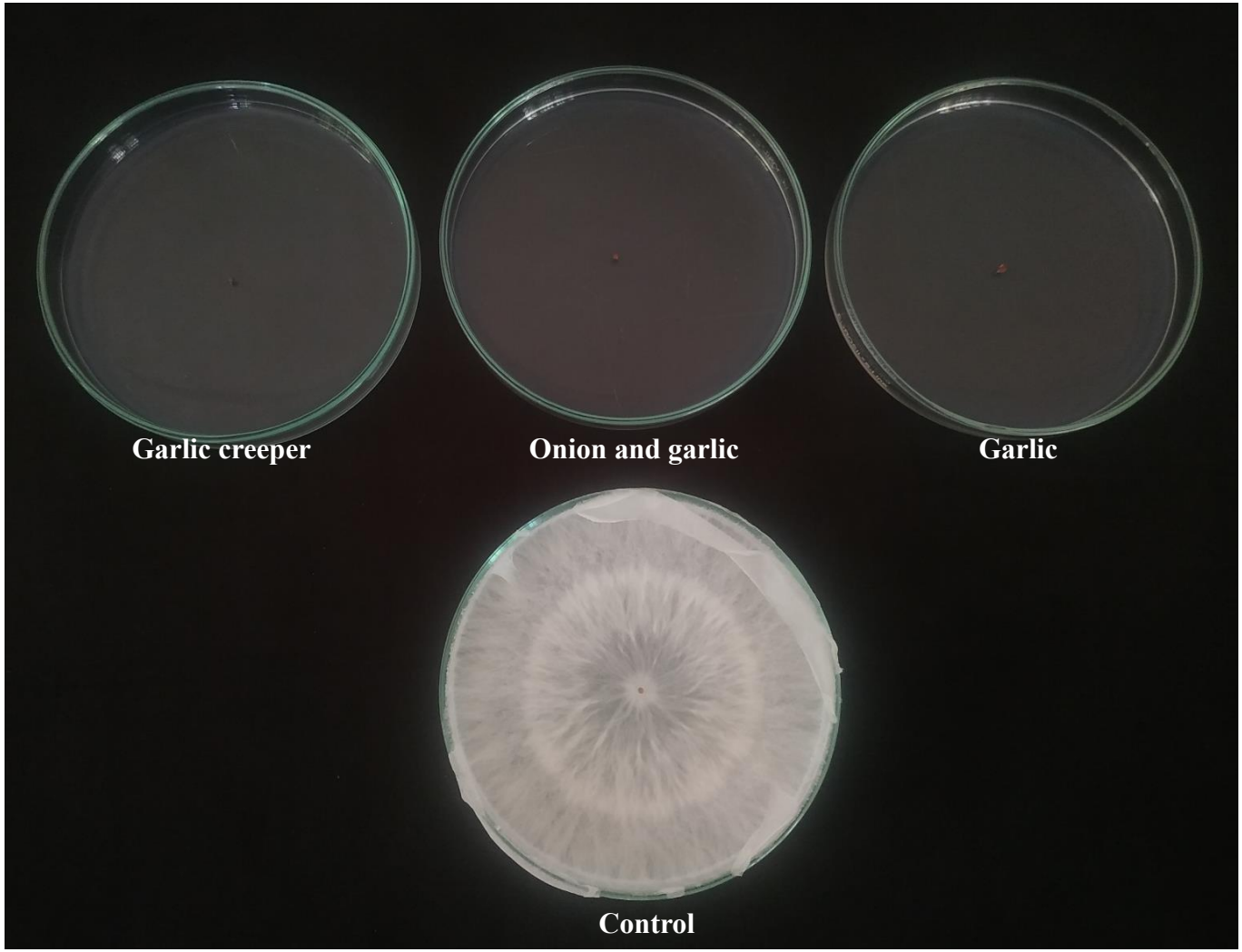


Plate 20. Complete inhibition of mycelial regeneration from sclerotia of *S. rolfsii* at 3 weeks after biofumigation

Table 37. Effect of soil biofumigation potential of plant extracts by confined container technique on germination of sclerotia of *S. rolfsii*

Treatment	1 week after biofumigation		2 weeks after biofumigation		3 weeks after biofumigation	
	MG*	PI	MG	PI	MG	PI
Mustard	4.40±0.173^{e}	47.04 (43.49±1.29)^{b}	0.00±0.000^{e}	100.00 (90.00±0.00)^{a}	0.00±0.000^{e}	100.00 (90.00±0.00)^{a}
Cabbage	6.93±0.120^{d}	22.96 (28.60±0.90)^{c}	3.23±0.145^{d}	64.07 (53.16±0.96)^{b}	0.00±0.000^{e}	100.00 (90.00±0.00)^{a}
Garlic creeper	0.00±0.000^{f}	100.00 (90.00±0.00)^{a}	0.00±0.000^{e}	100.00 (90.00±0.00)^{a}	0.00±0.000^{e}	100 (90.00±0.00)^{a}
Castor	8.33±0.120^{b}	7.04 (15.67±1.43)^{e}	5.10±0.153^{c}	43.33 (41.15±0.98)^{c}	4.17±0.088^{c}	53.7 (47.10±0.56)^{c}
Cauliflower	7.27±0.120^{c}	19.25 (25.99±0.98)^{d}	7.07±0.133^{b}	21.48 (27.58±1.02)^{d}	7.47±0.088^{b}	17.03 (24.35±0.74)^{d}
Onion and garlic	0.00±0.000^{f}	100.00 (90.00±0.00)^{a}	0.00±0.000^{e}	100.00 (90.00±0.00)^{a}	0.00±0.000^{e}	100.00 (90.00±0.00)^{a}
Garlic	0.00±0.000^{f}	100.00 (90.00±0.00)^{a}	0.00±0.000^{e}	100.00 (90.00±0.00)^{a}	0.00±0.000^{e}	100.00 (90.00±0.00)^{a}
Onion	0.00±0.000^{f}	100.00 (90.00±0.00)^{a}	0.00±0.000^{e}	100.00 (90.00±0.00)^{a}	3.20±0.153^{d}	64.44 (53.38±1.01)^{b}
Control	9.00±0.000^{a}	0.00 (0.00±0.00)^{f}	9.00±0.000^{a}	0.00 (0.00±0.00)^{e}	9.00±0.000^{a}	0.00 (0.00±0.00)^{e}
CD (0.05)	0.270	2.342	0.249	1.707	0.197	1.371
SEm±	0.090	0.782	0.083	0.570	0.066	0.458

MG – Diametre of mycelial growth in cm on 4th day; *Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level, values in parenthesis are arcsine transformed values

Table 38. Effect of soil biofumigation potential of oil cakes by confined container technique on germination of sclerotia of *S. rolfsii*

Treatment	1 week after biofumigation		2 weeks after biofumigation		3 weeks after biofumigation	
	MG*	PI*	MG	PI	MG	PI
Castor cake	3.37±0.133[b]	62.59 (52.28±0.88)[b]	0.10±0.058[b]	98.88 (85.09±2.56)[a]	0.13±0.088[b]	98.51 (84.44±3.06)[a]
Mustard cake	0.00±0.000[c]	100.00 (90.00±0.00)[a]	0.03±0.033[b]	99.62 (87.96±2.04)[a]	0.03±0.033[b]	99.62 (87.96±2.04)[a]
Pongamia cake	9.00±0.000[a]	0.00 (0.00±0.00)[c]	9.00±0.000[a]	0.00 (0.00±0.00)[b]	9.00±0.000[a]	0.00 (0.00±0.00)[b]
Control	9.00±0.000[a]	0.00 (0.00±0.00)[c]	9.00±0.000[a]	0.00 (0.00±0.00)[b]	9.00±0.000[a]	0.00 (0.00±0.00)[b]
CD (0.05)	0.221	1.457	0.110	5.416	0.156	6.088
SEm±	0.067	0.440	0.033	1.635	0.047	1.838

MG – Diameter of mycelial growth in cm on 4th day, PI – Percentage inhibition of sclerotial formation

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

antifungal potential against both the mycelia and sclerotia of *S. rolfsii*. Giving prime importance to biofumigation potential, further studies were conducted to reveal the lowest concentration of these two best effective treatments under *in vitro* conditions.

4.3.4.1. In vitro Studies to Identify the Lowest Effective Concentration of Best Treatments

Different concentrations of garlic (0.1, 0.3, 0.5, 0.7 and 0.9 g) as well as garlic creeper (1.0, 2.5 and 5.0 g) were tested *in vitro* for their biofumigation potential using paired plate technique in inhibiting the mycelial growth of *S. rolfsii*. Garlic at 0.3 g completely inhibited the mycelial growth of the fungus whereas, garlic creeper at 2.5 g resulted in cent per cent inhibition of the fungal mycelium (Table 39).

4.4. COMPATIBILITY OF TRICHODERMA VIRIDE (KAU ISOLATE) WITH THE BEST TWO TREATMENTS *IN VITRO*

The compatibility of *T. viride* (KAU isolate) with the two best biofumigants was tested based on their antifungal and biofumigation potential.

4.4.1. Compatibility Based on Antifungal Potential

When the treatments were tested for their compatibility with *T. viride* based on their antifungal potential, both garlic and garlic creeper were found to be compatible with the biocontrol agent at 0.3 and 2.5 per cent respectively (Table 40; Plate 21) and up to a maximum of 15 and 20 per cent respectively (Table 41 and 42; Plate 22).

4.4.2. Compatibility Based on Biofumigation Potential

When the compatibility of the two treatments individually with *T. viride* was tested based on their biofumigation potential, garlic and garlic creeper were found to be compatible with the biocontrol agent at 0.3 and 2.5 g respectively (Table 43; Plate 23) and up to a maximum of 15 and 20 g respectively (Table 44 and 45; Plate 24).

