STUDIES ON THE *Sclerotium rolfsii* Sacc. INCITANT OF STEM ROT OF GROUNDNUT (*Arachis hypogaea* L.)

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B.Sc. (Ag.)

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(PLANT PATHOLOGY)

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BY

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B.Sc. (Ag.)

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DECLARATION

I, **BACHU RAGHAVENDRA** hereby declare that the thesis entitled **"STUDIES ON THE *Sclerotium rolfsii* Sacc. INCITANT OF STEM ROT OF GROUNDNUT (*Arachis hypogaea* L.)"** submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place : Tirupati

Date :

(BACHU RAGHAVENDRA)

I.D.No. TAM/2016-29

CERTIFICATE

This is to certify that **Mr. BACHU RAGHAVENDRA** has satisfactorily prosecuted the course of research and that the thesis entitled **"STUDIES ON THE *Sclerotium rolfsii* Sacc. INCITANT OF STEM ROT OF GROUNDNUT (*Arachis hypogaea* L.)"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON THE *Sclerotium rolfsii* Sacc. INCITANT OF STEM ROT OF GROUNDNUT (*Arachis hypogaea* L.)"** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Acharya N. G. Ranga Agricultural University, Guntur is a record of bonafide research work carried out by **Mr. BACHU RAGHAVENDRA** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
<	:	lesser than
>	:	greater than
°C	:	Degree Celsius
µg	:	Micro gram
µl	:	Microlitre
µm	:	Micrometre
a.i	:	Active ingredient
CD	:	Critical difference
CFU	:	Colony Forming Units
cm	:	Centimetre
CRD	:	Complete Randomised Design
CV	:	Coefficient of Variance
EC	:	Emulsifiable Concentrate
et al.	:	and other people
etc.	:	and so on; and other people/ things
Fig.	:	Figure
g	:	gram (s)
h	:	Hour(s)
ha	:	Hectare
i.e.,	:	That is
IDM	:	Integrated Disease Management
kg	:	Kilogram
L	:	litre
mg	:	Milligram
ml	:	Millilitre
mm	:	Millimetre

No.	:	Number
P. fluorescens	:	*Pseudomonas fluorescens*
PDA	:	Potato dextrose agar
pH	:	Power of hydrogen ion concentration
ppm	:	parts per million
psi	:	Pounds per square inch
S Em	:	Standard error of mean
S. rolfsii	:	*Sclerotium rolfsii*
S.No	:	Serial Number
Sp. or Spp.	:	Species (singular or plural form)
T. viride	:	*Trichoderma viride*
U.V	:	Ultra violet
viz.	:	Namely
WP	:	Wettable powder

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ABSTRACT

The present investigation was carried out to study the groundnut stem rot causing pathogen *Sclerotium rolfsii* Sacc. The causal organism was isolated from the infected portion of the stem collected from the fields of S.V. Agricultural College, Tirupati. Pathogenicity of the fungus was tested by soil infestation method.

Effect of different inoculum densities of *S. rolfsii* on the development of stem rot of groundnut was assessed using soil infestation method in pot culture under green house. At 14 days after sowing, highest germination (45.83%), root length (12.37 cm) and shoot length (15.16 cm) was observed at 1 per cent inoculum density. Least germination (16.66%), root length (7.50 cm), shoot length (8.33 cm) was observed at 5 per cent inoculum density. Germination percentage, root length and shoot length of groundnut was gradually decreased with increase of inoculum densities from 1 to 5 per cent. Disease symptoms were not observed in any of the treatments, however development of sclerotial bodies were observed at 4 and 5 per cent inoculum.