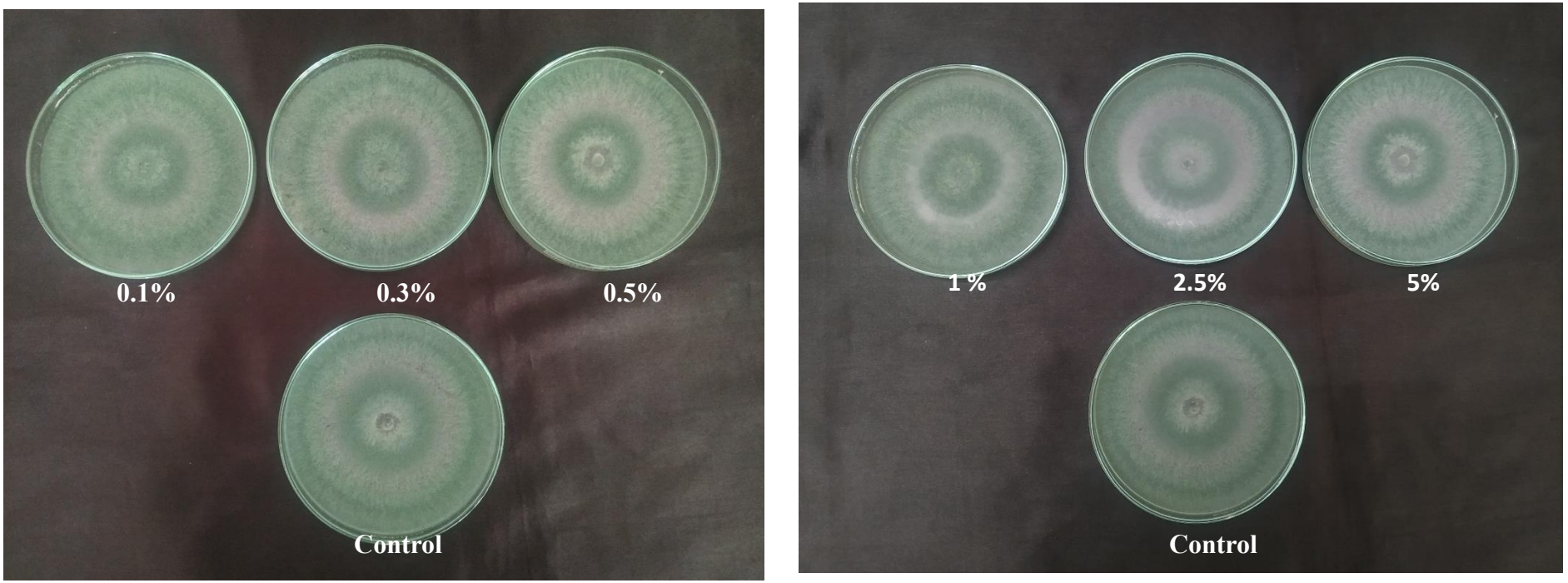
Plate 39. *In vitro* studies to identify the lowest concentration of garlic bulb and garlic creeper leaf extracts against *S. rolfsii* by paired plate technique

Sl. No.	Treatments	Concentration of treatments (g)	Mycelial growth (diameter in cm)*	Percentage inhibition* (%)
1	Garlic bulb extract	0.90	0.00±0.00^{c}	100.00 (90.00±0.00)^{a}
2	Garlic bulb	0.70	0.00±0.00^{c}	100.00 (90.00±0.00)^{a}
3	Garlic bulb	0.50	0.00±0.00^{c}	100.00 (90.00±0.00)^{a}
4	Garlic bulb	0.30	0.00±0.00^{c}	100.00 (90.00±0.00)^{a}
5	Garlic bulb	0.10	0.87±0.03^{b}	90.37 (71.90±0.36)^{b}
6	Garlic creeper leaves	5.00	0.00±0.00^{c}	100.00 (90.00±0.00)^{a}
7	Garlic creeper leaves	2.50	0.00±0.00^{c}	100.00 (90.00±0.00)^{a}
8	Garlic creeper leaves	1.00	4.67±0.03^{a}	48.14 (43.92±0.21)^{c}
	CD (0.05)		0.050	0.449
	SEm±		0.017	0.148

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level
Values in parenthesis are arcsine transformed values

Table 40. *In vitro* antifungal compatibility of garlic bulb and garlic creeper leaf extracts with *T. viride* by poisoned food technique

Sl. No	Treatment	Mycelial growth (Diameter in cm)	Percentage Inhibition (%)
1	Garlic 0.1%	9	0.00
2	Garlic 0.3%	9	0.00
3	Garlic 0.5%	9	0.00
4	Garlic creeper 1%	9	0.00
5	Garlic creeper 2.5%	9	0.00
6	Garlic creeper 5%	9	0.00
7	Control	9	0.00



Garlic **Garlic creeper**

Plate 21. *In vitro* compatibility of *T. viride* with lower concentrations of garlic bulb and garlic creeper leaf extracts at 4th day of growth

Table 41. *In vitro* antifungal compatibility of garlic bulb and garlic creeper leafs extract with *T. viride* by poisoned food technique

Treatments	Quantity of macerated tissue of garlic and garlic creeper for compatibility with *T. viride* (g)*					
	1	5	10	15	20	25
	MG	MG	MG	MG	MG	MG
Garlic	8.96±0.03	8.96±0.03	8.93±0.06	8.86±0.03^{b}	8.66±0.03^{b}	7.13±0.06^{c}
Garlic creeper	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00^{a}	9.00±0.00^{a}	8.60±0.05^{b}
Control	9.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}
CD (0.05)	NS	NS	NS	0.068	0.068	0.180
SEm±	0.019	0.019	0.039	0.019	0.019	0.051

MG – Diametre of mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Table 42. *In vitro* antifungal compatibility of garlic bulb and garlic creeper leaf extract with *T. viride* by poisoned food technique

Treatments	Quantity of macerated tissue of garlic and garlic creeper for compatibility with *T. viride* (g)*					
	1	5	10	15	20	25
	PI	PI	PI	PI	PI	PI
Garlic	0.37 (2.02±2.02)	0.37 (2.02±2.02)	0.74 (2.86±2.86)	1.48 (6.89±0.84)[a]	3.7 (11.06±0.55)[a]	20.74 (27.07±0.52)[a]
Garlic creeper	0.00 (0.00±0.00)	0.00 (0.00±0.00)	0.00 (0.00±0.00)	0.00 (0.00±0.00)[b]	0.00 (0.00±0.00)[b]	4.44 (12.10±0.90)[b]
Control	0.00 (0.00±0.00)	0.00 (0.00±0.00)	0.00 (0.00±0.00)	0.00 (0.00±0.00)[b]	0.00 (0.00±0.00)[b]	0.00 (0.00±0.00)[c]
CD (0.05)	NS	NS	NS	1.711	1.120	2.114
SEm±	1.163	1.163	1.648	0.485	0.317	0.599

PI – percentage inhibition of mycelial growth on 4th day

*Mean ± SD of three replications, values with similar super scripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

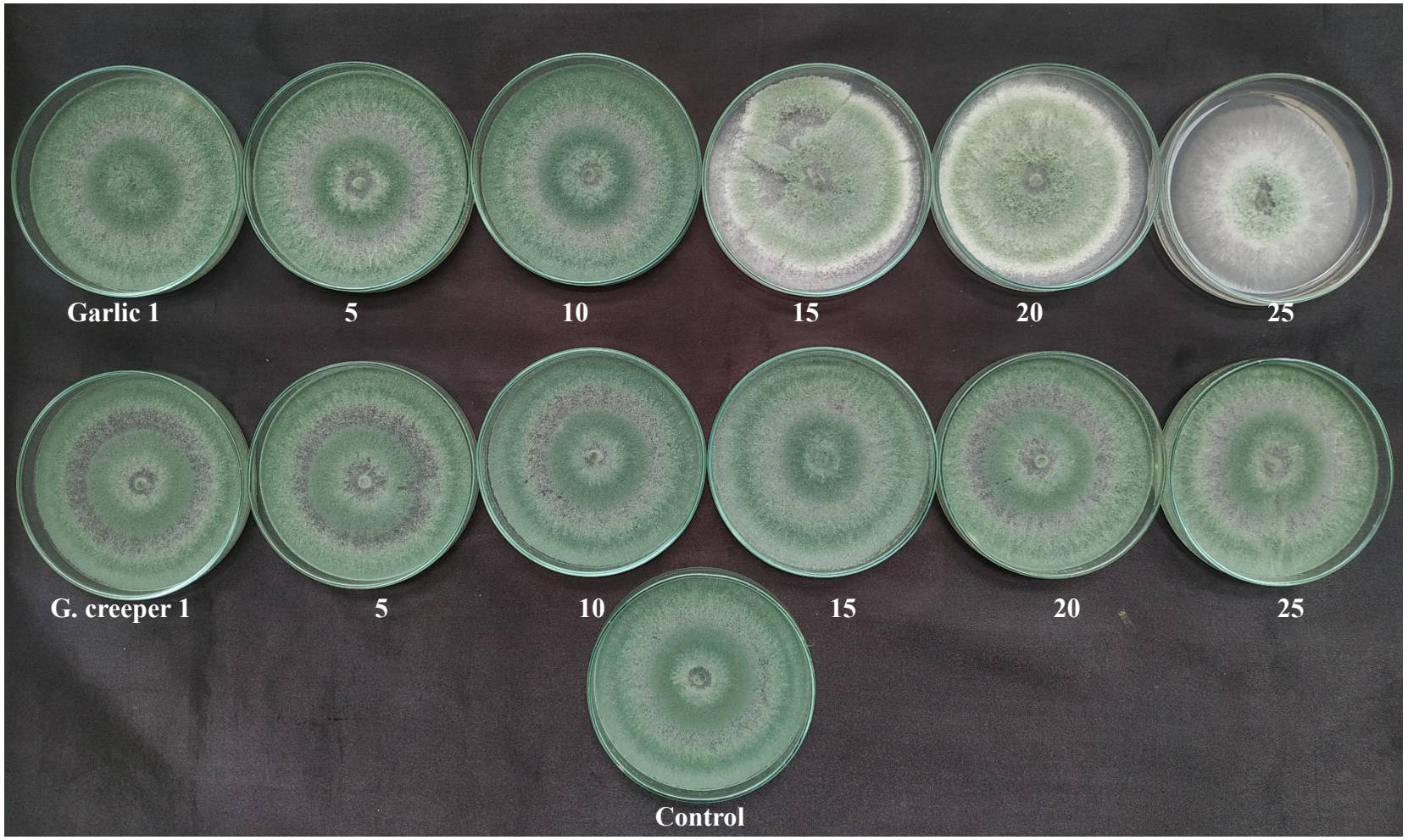
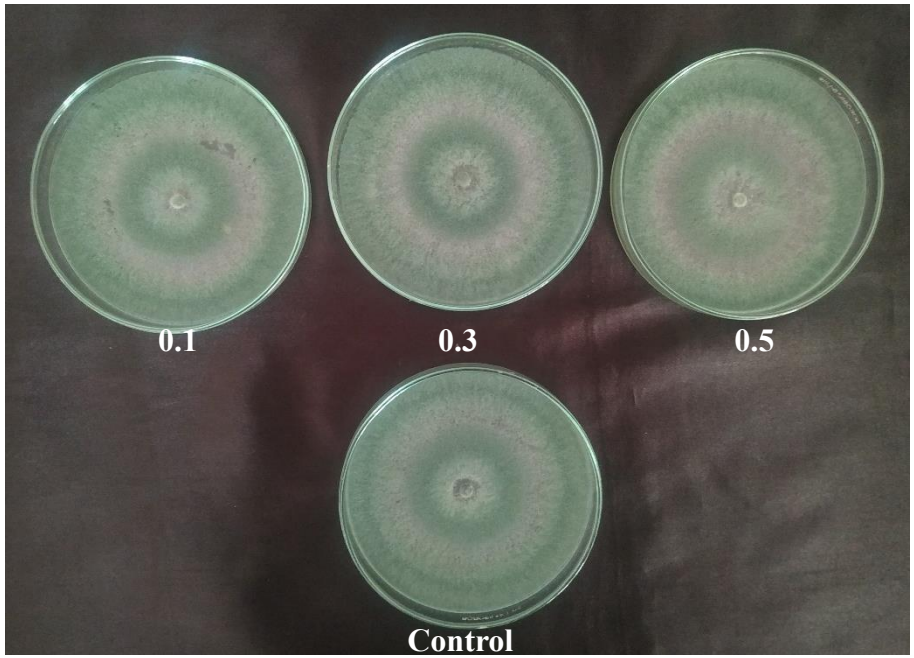