Influence of soil temperature, soil pH and soil moisture levels were observed on the viability of sclerotia. Maximum sclerotial germination (100%) was observed at soil temperature of 27^{o} and 30^{o}C and lowest at 20^{o}C. From the study it was observed that optimum soil temperature for survival of sclerotia is 27-30^{o}C and the germination is affected if temperature is decreased below 25^{o}C and also above 35^{o}C. Soil pH 7.2 and 7.5 was found to be best (100%) for sclerotial germination and pH 9.0 was found to be less favourable showing least (76.66%) sclerotial germination. Soil moisture levels of 50% (control) and 40% were found to be best for sclerotial germination (100%). Least germination (76.67%) was observed at 100% moisture, indicating that increase in soil moisture decreases the sclerotial viability.

The antagonistic effect of three *Trichoderma viride* isolates (Tr-DM, Tr-SNG and Tr-RGP) and three *Pseudomonas fluorescens* isolates (Pf-DM, Pf-SNG and Pf-RGP) were assessed against *S. rolfsii* by dual culture technique. Among the *T. viride* isolates, maximum inhibition (87.19%) was observed with the DM-Tr and among *P. fluorescens* isolates maximum inhibition (28.25%) was observed with the Pf-DM isolate and they were significantly different from each other.

Efficacy of ten fungicides belonging to systemic and non-systemic groups were observed at various concentrations against mycelial growth of *S. rolfsii* by using poison food technique. Among different fungicides tested, fungus was most sensitive to systemic fungicides azoxystrobin, tebuconazole, hexaconazole, carboxin and non-systemic fungicide mancozeb showing complete mycelial growth inhibition (100%) at all the concentrations tested. Among systemic fungicides, carbendazim and among non-systemic fungicides, copper oxychloride was found to be less effective in inhibiting the mycelial growth of *S. rolfsii.*

Efficacy of different herbicides viz., pendimethalin, quizalofop ethyl, imazethapyr were observed at various concentrations against mycelial growth of *S. rolfsii* by using poison food technique. Among them, quizalofop ethyl showed maximum inhibition of mycelial growth (100%) at higher concentration (1000 ppm) and imazethapyr was found to be less effective in inhibiting the fungus.

Studies conducted to identify the effective dosage of *T. viride* (Tr-DM) isolate on germination of sclerotial bodies of *S. rolfsii* and observed that at higher concentration (1×10^8) maximum sclerotial inhibition was observed i.e., 66.66% inhibition over control and least sclerotial inhibition (43.33%) was observed at 1×10^4 concentration, this indicates that increase in spore concentration increases sclerotial inhibition. Similarly, sclerotial germination was tested against effective *P. fluorescens* isolate (Pf-DM) suspension at the concentrations from 10^{-4} to 10^{-8} CFU/ml and the inhibition of sclerotial germination was 66.66 per cent at 10^{-4} and 10^{-5} concentrations, 46.66 per cent at 10^{-6} and 10^{-7} concentrations. Lower sclerotial germination (36.66%) was observed at concentration of 10^{-8} (lower concentration). There was a significant increase in per cent inhibition of sclerotial germination with increase in concentration of the bacterial suspension.

All the above mentioned fungicides were tested at recommended dosage against the sclerotial germination in soil. Among different fungicides tested, highest inhibition (100%) was observed with the fungicides mancozeb, tebuconazole, hexaconazole and carboxin and least inhibition (16.66%) was observed with validamycin. Similarly, different herbicides were tested and quizalofop ethyl was highly effective in inhibiting the germination of sclerotia recording 36.66 per cent inhibition.

Compatibility among effective fungicides, herbicide and their combinations on the effective *T. viride* isolate (Tr-DM) has revealed that mancozeb was highly compatable with effective *T. viride* isolate (Tr-DM) and did not show mycelial growth inhibition, hence this fungicide was used for further pot culture study.