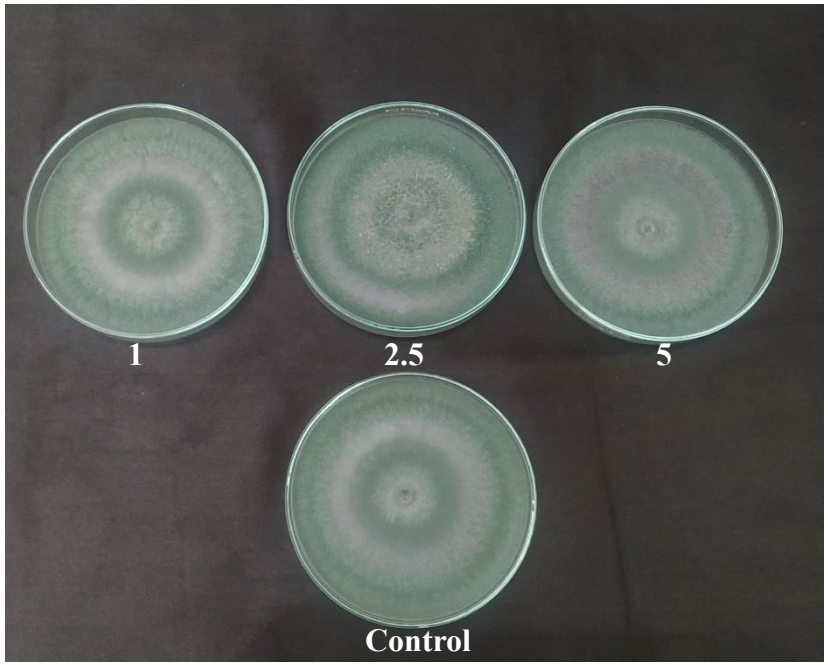
Plate 22. *In vitro* compatibility of *T. viride* with higher concentrations garlic bulb and garlic creeper leaf extracts at 4th day of growth

Table 43. *In vitro* biofumigation compatibility of garlic bulb and garlic creeper leaf extract with *T. viride* by paired plate

Sl. No.	Treatments	Mycelial growth (Diameter in cm)*	PI (%)
1	Garlic - 0.1g	9	0.00
2	Garlic - 0.3g	9	0.00
3	Garllic - 0.5g	9	0.00
4	Garlic creeper - 1g	9	0.00
5	Garlic creeper - 2.5g	9	0.00
6	Garlic creeper 5g	9	0.00
7	Control	9	0.00



Garlic



Garlic creeper

Plate 23. *In vitro* biofumigation compatibility of *T. viride* with lower concentrations of garlic bulb and garlic creeper leaf extracts at 4th day of growth

Table 44. *In vitro* biofumigation compatibility of garlic bulb and garlic creeper leaf extract with *T. viride* by paired plate technique

Treatments	Quantity of macerated tissue of garlic and garlic creeper for compatibility with *T. viride* (g)*					
	1	5	10	15	20	25
	MG	MG	MG	MG	MG	MG
Garlic	8.97±0.033	8.97±0.033	8.93±0.067	8.40±0.153^{b}	7.23±0.033^{b}	4.43±0.067^{c}
Garlic creeper	9.00±0.000	9.00±0.000	9.00±0.000	9.00±0.000^{a}	9.00±0.000^{a}	8.37±0.088^{b}
Control	9.00±0.000	9.00±0.000	9.00±0.000	9.00±0.000^{a}	9.00±0.000^{a}	9.00±0.000^{a}
CD (0.05)	NS	NS	NS	0.311	0.068	0.225
SEm±	0.019	0.019	0.039	0.088	0.019	0.064

MG – Diametre of mycelial growth in cm on 4th day

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Table 45. *In vitro* biofumigation compatibility of garlic bulb and garlic creeper leaf extract with *T. viride* by paired plate technique

Treatments	Quantity of macerated tissue of garlic and garlic creeper used for compatibility with *T. viride* (g)*					
	1	5	10	15	20	25
	PI	PI	PI	PI	PI	PI
Garlic	0.37 (2.02±2.02)	0.37 (2.02±2.02)	0.74 (2.86±2.86)	6.66 6.66±1.70)[a]	19.62 (26.28±0.27)[a]	28.51 32.26±0.47)[a]
Garlic creeper	0.00 (0.00±0.00)	0.00 (0.00±0.00)	0.00 (0.00±0.00)	0.00 (0.00±0.00)[b]	0.00 (0.00±0.00)[b]	7.03 (15.30±1.09)[b]
Control	0.00 (0.00±0.00)	0.00 (0.00±0.00)	0.00 (0.00±0.00)	0.00 (0.00±0.00)[b]	0.00 (0.00±0.00)[b]	0.00 (0.00±0.00)[c]
CD (0.05)	NS	NS	NS	3.460	0.550	2.408
SEm±	1.163	1.163	1.648	0.981	0.156	0.683

PI – Percentage inhibition

*Mean ± SD of three replications, values with similar super scripts are not significantly different at 5% level

Values in parenthesis are arcsine transformed values

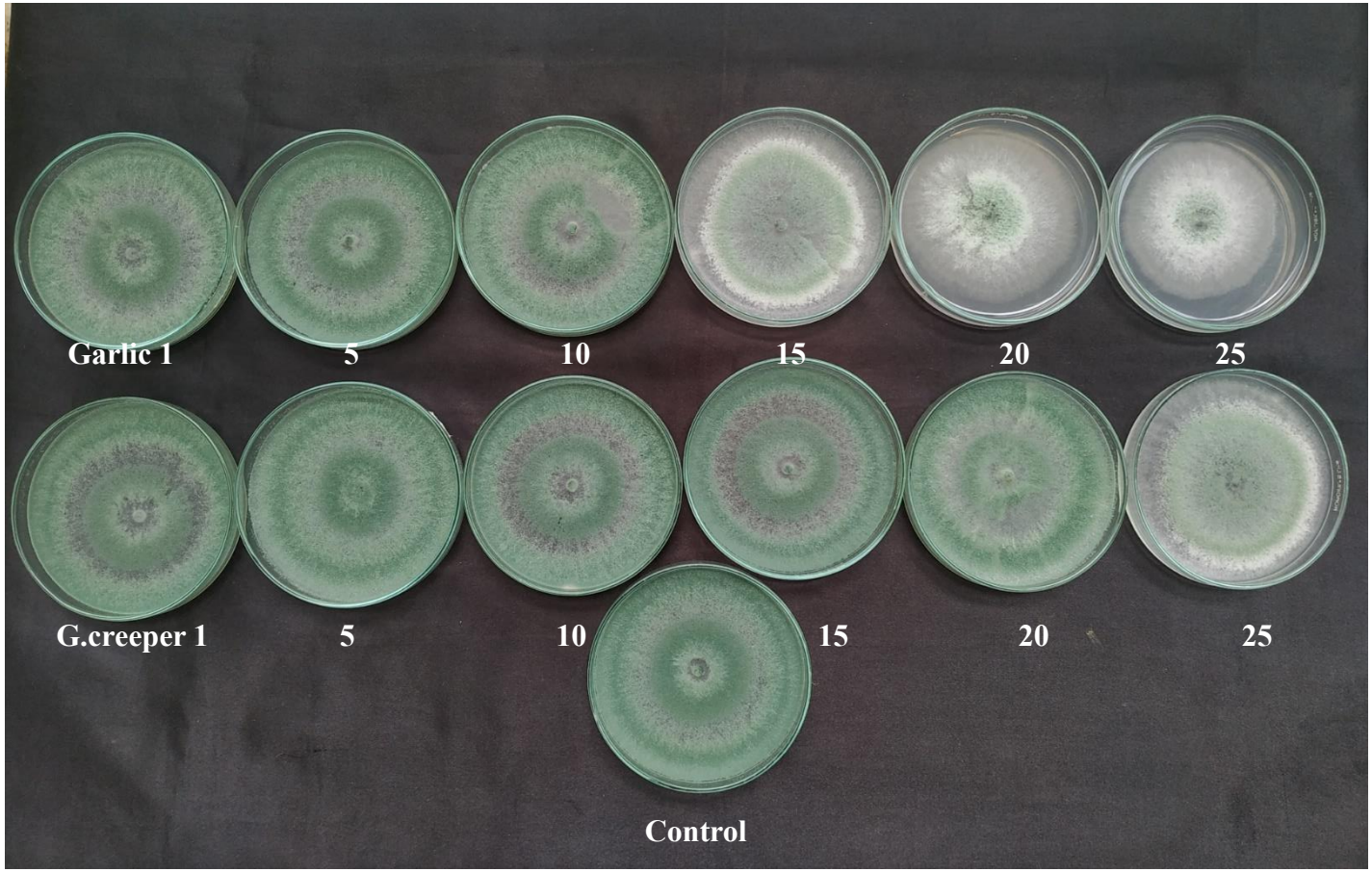


Plate 24. *In vitro* biofumigation compatibility of *T. viride* with higher concentrations of garlic bulb and garlic creeper leaf extracts at 4th day of growth

4.5. *IN VIVO* MANAGEMENT STUDIES

4.5.1. Treatments Selected for *in vivo* Studies

Nine treatments including the best two effective biofumigants (garlic bulb and garlic creeper leaves) selected from *in vitro* studies individually as well as in combination with the biocontrol agent *viz*., *T. viride* (KAU isolate) were tested for their biofumigation potential against basal stem rot and blight disease of vegetable cowpea (var. Gitika) under *in vivo* conditions at IFSRS, Karamana (Plate 25).