Efficacy of effective herbicide, fungicide and bioagent was tested against *S. rolfsii* through pot culture under greenhouse conditions. Among different treatments, T_6 (*T. viride* @ 10 g/kg soil+ mancozeb @ 0.3% a.i/kg soil + quizalofop ethyl @ 0.3% ml a.i./kg soil) did not show disease incidence upto 30 DAS with highest groundnut seed germination (91.67%), root length (22.33 cm) and shoot length (32.67 cm). In addition, disease incidence was not observed with T_8 (control), T_6 (*T. viride* @ 10 g/kg soil+ mancozeb @ 0.3% a.i/kg soil + quizalofop ethyl @ 0.3% ml a.i. /kg soil), T_4 (mancozeb @ 0.3% a.i/kg soil + quizalofop ethyl @ 0.3% ml a.i. /kg soil), T_3 (mancozeb @ 0.3% a.i/kg soil) and T_1 (quizalofop ethyl @ 0.3% ml a.i./kg soil). Highest dry weight of the plant was recorded at T_8 (control) (3.66 gm) followed by T_4 (mancozeb @ 0.3% a.i/kg soil + quizalofop ethyl @ 0.3% ml a.i./kg soil), and T_6 (*T. viride* @ 10 g/kg soil+ mancozeb @ 0.3% a.i/kg soil + quizalofop ethyl @ 0.3% ml a.i./kg soil) (3.26 gm). From the study it was clear that integrated treatment T_6 (*T. viride* @ 10 g/kg soil+ mancozeb @ 0.3% a.i/kg soil + quizalofop ethyl @ 0.3% ml a.i. /kg soil) is highly effective with high groundnut seed germination, root length, shoot length, dry weight of the plant and least disease incidence.

Chapter ~ I

Introduction

Chapter – I

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop suitable for cultivation in tropical areas of the world. It is regarded as "King of oilseed crops" on account of its diversified uses. Groundnut is third largest oil seed crop grown in world and second in India. Groundnut seeds are rich in oil (43-55%) as well as protein (25-28%) and also contains 18 per cent carbohydrates. It can supply about 5.6 and 5.8 calories per gram of kernel in the raw and roasted forms respectively. It is also very good source of minerals (calcium, magnesium and iron) and vitamins (B1, B2 and Niacin). Groundnut being a legume crop, it fixes a large amount of nitrogen and improves the fertility status of the soil. Groundnut cake is used as animal feed and the shell some times used as fodder.

The groundnut production in India was 71.8 lakh tons in 2015-16. There were 9 States having groundnut production of more than 1 lakh ton viz. Gujarat, Rajasthan, Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, West Bengal and Telangana in 2015-16. Gujarat was the leading producer of groundnut with 28.9 lakh tons in 2015-16 followed by Rajasthan 10.41 lakh tons and Andhra Pradesh ranks 4th leading producer in India with 7.88 lakh tons in 2015-16. It increased by 2.95 lakh tons as compared to the groundnut production of 4.93 lakh tons in the year 2014-15. Thus, the annual growth recorded in the year 2015-16 was 59.84%. (Source: Production of Groundnut from 2013-14 to 2015-16 from Ministry of Agriculture and Farmers Welfare).

The low productivity of groundnut in India was attributed to several biotic and abiotic factors. Groundnut is susceptible to wide range of microorganisms which include fungi, viruses, mycoplasma, nematodes and bacteria (Khirood and Paramjit Kaur, 2013). Among them, stem rot caused by

Sclerotium rolfsii Sacc. is a major problem and is an economically important soil borne pathogen. It causes pod yield losses of 10-25%, but under severe diseased conditions yield losses may range up to 80% (Rodriguez Kabana *et al.,* 1975). Stem rot is a persistent soil borne disease throughout India and its incidence is increasing even at maturity stage of the groundnut crop. Though *S. rolfsii* survives both on seed and in soil, a greater threat is posed by soil borne inoculum (Kumar *et al.,* 2013). *S. rolfsii* forms brownish sclerotia that can survive for long periods in the soil and retain their viability by tolerating biological and chemical degradation due to the presence of melanin in the outer membrane (Chet, 1975). *S. rolfsii* is a necrotrophic soil borne fungal pathogen, which has a very wide host range and distributed worldwide in warm climates (Aycock, 1966).