4.5.2. Preparation of Inoculum of the Virulent Isolate (S_1) of *S. rolfsii*

All the treatments except T_8 (uninoculated control) were equally soil inoculated with *S. rolfsii* (2% inoculum).

4.5.3. Treatment Application

The four treatments *viz*., soil application of garlic bulb extract (3 g / kg soil) at two weeks before transplanting followed by soil plastering with cow dung slurry for two weeks (T_1), soil application of garlic creeper leaf extract (25 g per kg soil) at two weeks before transplanting followed by soil plastering with cow dung slurry for two weeks (T_2), soil application of garlic bulb extract (3 g / kg soil) at two weeks before transplanting followed by soil plastering with cow dung slurry for two weeks and soil application of cow dung and neem cake (9:1) enriched with *T. viride* one month after planting (T_3) and soil application of garlic creeper leaf extract (25 g / kg soil) at two weeks before transplanting followed by soil plastering with cow dung slurry for two weeks and soil application of cow dung and neem cake (9:1) enriched with *T. viride* one month after planting (T_4) did not exhibit any symptoms and were effective in completely managing the disease. The plants in the inoculated control started exhibiting the symptoms on 4 days after transplanting (DAT), whereas it took 17 days for symptom expression in plants treated with the fungicide *viz*., mancozeb. The disease started to exhibit in plants applied with the biocontrol agents *viz*., *T. viride* and *Pseudomonas fluorescens* (KAU isolates) in soil on nine and eight DAT respectively.

Plate 25. ***In vivo*** **experiment on biofumigation potential of plant extracts and biocontrol agents in vegetable cowpea var. Gitika**

The presence of sclerotia on the stem base was observed only in inoculated control plants on 12 DAT. The four treatments *viz*., T_1, T_2, T_3 and T_4 resulted in cent per cent reduction in disease incidence and disease intensity; and sclerotia were not observed in any of these four treatments, proving that they could inhibit both the mycelial growth and the formation of sclerotia of the pathogen. The inoculated control plants recorded 88.89 per cent disease incidence and 66.66 per cent disease intensity. The plants treated with mancozeb recorded 33.33 and 3.70 per cent disease incidence and intensity respectively, whereas significant disease incidence and intensity were recorded in plants applied with *T. viride* (44.44 and 10.18% respectively) and *P. fluorescens* (44.44 and 16.67% respectively) (Table 46).

The biometric observations *viz*., vine length and number of trifoliate leaves of vegetable cowpea plants in all the treatments were recorded at 60 days after sowing (DAS). The maximum vine length (4m) and trifoliate leaves (66.33) were recorded in T_3. The next highest vine length (3.75m) was recorded in uninoculated control (T_8), but the highest number of trifoliate leaves (64.33) was observed in fungicide applied plants (T_9). The inoculated control revealed the least vine length (2.10m) and trifoliate leaves (18.33) (Table 47). Comparison of the pods of the plants of inoculated control treatment (T_9) as well as the best effective treatment (T_3) at 60 days after sowing revealed that there was significant reduction in the pod length and quality in the inoculated control plants (Plate 26 and 27).

Thus, based on the disease incidence, intensity and biometric observations, the study revealed that soil application of garlic bulb extract at the rate of 3 g per kg soil two weeks before planting followed by soil plastering with cowdung slurry for two weeks and soil application of *T. viride* multiplied in cowdung and neem cake mixture (9:1 ratio) at the rate of 500 g per plant at one month after transplanting was the most effective treatment in completely managing the basal stem rot and blight disease of vegetable cowpea under *in vivo* conditions.

Table 46. Effect of garlic bulb, garlic creeper leaf and biocontrol agents on incidence and severity of basal stem rot and blight disease of vegetable cowpea var. Gitika

Sl. No.	Treatments	Days for symptom development	Days for production of sclerotia	Disease incidence (%)	Disease intensity* (%)
1.	Garlic bulb (3g/kg soil) + soil plastering with cowdung slurry	-	-	0	$(0.00\pm0.00)^{c}$
2.	Garlic creeper leaves (25g/kg soil) + soil plastering with cowdung slurry	-	-	0	$(0.00\pm0.00)^{c}$
3.	T1 + application of *T. viride*	-	-	0	$(0.00\pm0.00)^{c}$
4.	T2 + application of *T. viride*	-	-	0	$(0.00\pm0.00)^{c}$
5.	Soil application of *T. viride* (KAU isolate) at one MAP alone	9 DAT	-	44.44	$(10.18\pm4.56)^{bc}$
6.	Soil application of *Pseudomonas fluorescens* (KAU isolate) 2% drench at one MAP alone	8 DAT	-	44.44	$(16.67\pm6.94)^{b}$
7.	Inoculated control (*S. rolfsii*)	4 DAT	12 DAT	88.89	$(66.66\pm9.52)^{a}$
8.	Uninoculated control	-	-	0	$(0.00\pm0.00)^{c}$
9.	Mancozeb @ $3gL^{-1}$	17 DAT	-	33.33	$(3.70\pm2.02)^{c}$
CD (0.05)		-	-	-	12.050
SE±m		-	-	-	4.265

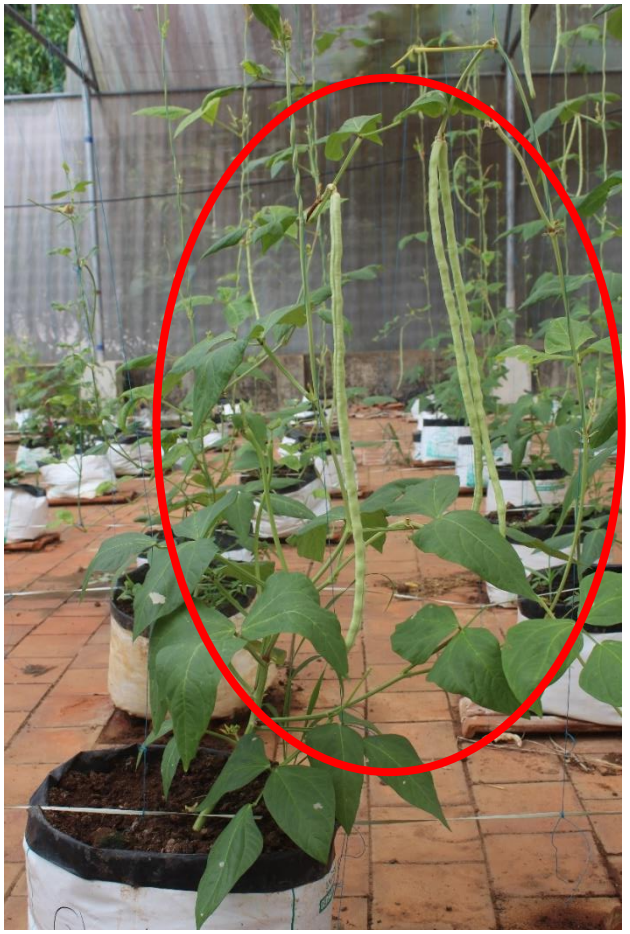
*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% levelValues in parenthesis are arcsine transformed values

Table 47. Effect of garlic, garlic creeper and biocontrol agents on biometric characters of vegetable cowpea var. Gitika on 60 DAS

Sl. No.	Treatments	Vine length* (m)	No. of leaves* (triplets)
1	Garlic bulb (3g/kg soil) + soil plastering with cowdung slurry	3.44 ± 0.17^{abc}	38.33 ± 2.03^{b}
2	Garlic creeper leaves (25g/kg soil) + soil plastering with cowdung slurry	3.07 ± 0.54^{c}	33.33 ± 7.22^{bc}
3	T1 + application of *T. viride*	4.00 ± 0.06^{a}	66.33 ± 0.33^{a}
4	T2 + application of *T. viride*	3.12 ± 0.17^{bc}	40.33 ± 0.88^{b}
5	Soil application of *T. viride* (KAU isolate) at one MAP alone	2.90 ± 0.03^{cd}	29.00 ± 1.73^{cd}
6	Soil application of Pseudomonas fluorescens (KAU isolate) 2 5 drench at one MAP alone	2.25 ± 0.03^{de}	20.00 ± 2.31^{de}
7	Inoculated control (*S. rolfsii*)	2.10 ± 0.22^{e}	18.33 ± 2.33^{e}
8	Uninoculated control	3.75 ± 0.13^{ab}	40.67 ± 1.86^{b}
9	Mancozeb @ $3gL^{-1}$	3.53 ± 0.14^{abc}	64.33 ± 3.18^{a}
	CD (0.05)	0.663	9.172
	SE±m	0..221	3.063

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level

Inoculated control



Best treatment (T_3)

Plate 26. Comparision of pods of vegetable cowpea plants in inoculated control and best treatment (T_3)

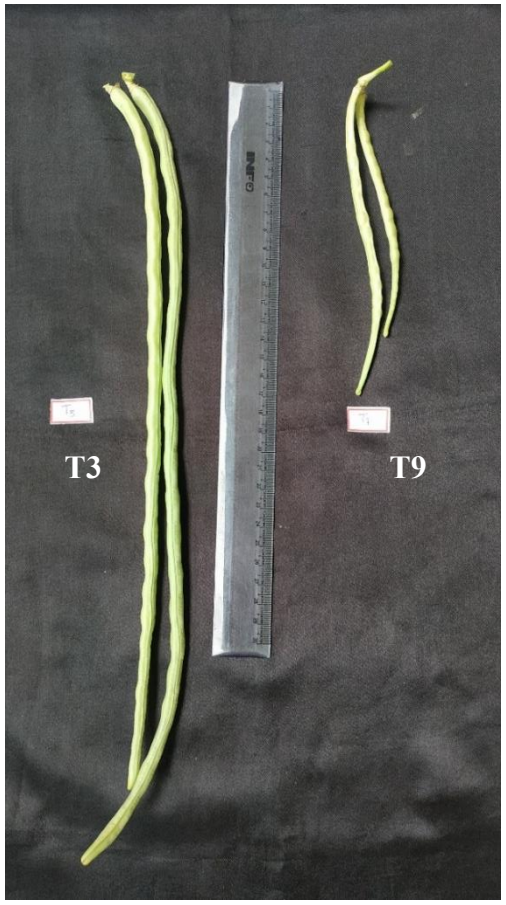


Plate 27. Comparison of pod length of vegetable cowpea in best treatment (T_3) and inoculated control (T_9)

4.6. ESTIMATION OF SOIL FUNGAL POPULATION DYNAMICS

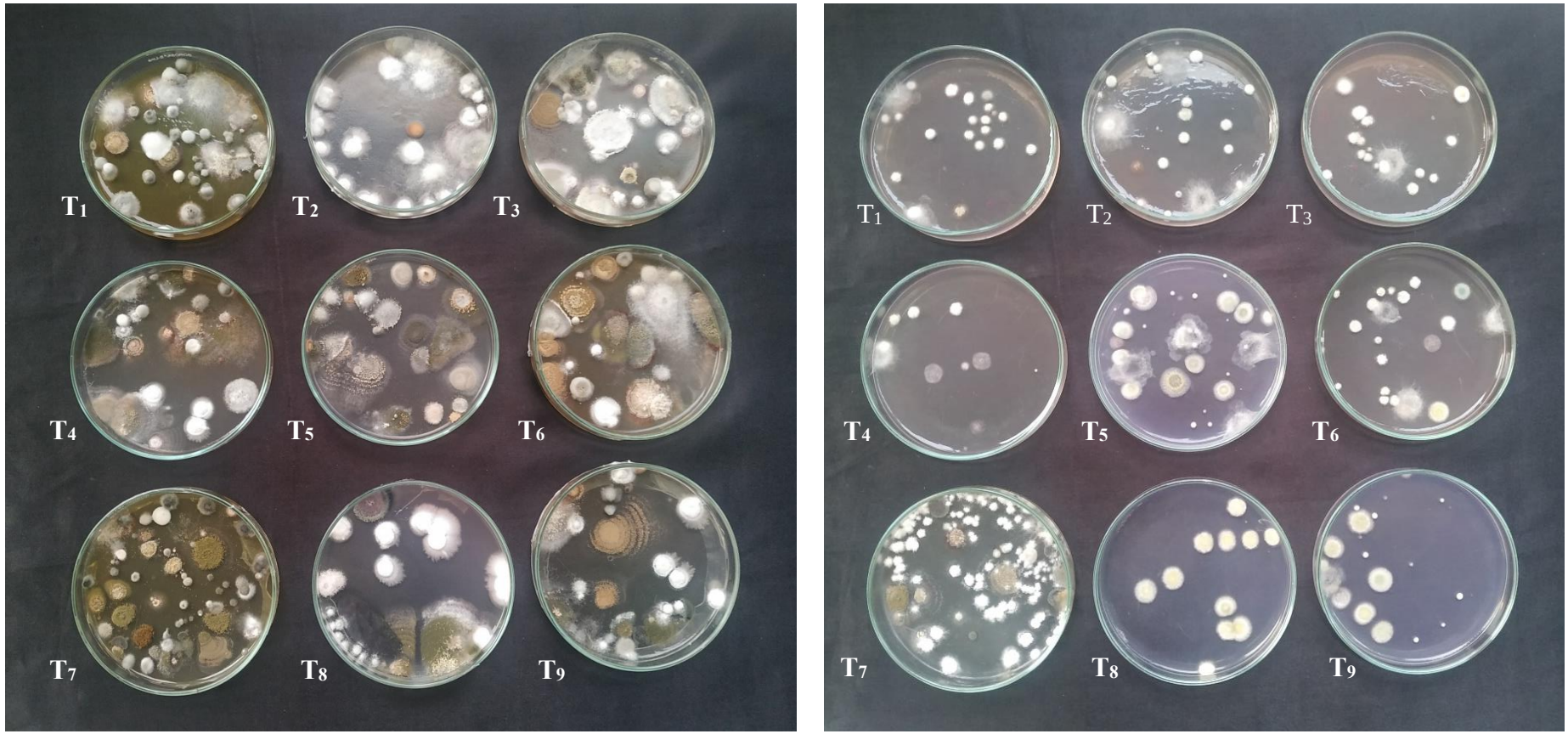
The soil fungal population dynamics was enumerated in each treatment before and one month after biofumigation to check the efficacy of biofumigation on soil fungal population under *in vivo* conditions. The study revealed that the application of biofumigants *viz*., garlic and garlic creeper individually as well as in combination with *T. viride* resulted in significant reduction in the soil fungal population at one month after biofumigation. The fungicide application also recorded a reduction in the population dynamics, before drenching (28.67 cfu x 10^{-3}) and after drenching (12.33 cfu x 10^{-3}). The inoculated control, on the other hand recorded a significant increase in the fungal population level, from an initial level of 47 cfu x 10^{-3} to 64 cfu x 10^{-3} at one month after biofumigation (Table 48; Plate 28).

Thus, the study revealed that basal stem rot and blight disease of vegetable cowpea caused by *S. rolfsii* can be effectively managed by soil application of garlic bulb extract @ 3 g per kg soil at two weeks before planting followed by soil plastering with cow dung slurry for two weeks and soil application of 500 g cow dung and neem cake (9:1) enriched *T. viride* at one month after planting which can be used as an eco-friendly management strategy for safe-to-eat vegetable cowpea production Soil application of garlic creeper leaves @ 25g per kg soil at two weeks before planting followed by soil plastering with cow dung slurry for two weeks and soil application of 500 g cow dung and neem cake (9:1) enriched *T. viride* at one month after planting was the next best effective treatment for the management of the disease. However, the effectiveness of application of cow dung and neem cake (9:1) enriched *T. viride* at one week after biofumigation, besides its application at one month after planting need to be studied. The effect of the biofumigants in inhibiting other soil borne fungal pathogens of vegetable cowpea also needs to be explored.

Table 48. Effect of biofumigation of garlic bulb and garlic creeper leaf extracts on soil fungal population dynamics

Sl. No.	Treatments	Soil fungal population in 10^3 (cfu g^{-1} soil)*	
		Before biofumigation	After biofumigation
1	Garlic bulb (3g/kg soil) + soil plastering with cowdung slurry for 2 weeks (basal application)	36.67 ± 5.70^{b}	11.00 ± 0.58^{d}
2	Garlic creeper leaves (25g/kg soil) + soil plastering with cowdung slurry for 2 weeks (basal application)	30.33 ± 0.88^{bc}	19.33 ± 0.33^{c}
3	T1 + soil application with cowdung and neem cake enriched with *T. viride* (KAU isolate) at one MAP	32.33 ± 0.88^{bc}	15.33 ± 0.88^{cd}
4	T2 + soil application with cowdung and neem cake enriched with *T. viride* (KAU isolate) at one MAP	17.33 ± 2.19^{e}	10.67 ± 0.88^{d}
5	Soil application with cowdung and neem cake enriched with *T. viride* (KAU isolate) at one MAP alone	29.67 ± 4.18^{bc}	31.33 ± 3.84^{b}
6	Soil application of *Pseudomonas fluorescens* (KAU isolate) 2% drench at one MAP alone	24.67 ± 4.91^{cde}	30.67 ± 6.69^{b}
7	Inoculated control (*S. rolfsii*)	47.00 ± 1.00^{a}	64.00 ± 2.52^{a}
8	Uninoculated control	19.00 ± 0.58^{de}	11.00 ± 0.58^{d}
9	Mancozeb @ 3g L^{-1}	28.67 ± 4.81^{bcd}	12.33 ± 1.33^{cd}
	CD(0.05)	4.72	4.79
	SE M (±)	0.7	1.3

*Mean ± SD of 3 replications, values followed by similar superscripts are not significantly different at 5% level



Before biofumigation

After biofumigation

Plate 28. Enumeration of population dynamics of soil fungal colonies before and after biofumigation

Discussion

5. DISCUSSION

Vegetable cowpea is a major vegetable crop in Kerala. The crop has been identified to be affected by many soil borne fungal pathogens resulting in severe yield losses. The major soil borne fungi affecting the crop include *Fusarium oxysporum* causing vascular wilt and basal swelling, *Rhizoctonia solani* inciting collar rot and web blight and *Pythium aphanidermatum* resulting in stem rot as well as damping off. During the past few years, the crop was observed to be affected by a new, soil borne disease resulting in wilting, yellowing of leaves, collar rot, stem shredding and final death of the plants (Sajeena *et al*., 2014). The disease resulted in complete death of the crop and was observed to affect the crop at all growth stages starting from seedling to harvesting. The disease has been observed to be of first incidence in vegetable cowpea in Kerala and it is of utmost importance to study the pathogen in detail and to develop an eco-friendly management strategy. Hence, the present study entitled "Biofumigation for the management of *Sclerotium rolfsii* in vegetable cowpea" was undertaken at Integrated Farming System Research Station, Karamana and Department of Plant Pathology, College of Agriculture, Vellayani during 2018-2020 to isolate the fungus from basal stem rot and blight affected vegetable cowpea samples and to assess the biofumigant potential of selected botanicals and oil cakes against the disease. The results obtained from the *in vitro* and *in vivo* studies are discussed in this chapter.

5.1. SYMPTOMOTOLOGY, ETIOLOGY AND PATHOGENICITY OF ISOLATES

In the present study, six isolates of *S. rolfsii* causing basal stem rot and blight disease were collected from commonly cultivated varieties of vegetable cowpea *viz*., Gitika, Vellayani Jyothika and NS 621from predominant vegetable cultivating areas. The disease was manifested as wilting, yellowing of leaves, defoliation and stem shredding in all the collected, disease affected vegetable cowpea plant samples. Anahosur (2000) described the symptoms of wilt caused by *S. rolfsii* in potato as brown coloured lesions at collar region resulting in yellowing and drooping of leaves as well as wilting and death of the plant. Fery and Dukes (2002) described the symptoms of southern blight of cowpea caused by *S. rolfsii* as initial general wilting of the whole plant resulting in yellowing and drying of the foliage and subsequent plant death.

Songvilay *et al*. (2013) gave the first report on basal stem rot of snake bean (*Vigna unguiculata* subsp. *sesquipedalis*) caused by *S. rolfsii* in Lao PDR in which they described the disease symptoms as bleached appearance of the stem region. The disease caused by *S. rolfsii* in potato and artichoke was manifested as brown coloured lesions on the stem followed by drooping and wilting of the leaves and ultimate death of the whole plants (Ayed *et al*., 2018).