S. rolfsii was first reported on tomato by Rolfs (1892) and later the pathogen was named as *Sclerotium rolfsii* by Saccardo (1911). Higgiens (1927) worked in detail on physiology and parasitism of *S. rolfsii*. This was the first detailed and comprehensive study in USA. It is distributed in tropical and sub-tropical regions of the world where high temperatures prevail. The fungus has a wide host range of 500 species in about 100 families including groundnut, green bean, lima bean, onion, garden bean, pepper, potato, sweet potato, tomato and water melon (Aycock, 1966).

Stem rot of groundnut has become one of the major constraints in recent years. Management of stem rot disease is difficult because of its soil borne nature and the chemical methods are very expensive and will not be that good affect against the pathogen. In view of unsatisfactory results by chemical methods, considerable attention has been given on the other non-chemical means of plant disease control i.e., the integration of biological methods which include the use of eco-friendly bio control agents.

Thus, keeping the wide occurrence of disease and its destructive nature of the pathogen, present investigation was undertaken with the following objectives.

Objectives

1. Evaluation of inoculum density of *S. rolfsii* on disease severity.
2. Influence of temperature, pH on the viability of sclerotia of *S. rolfsii.*
3. Evaluation of efficacy of different bioagents, fungicides, herbicides against *S. rolfsii* and on sclerotial germination *in vitro*
4. Integrated management of stem rot of ground nut *in vivo* in pot culture

Chapter ~ II

Review of Literature

Chapter – II

REVIEW OF LITERATURE

Groundnut (*Arachis hypogaea* L.) is the sixth most important oilseed crop in the world and it is called as the 'king' of oilseeds. It is one of the most important food and cash crops of our country. It contains 48-50% of oil and 26-28% of protein, and is a rich source of dietary fiber, minerals, and vitamins. Groundnut crop suffers from 15 foliar and 7 seed borne diseases (Subrahamanyam *et al*., 1990 and Reddy *et al*., 1999). Among them, stem rot (*Sclerotium rolfsii* Sacc.) is one of the economically important soil borne disease and major constraints to production wherever groundnut is grown. In India, stem rot occurs in all groundnut growing states and it is most severe in Maharashtra, Gujrat, Madhya Pradesh, Karnataka, Andhra Pradesh, Orissa and Tamil Nadu (Mehan *et al*., 1995). This disease occurs at any stage of crop growth and can even damage yield over 25 per cent and it can reach over 80 per cent in heavily infected fields (Mehan and Mc Donald, 1990). Keeping the wide occurrence of disease and its destructive nature of the pathogen, present investigation entitled "Studies on the *Sclerotium rolfsii* Sacc. incitant of stem rot of groundnut (*Arachis hypogaea* L.)" was undertaken.

2.1 THE PATHOGEN

The first report of the disease caused by *Sclerotium rolfsii* was the tomato blight in Florida (1892) and later Saccardo (1911) named it as *S. rolfsii.* He characterized the fungus as an imperfect form without asexual spores. Shaw and Ajrekar (1915) isolated an organism from rotted potatoes and identified as *Rhizoctonia destruens* Tassi. But, later studies revealed that, the fungus involved was *S. rolfsii* (Ramakrishan and Damodaran, 1930).

2.1.1 Taxonomy

Sclerotium rolfsii (Sacc.) is a member of

Kingdom	-	Mycota
Division	-	Eumycota
Sub division	-	Deuteromycotina
Form – class	-	Aganomycetes
Form – order	-	Aganomycetales
Form – family	-	Aganomycetaceae

2.1.2 Economic Importance

S. rolfsii is an economically important pathogen on numerous crops worldwide (Aycock, 1966). It is an omnivorous and destructive parasite of many plants. It has an extensive host range, at least 500 species in 100 families are susceptible and the most common hosts are legumes, crucifers and cucurbits (Chupp and Sherf, 1960). Weber (1931) and Garret (1956) reported that the fungus survived in the soil for years together by producing sclerotial bodies and causing the disease on various hosts. It is a destructive parasite of many plants causes yield losses of 10-25 per cent and under severe diseased conditions it reaches above 25 per cent and upto 80-90 per cent. (Rodriguez-Kabana *et al*., 1975; Grichar and Boswell, 1987). Adiver (2003) reported the yield loss of 15-70 per cent in groundnut due to leaf spot, rust and stem rot singly or in combination.