S. rolfsii is studied to produce several enzymes such as xylanase, mannanase, arabinosidase, acetyl esterse, galactosidase and oxalic acid, which are directly correlated to its ability to disintegrate the plant tissues at stem portion. These enzymes and oxalic acid together disintegrates the host outer cell layer. The entry of the fungus into the cortex region resulted in stem girdling and plant death (Kwon and Park, 2002). The fungus, then produces mycelia and sclerotia over the disintegrated portion, which will help in widening its infection from stem to further roots (Tanimu *et al*., 2018). The disintegration of the stem, collar and root regions will block the absorption of water and nutrients from soil and hence, the disease gets manifested as wilting of the plants, yellowing and drying of the leaves, shredding of the infected stem portion and ultimate death of the plants.

In this study, the fungus, *S. rolfsii* appeared to have white coloured, fan or thread like mycelial growth. Mustard shaped, tan brown coloured sclerotia which are the vegetative resting structures of the fungus were observed at the collar region. Aycock (1966) also described the fungus to be present in the collar region of the infected plants as white fan or thread shaped mycelia along with mustard shaped, cream to tan coloured sclerotia. Anahosur (2000) observed the presence of cottony white, thread like fungal mycelium which was found to girdle the stem base initially and to move to the root portion subsequently. Songvilay *et al*. (2013) reported that abundant small, round, brown coloured sclerotia also in the soil and debris of the affected cowpea plants, in addition to their presence on the stem base. The results of Kator *et al.* (2015) that *S. rolfsii* was present as white cottony thread like mycelia along with small, brown sclerotia, initially in the stem region and subsequently on the roots of the infected cowpea plants are also in accordance with our results. Several studies also revealed that white, fan or thread like mycelial growth with numerous mustard shaped, brown

coloured sclerotia is the characteristic growth pattern of the fungus on its infected host plants (Anahosur, 2000; Han *et al*., 2012; Hansda *et al*., 2014; Sun *et al*., 2020).

In our study, the disease was observed throughout the different growth stages of the crop *viz*., seedling, vegetative, flowering, pod formation and harvesting stages. Muthukumar and Venkatesh (2013) reported that the fungus can infect all growth stages of pepper mint, when soil inoculum was present. However, the most susceptible stage of the crop was revealed to be 20 days after emergence (DAE) followed by 60 and 80 DAE. The reports of Ayed *et al*. (2018) that the disease incited by *S. rolfsii* in potato and artichoke affected all the plant growth stages is also in accordance with our study.

In this research work, six isolates of *S. rolfsii* were isolated and screened to identify the most virulent isolate for further studies. The mycelia of all the isolates appeared dull to pure white in colour. The colony growth of the isolates varied from thread like to dense and fluffy. All the isolates showed the presence of sclerotia in PDA medium. The sclerotia appeared smooth round to irregular round in shape. The sclerotial colour varied from dark brown, reddish brown, white to golden brown. The sclerotial arrangement appeared as dense towards rim, dense at centre, scattered as well as scattered and sparse. Similar results have been reported on the mycelial growth and sclerotial characters of *S. rolfsii* in PDA medium. The cultural characters of eight isolates of the fungus were studied in PDA medium which revealed that the mycelial colour had a variation from dull or light white to extra white. The mycelia varied in appearance from loose and sparse to dense and fluffy. The sclerotial colour ranged from dull brown to dark brown (Kumar *et al*., 2014). Studies conducted by Sivakumar *et al.* (2016) on different isolates of the fungus also revealed that the mycelial colour varied from light to dull white with profuse cottony mycelial growth. The various colours exhibited by the sclerotia were light brown, brown, chocolate and dark brown. Sun *et al.* (2020) screened five isolates of *S. rolfsii* in PDA medium and observed that the mycelia were white in colour and the sclerotia were initially white, later turning to brown colour.

The isolates took a minimum of two days to a maximum of five days for lesion development on the artificially inoculated vegetable cowpea seedlings. Tanimu *et al*. (2018) reported that *S. rolfsii* will produce mycelia on the plant surface prior to entry

and tissue disintegration. The mycelial production on plants requires two to ten days duration and this may be the reason for the symptom development in the plants between a duration of two to five or more days. Sun *et al.* (2020) revealed that artificial inoculation of mung bean plants with *S. rolfsii* by hypocotyl puncture and mycelia root drenching took four to seven days respectively for symptom expression. They reported a correlation between the production of oxalic acid and the virulence of the isolates; and proposed that the isolates producing more quantity of oxalic acid in a shorter duration of time can result in early symptom expression.

5.2. IDENTIFICATION OF THE PATHOGEN

Morphological and cultural characters as well as internal transcribed spacer (ITS) region based molecular characterization were undertaken to identify and confirm the pathogen as *S. rolfsii*. Hyaline and septate mycelium, granular cytoplasm, characteristic clamp connections as well as the presence of moniloid cells, together with conspicuous thread like, white, mycelial growth along with mustard like, dark brown sclerotia supported the morphological identification of the fungus as *S. rolfsii*. Confirmatory identification was undertaken by amplification of the rDNA using ITS1 /ITS4 universal primers. Mahadevakumar *et al*. (2016) reported the identity of the fungus causing fruit rot of *Cucurbita maxima* as *S. rolfsii* based on rDNA amplification using ITS1 / ITS4 universal primers. Similar results were reported by Morales *et al.* (2018) who identified the causal organism of southern blight of sesame as *S. rolfsii* based on ITS /ITS4 universal primers and subsequent blasting the sequence in NCBI. Sun *et al.* (2020) also identified the fungus inciting southern blight of mungbean as *S. rolfsii* based on amplification of rDNA using ITS1 / ITS4 universal primers.

5.3. OPTIMUM pH, MOISTURE AND INOCULUM LEVEL OF S. ROLFSII

In the current study, *S. rolfsii* completed its mycelial growth within three DAI in PDA medium of pH 6.0 and the maximum number of sclerotia was recorded in the medium of pH 7 at 15 DAI. Previous studies on the effects of pH on *S. rolfsii* revealed that optimum growth of the fungus occurred at low pH (3.0-5.5) and that above ~~a~~ pH 8, no growth was observed (Aycock, 1966). Punja (1982) reported that sclerotia could not germinate in agar medium having a pH of 7. It was also observed that at a soil pH of

above 8, the disease incidence was significantly lower than that at acidic soil. However, Punja and Grogan (1981) later revealed that sclerotia could germinate and incite disease at pH above 7 in agar medium. Zope *et al.* (2014) opined that the optimum pH level for the production of mycelium and sclerotia of *S. rolfsii* ranged from 5.5 to 7.5. Recently, Sravani and Chandra (2020) reported that maximum mycelial growth was recorded at pH 6, proving that the fungus required slightly acidic condition for its mycelial growth and sclerotial production, which is in concurrence with the results of our study.

In this research study, disease development and symptom expression were favoured in the moisture range between 35 to 50 per cent. Beute and Rodriguez-Kabaa (1981) reported that the mycelia of *S. rolfsii* got destroyed rapidly in moist soil, but were observed to survive for a period of six months in dry conditions of soil. Tarafdar *et al*. (2018) revealed that the survival of the fungus decreased with increase in soil moisture and was high in well-drained as well as sandy soils. However, the authors opined that the disease incidence had a direct relation with soil moisture.

Among the different inoculum levels of *S. rolfsii* tested for symptom expression, three days were taken for symptom expression in the seedlings when 2 to 11 per cent of inocula were used, the least inoculum level being two per cent and hence it was selected for further studies. Muthukumar and Venkatesh (2013) is of the opinion that, five per cent fungal inoculum produced the maximum collar rot incidence in pepper mint followed by 4 per cent inoculum load of *S. rolfsii*.

In our study, it was revealed that mancozeb alone and in combination with carbendazim completely inhibited the mycelial growth of *S. rolfsii*, whereas wettable sulphur, carbendazim and copper oxychloride had no inhibitory effect at all on the fungus. Mancozeb at 200 ppm was reported to completely inhibit the mycelial growth and sclerotial formation of *S. rolfsii* inciting rot of cocoyam plants when tested under *in vitro* conditions (Yaqub and Shahzad, 2006). Chaurasia *et al*. (2014) tested *in vitro* efficacy of mancozeb against *S. rolfsii* causing foot rot of brinjal and reported that mancozeb 0.5 per cent completely inhibited the radial growth of the fungus; and the effect was fungicidal and not fungistatic. They also clearly stated that wettable sulphur, copper oxychloride and carbendazim did not have any significant inhibitory effect

against the fungus which is in consensus with the results of Mahato *et al*. (2011). The results of Salvi *et al*. (2017) that mancozeb could completely inhibit the mycelial growth and sclerotial production of *S. rolfsii* causing collar rot and root rot of pigeon pea is also in accordance with the results of the present study.

It has been already reported that the pathogenicity of the fungus was partially due to the synthesis of polygalacturanase and cellulase. Studies revealed that mancozeb did not inhibit the activity of these two enzymes. Hence, the fungicidal activity was proposed to be either stimulation of host defence systems or metabolization of the fungicide into more active compounds, which in turn had the inhibitory action. It is already known that mancozeb inactivates sulfhydryl groups of amino acids and enzymes in the fungal cells, thereby causing disruption of lipid metabolism and respiration. Gisi *et al*. (1985) revealed that contact fungicides will disrupt cellular function, and denature proteins and enzymes in the pathogens. In consensus with our study, Ohazurike (1996) revealed that mancozeb at 200 ppm was the best fungicide in preventing cocoyam rot caused by *S. rolfsii*. Roy *et al*. (2013) reported that the application of mancozeb (75% WP) on chilli seedlings inoculated with *S. rolfsii* resulted in significant increase in defence related enzymes especially peroxidase and esterase.

5.4. *IN VITRO* MANAGEMENT STUDIES

Among the plant extracts and oil cakes tested *in vitro*, garlic at one per cent and garlic creeper at five per cent led to complete inhibition of both the mycelia and sclerotia of *S. rolfsii* based on their biofumigation and antifungal potential. Studies conducted to identify the lowest concentration of garlic bulb and garlic creeper leaves against the fungus revealed that garlic bulb extract at 0.3 per cent and garlic leaf extract at 2.5 per cent completely inhibited the mycelial growth as well as the sclerotia formation of the fungus.

Slusarenko *et al*. (2008) made a detailed study on the antimicrobial properties of garlic (*Allium sativum*). Garlic tissue substrates have alliin (S-allyl- L-cysteine sulphoxide). When garlic tissues get disrupted, the enzyme *viz*., alliin lyase acts on alliin to produce the volatile antimicrobial, membrane permeable substance *viz*., alliciin (diallylthiosulphinate), which takes part in thiol disulphide exchange reactions with the free thiol groups of fungal proteins.

Chaturvedi *et al*. (1987) reported that the leaves of garlic creeper (*Adenocalymma alliaceum*) belonging to bignoniacea contained a volatile oil having antifungal potential against *Drechslera oryzae.* It was revealed to stimulate the nitrate reductase activity of the host plants, thereby preventing disease incidence. Zoghbi *et al*. (1984) suggested leaves of this plant as a good substitute for garlic. The methanolic extract of garlic creeper at 2.5 and 10.0 per cent inhibited the growth of *Pythium aphanidermatum* and *Macrophomina phaseolina* respectively (Girijashankar and Thayumanavan, 2005). Chloroform extracts of the leaves of garlic creeper were highly effective in inhibiting the mycelial growth and spore germination of *Colletotrichum gloeosporioides* (anthracnose) and *Botrytis theobromae* (stem end rot) in mango (Aswini *et al*., 2010). They also reported that thin layer chromatography of the leaf extracts revealed the presence of phenolic compounds *viz*., tannic acid and resorcinol, which had antimicrobial activity by reacting on sulfhydryl groups of pathogen enzymes. Besides, they were also reported to increase the activities of defence related enzymes such as peroxidase, poly phenol oxidase and phenyl alanine ammonia lyase. Jadesha *et al*. (2013) revealed that cold water and methanol extracts of leaves of garlic creeper and zimmu completely inhibited the growth of *Colletotrichum musae in vitro*.

5.5. COMPATIBILITY STUDIES WITH TRICHODERMA VIRIDE

In vitro studies revealed that garlic (0.3%) and garlic creeper (2.5%) were found to be compatible with the biocontrol agent *T. viride* and even up to a concentration of 15 per cent in case of garlic bulb extract and 20 per cent of garlic creeper leaf extract. A successful integrated disease management programme should incorporate the beneficial effects of botanicals, biocontrol agents as well as selected fungicides if needed, for which the biocontrol agent should be compatible with botanicals and fungicides. Thus, the results of the present study has great significance, since garlic and garlic creeper even at high concentrations are not inhibiting the growth of *T. viride*. Chattopadhyay *et al*. (2007) reported that an isolate of *Trichoderma harzianum* was compatible with one per cent concentration of garlic extract and was effective in inhibiting the growth of *Sclerotinia sclerotiorum* causing rot of Indian mustard. Madhavi *et al.* (2011) reported that *T. viride* was found be highly compatible with the

fungicide, mancozeb which still adds significance to our study, as mancozeb was the only fungicide revealed to inhibit the growth and sclerotial production of *S. rolfsii*.

Sanchi *et al.* (2004) tested the efficacy of combined application of *T. harzianum*-T39 and *Brassica carinata* seed meal against *S. sclerotiorum* and *Sclerotinia* minor and revealed that isothiocyanates (ITCs) released from the meal did not reduce the ability of colonization as well as the potential of diffusible and volatile principles released from the biocontrol agent, thus proving the compatibility of the biocontrol agent with ITCs.

Galletti *et al.* (2008) remarkably described the mechanism of compatibility of *Trichoderma* spp with ITCs. The biocontrol agents were revealed to produce certain volatile compounds which could interact with the ITCs produced by biofumigants. Since the colonizing ability of the biocontrol agent will not be inactivated, there will be profuse mycelial growth. The concentration of volatile ITCs will be reduced to a lower level either by adsorption on mycelial surface or get disintegrated in the mycelium. The biocidal principles (ITCs) will be degraded to a level not toxic to the biocontrol agent, but toxic to the soil borne phyto pathogens.

5.6. *IN VIVO* MANAGEMENT STUDIES

The present *in vivo* study revealed that soil application of garlic bulb extract at the rate of 3 g per kg soil two weeks before planting followed by soil plastering with cowdung slurry and soil application of *T. viride* multiplied in cowdung and neem cake (9:1 ratio) at the rate of 500 g per plant one month after transplanting was the most effective treatment in managing basal stem rot and blight disease of vegetable cowpea as well as in inducing plant growth promotion. Kojima and Oawa (1971) studied the effect of ITCs and their anologues on microorganisms especially on oxygen uptake in yeast. Manici *et al*. (1997) proposed several theories on the mechanism of action of ITCs on phytopathogens. There may be an oxidative disintegration of disulphide bridges resulting in the inactivation of intracellular enzymes. The resultant hydrolysis products of ITCs may inactivate pathogen enzymes. The oxygen uptake of the pathogens will be prevented by inhibiting oxidative phosphorylation. Galletti *et al*. (2008) proposed that ITCs inhibited a wide range of soil borne phytopathogens and

proposed that ITCs released from the biofumigants will interact and deactivate the sulphhydryl groups, disulphide bonds, amino groups of the proteins as well as amino acid residues of the mycelium of the fungal pathogens.

Biocontrol agents *viz*., *Trichoderma* spp. can have an added effect if combined with biofumigants. Studies revealed that a slight temporal separation is required between the applications of biofumigants and the biocontrol agents to have synergistic effects. *Trichoderma* spp. will have competitive effect on pathogens for nutrients and space. They are reported to induce the production of defence related enzymes such as peroxidase, polyphenoloxidase, glucanase and chitinase. Antibiotics will also supplement their inhibitory action (Abd-El-Khair *et al*., 2010).

In the present study, it was revealed that garlic bulb extract exhibited growth promotion activities in vegetable cowpea revealed from an increase in the vine length and number of trifoliate leaves. Cheng *et al.* (2016) revealed an increase in the germination of seeds, root growth, level of phytohormones and gene expression of auxin biosynthesis pathways in tomato supporting the growth enhancement potential of garlic extract. Hayat *et al*. (2018) reported the growth promotion activities of aqueous extracts of garlic bulb in vegetables, wherein an increase in the plant height, number of leaves and root growth were observed as well as enhancement in the content of chlorophyll and carotenoids. They also proposed a priming effect of the extract against phytopathogens due to the activation of defence related enzymes such as super oxide dismutase and peroxidase. The presence of starch, vitamins, allicin and diallyl sulphides (organo sulphur compounds) enabled the growth promotion in vegetables. These studies indicate a positive correlation between the use of garlic extract on improving the plant growth parameters besides plant disease management attributes of the extract.

5.7. ESTIMATION OF SOIL FUNGAL POPULATION DYNAMICS

The effect of biofumigants on the reduction of soil fungal population was studied one month after biofumigation. Garlic bulb extract individually and in combination with *T. viride* resulted in a significant reduction of the soil fungal population, whereas an increase was observed in untreated inoculated control. Vargas (2013) reported a reduction in the growth of mycelium of *Rhizoctonia solani* due to the

production of ITCs and proposed that the inhibitory effect varied with the type of ITCs. Hu *et al*. (2015) reported the change in the soil fungal population in presence of ITCs. The authors worked with allyl ITCs and revealed a variation in the soil fungal colonies due to the release of chemicals and other suppressive impacts. Aromatic isothiocyanate volatiles (ITVs) were reported to have a longer incubation period of biofumigation resulting in great impacts due to active principles as well as due to the degradation products of hydrolysis after soil incorporation. The production of volatile compounds as well as the direct impact on the pathogens can be concluded as the reason for the reduction of the soil fungal population after biofumigation.

The present study was an attempt to identify eco-friendly and cost effective management strategy against a newly emerging soil borne fungus, *S. rolfsii* causing basal stem rot and blight disease in vegetable cowpea. The study revealed that garlic bulb extract at 0.3 per cent concentration was effective in managing the disease and was compatible with the biocontrol agent *viz*., *T. viride* which was revealed to enhance the biocontrol potential of the garlic extract. The combination was also revealed to have an enhancement in various growth parameters of the crop. Thus, the study was successful in developing an effective, disease management strategy which included soil application of garlic bulb extract @ 3g per kg soil at two weeks before planting followed by soil plastering with cow dung slurry for two weeks and soil application of 500 g cow dung and neem cake (9:1) enriched *T. viride* at one month after planting which can result in for safe-to-eat vegetable cowpea production.

The present study opens up future lines of works including identification of the active fungicidal principles in garlic bulb and garlic creeper leaves, compatibility of the biofumigants with other beneficial microorganisms and testing the bioactive principles for inhibition of other soil borne fungal pathogens of vegetable cowpea, which will aid in the development of an eco-friendly management strategy for the major soil borne diseases of vegetable cowpea.

Summary

6. SUMMARY

The research study entitled “Biofumigation for the management of *Sclerotium rolfsii* in vegetable cowpea” was conducted at Integrated Farming System Research Station, Karamana and Department of Plant Pathology, College of Agriculture Vellayani during 2018-2020 to identify the biofumigation potential of selected botanicals and oil cakes for the management of basal stem rot and blight of vegetable cowpea caused by *S. rolfsii* which is a new and emerging disease of the crop in Kerala.

Basal stem rot and blight disease of vegetable cowpea affected all the growth stages of the crop starting from seedling to harvesting stage. The characteristic symptoms of the diseases included wilting, yellowing of leaves, defoliation, stem shredding, drying and ultimate plant death. White, fan or thread like mycelial growth was observed at the collar region. Mustard shaped, initially white and later turning to tan coloured sclerotia were observed at the collar region.

Among the six isolates of *S. rolfsii* tested, the isolate, Sr_1 completed its mycelial growth within 72 HAI in PDA medium. The minimum days for sclerotial initiation (4 days) and the maximum number of sclerotia (332) were also recorded in Sr_1. The arrangement of sclerotia appeared as dense towards rim and the degree of formation of sclerotia was graded as excellent. Thus, the isolate, Sr_1 was identified as the most virulent isolate. Leaf inoculation using the mycelial discs of the fungus on the under surface of detached leaves resulted in yellowing and blighting of the leaves on one DAI, revealing that the fungus affects leaves as well.

The presence of hyaline and septate mycelium, granular cytoplasm, characteristic clamp connections, thread like, white, mycelial growth with the presence of mustard like, dark brown coloured sclerotia confirmed the pathogen to be *S. rolfsii*. Molecular identification based on the ITS region of the rDNA of *S. rolfsii* revealed 99.68 per cent identity with four strains of *S. rolfsii* (teleomorph: *Athelia rolfsii*)

The study revealed that the mycelial growth as well as the production of sclerotia was comparatively less in the alkaline pH range. The disease development and symptom expression was favoured in the moisture range of 35 to 50 percentage at which

the disease occurred within 5 to 8 days. Two per cent inoculum of *S. rolfsii* multiplied in sand oats medium resulted in early disease expression and hence, was selected for further studies.

Mancozeb alone and in combination with carbendazim completely inhibited the mycelial growth of *S. rolfsii*, whereas the other fungicides *viz*., carbendazim, copper oxychloride and wettable sulphur had no inhibitory effect at all on the fungus.

Garlic extract at one per cent followed by garlic creeper at five per cent resulted in complete inhibition of the mycelial growth of the fungus. Garlic at one per cent alone resulted in complete inhibition of the mycelial regeneration from sclerotia followed by garlic creeper at ten per cent. Thus, garlic (1%) followed by garlic creeper (5 and 10%) were the best effective antifungal extracts for inhibiting the mycelial growth as well as the sclerotia of the fungus.

Garlic extract (1 g) completely inhibited the mycelial growth of *S. rolfsii* when tested for its biofumigation potential. The next best effective treatment was garlic creeper (5 g) which also resulted in complete mycelial inhibition. Garlic extract at one percent completely inhibited the mycelial regeneration from sclerotia of the fungus up to the three week period when tested by confined container technique.

Garlic at one per cent and garlic creeper at five per cent concentrations were selected as the two best treatments based on their biofumigation and antifungal potential against both the mycelia and sclerotia of *S. rolfsii*. Garlic at 0.3 g and garlic creeper at 2.5 g completely inhibited the mycelial growth of the fungus and these concentrations were used for further studies.

Both garlic and garlic creeper were compatible at 0.3 and 2.5 per cent respectively and up to a maximum of 15 and 20 per cent respectively. They were found to be compatible with the biocontrol agent at 0.3 and 2.5 g respectively and even at higher concentrations.

The four treatments *viz*., soil application of garlic bulb extract (3 g / kg soil) at two weeks before transplanting, followed by soil plastering with cow dung slurry for two weeks (T_1), soil application of garlic creeper leaf extract (25g per kg soil) at two

weeks before transplanting, followed by soil plastering with cow dung slurry for two weeks (T_2), soil application of garlic bulb extract (3 g / kg soil) at two weeks before transplanting, followed by soil plastering with cow dung slurry for two weeks and soil application of cow dung and neem cake (9:1) enriched with *T. viride* at one month after planting (T_3) and soil application of garlic creeper leaf extract (25 g / kg soil) at two weeks before transplanting, followed by soil plastering with cow dung slurry for two weeks and soil application of cow dung and neem cake (9:1) enriched with *T. viride* at one month after planting (T_4) did not exhibit any symptoms and were effective in completely managing the disease. The inoculated control plants revealed 88.89 per cent disease incidence and 66.66 per cent disease intensity. The maximum vine length (4m) and trifoliate leaves (66.33) were recorded in T_3. There was significant reduction in the pod length and quality of pods in the inoculated control plants at 60 days after sowing. Garlic and garlic creeper individually as well as in combination with *T. viride* resulted in significant reduction in the soil fungal population at one month after biofumigation.

The present study revealed that basal stem rot and blight disease of vegetable cowpea, can be effectively managed by soil application of garlic bulb extract @ 3 g per kg soil at two weeks before planting followed by soil plastering with cow dung slurry for two weeks and soil application of 500 g cow dung and neem cake (9:1) enriched *T. viride* at one month after planting. Soil application of garlic creeper leaves @ 25 g per kg soil at two weeks before planting followed by soil plastering with cow dung slurry for two weeks and soil application of 500 g cow dung and neem cake (9:1) enriched *T. viride* at one month after planting was the next best effective treatment for the management of the disease. Garlic bulb extract (0.3%) as well as garlic creeper leaf extract (2.5%) resulted in enhancement of vine length and number of trifoliate leaves compared to other treatments, revealing their growth promotion besides fungicidal activity.

The research study demostrated the biofumigation and antifungal potential of garlic bulb and garlic creeper leaf extracts against *S. rolfsii* and their growth promotion activities in vegetable cowpea. The present work revealed the effectiveness of integration of biofumigants and biocontrol agents for the management of the emerging

disease of vegetable cowpea caused by *S. rolfsii*, which can be used as an eco-friendly management strategy for safe-to-eat vegetable cowpea production.

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Appendices

Appendix- I

COMPOSITION OF MEDIA USED

1. PDA (POTATO DEXTROSE AGAR)

Potato	- 200 g
Dextrose ($C_6H_{12}O_6$)	- 20 g
Agar-agar	- 20 g
Distilled water	- 1000 ml

Potato extract was filtered and collected after boiling in 500 ml of distilled water. Twenty grams of agar-agar was separately dissolved in 500 ml of distilled water. The potato extract was mixed in molten agar and 20 g dextrose was dissolved into the mixture. Final volume was made upto 1000 ml with distilled water and the media was sterilized at 15 psi and 121^0C for 15-20 minutes.

2. Double strength PDA

Potato	- 400 g
Dextrose ($C_6H_{12}O_6$)	- 40 g
Agar-agar	- 40 g
Distilled water	- 1000 ml

Appendix- II

COMPOSITION OF LACTOPHENOL COTTON BLUE STAIN

Composition

Anhydrous lactophenol - 67.0 mL

Distilled water - 20.0 mL

Cotton blue - 0.1 g

Anhydrous lactophenol prepared by dissolving 20 g phenol in 6 mL lactic acid and in 3 mL glycerol.

Appendix- III

SEQUENCE OF *SCLEROTIUM ROLFSII*

Accession number MT560347.

ATGCAAAGGAGTTGTGCTGGTAATAAATATTGCATGTGCACACTCTGAAGCTATATAACATATACACC
TGTGAACCAACTGTAGTCTGGAGAAATCCTGACTATGATTACTCTATATAACTCTTATTGTATGTTACA
TAGAACGATCTCATATTGAAGCTTTGTTTTTTTTTACAAGTTTCTCTTAATTGAAAAATACACAACTTTC
AACAACGGATCTCTTGGCTCTTGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTG
CAGAATCCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAGGGGCATGCCT
GTTTGAGAGTCATTAAATTCTCAACCTTACAAATTTTTGTATTTGTCAAGGCTTGGATGTGAGAGTTG
CTAGTTAAGAATATCTGACTGGCTCTCTTTAAAACTATTAGTAGGACATATAGAAATGCCTGCGGTTG
GTGTGATAATATGTCTACGCCTATACCAAAGGGGATTCTAGCTTGTATGCACTACTTATAAAATCATG
CGCATATATCTAGCATATAAGTGCATACATTGACCATTTGACCTCAAATCAGGTAGGACTACCCGCTG
AACTTAAGCATATCAATAAAGCGGAGGGAAAAGGAATCATTATTGAATTCATATATGCAAAGGAGTT
GTGCTGGTAA

Abstract

BIOFUMIGATION FOR THE MANAGEMENT OF *Sclerotium rolfsii,* IN VEGETABLE COWPEA

by

PETETI TEJA SRI

(2018-11-074)

ABSTRACT

Submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

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KERALA, INDIA

2020

ABSTRACT

The study entitled ‘Biofumigation for the management of *Sclerotium rolfsii* in vegetable cowpea’ was conducted at College of Agriculture, Vellayani and Integrated Farming System Research Station (IFSRS), Karamana during 2018 - 2020 with the objective to develop an eco-friendly strategy for the management of *S. rolfsii,* an emerging soil borne fungus of vegetable cowpea using the biofumigation potential of various plants and oil cakes.

Six isolates of *S. rolfsii* were collected from different vegetable cowpea growing tracts. The isolate, Sr_1 from var. Gitika (Pathanamthitta district) was identified as the most virulent based on virulence rating *viz.,* days taken for symptom development, lesion length, mycelial growth and sclerotial formation; and was used for further studies. Leaf inoculation of the fungus also produced leaf and stem blight, and wilting indicating that the fungus can also attack leaves besides the collar region. The morphological characters including the presence of mycelial septation, granular cytoplasm and clamp connections as well as the molecular identification based on ITS region of rDNA confirmed the fungus as *Sclerotium rolfsii*.

S. rolfsii is soil borne in nature and hence, the pH and moisture of soil as well as the inoculum level of the fungus will determine the incidence of basal stem rot and blight disease. *In vitro* studies revealed that soil pH of 6.0 and the soil moisture levels of 35 to 50 per cent were the most ideal levels for the mycelial growth and production of sclerotia of the fungus. Two per cent inoculum multiplied in sand-oats medium was standardised as the inoculum level enough for cent per cent basal stem rot and blight resulting in complete crop loss.

The antifungal and biofumigation potentials of plant extracts *viz.,* mustard, cabbage, garlic creeper, castor, cauliflower, onion and garlic bulbs as well as oil cakes namely castor cake, mustard cake and pongamia cake were tested against *S. rolfsii* by poisoned food technique and paired plate technique respectively. Confined container technique was used to test the inhibition of sclerotia. Among the plant extracts, garlic bulb at one per cent followed by garlic creeper leaves at five per cent completely inhibited the mycelial growth and mycelial regeneration from sclerotia proving its antifungal and biofumigation potentials. Subsequent *in vitro* studies revealed that garlic

bulb at 0.3 per cent and garlic creeper leaves at 2.5 per cent also completely inhibited the mycelial growth and mycelial regeneration from sclerotia.

The compatibility of *Trichoderma viride* (KAU isolate) with garlic bulb and garlic creeper leaf extracts was tested under *in vitro* conditions. The study revealed that the biocontrol agent was compatible with garlic bulb extract even at 15 per cent and garlic creeper leaves at 20 per cent.

A pot culture experiment was conducted to assess the efficacy of selected treatments for the management of the disease in the polyhouse of IFSRS, Karamana in CRD using nine treatments with three replications. Soil application of garlic bulb extract @ 3g per kg soil at two weeks before planting followed by soil plastering with cow dung slurry for two weeks and soil application of 500 g cow dung and neem cake (9:1) enriched *T. viride* at one month after planting resulted in complete disease management compared to the inoculated control (Disease incidence: 88.89 % and Disease severity: 66.66 %). Soil application of garlic creeper leaves @ 25g per kg soil at two weeks before planting followed by soil plastering with cow dung slurry for two weeks and soil application of 500 g cow dung and neem cake (9:1) enriched *T. viride* at one month after planting was the next best effective treatment for the management of the disease. Studies on the dynamics of soil fungal population before and after biofumigation revealed its significant reduction.

Thus, the present study revealed that basal stem rot and blight disease of vegetable cowpea, an emerging disease can be effectively managed by soil application of garlic bulb extract @ 3g per kg soil at two weeks before planting followed by soil plastering with cow dung slurry for two weeks and soil application of 500 g cow dung and neem cake (9:1) enriched *T. viride* at one month after planting; which can be used as an ecofriendly management strategy for safe-to-eat vegetable cowpea production.