2.1.3 Morphology, Biology

According to West (1961) the fungus belonging to the group of non-spore producing fungi and production of small round sclerotia is an important morphological characteristic feature of the organism. Initially the fungal

mycelium is silky white in pure culture but gradually loses its luster and becomes somewhat dull in appearance. The mycelium is radiating with abundant aerial hyphae. The mycelium completely disappears over a period of three months leaving only sclerotial bodies. According to (Subramanian, 1964 and Mehan *et al*., 1995) sclerotia are initially white, later becomes light brown to dark brown at maturity.

The fungus initially developed as white aggregates or knots of mycelium, then it differentiate to form the mature sclerotium within 2-3 weeks. The fungus produces sclerotia abundantly on Potato Dextrose Agar (PDA) and also on a substrate like autoclaved oat kernels moistened with 1.5 per cent water agar (Punja and Grogan, 1981). *S. rolfsii* can over winter as mycelium in infected tissues or plant debris. Sclerotia are disseminated by cultural practices, infected transplant seedling, water (especially through irrigation), and wind and possibly on seeds. In addition, a small per cent of sclerotia may survive passage through sheep and cattle and thus could be spread through fertilizer (Stephen *et al.*, 2001).

2.1.4 Symptomatology

Stem rot disease starts with pre-emergence rotting of seeds and is characterized by rotten and softened seeds that are covered with profuse, white mycelial growth of the fungus. The pathogen attacks the germinated seedlings and causes wilt. Although all the plant parts are susceptible to the pathogen, stem infection is the most common and destructive one. The disease starts with yellowing and wilting of branches at the base of the stem near the soil line, later sheaths of white mycelium develop at or near the soil line around the affected areas of the stem. Abundant sclerotia, initially white that later turn brown, develop on the infected areas (Nyvall, 1989). The entire plant is killed, but in some cases two or three branches are affected. Infection of pegs, pods and roots occur either independently or together with stem infection. Orange or brown coloured lesions may be found on the pods.

Severely infected pods are completely covered with a white mycelial mat and eventually decay. In some cases the seeds from the diseased pods show a characteristic bluish grey discolouration of the test a known as blue damage (Subramanian, 1964 and Beattle, 1954). Stem rot pathogen produces a variety of symptoms in groundnut such as seed rot, seedling blight, collar and stem rot, peg and pod rot. Seed rot and blight are less common than stem and pod rot damage (Asghari and Mayee, 1991). Anahosur (2001) observed dark brown lesion on the stem just below the soil surface followed by drooping and wilting of infected leaves and gradually wilting of the whole plant. He also observed whitish mycelial growth with sclerotial bodies resembling mustard seeds on collar region and roots.

2.1.5 Pathogenicity Test

Datar and Bindu (1974) proved the pathogenicity of *S. rolfsii* on sunflower by soil inoculation method by growing the fungus on sterilized maize bran medium and they mixed maize bran medium with the sterilized soil one week before sowing. Mishra and Bais (1987) used 15 days old fungal cultures grown on the sand corn meal medium for proving pathogenicity of root rot of barley caused by *S. rolfsii*, by mixing upper 4-5 cm layer of soil with the inoculum. Siddaramaiah (1988) proved the pathogenicity of *S. rolfsii* on cardamom in pot culture. They inoculated 25 days old sclerotial cultures which was grown on the sand corn meal medium and observed the symptoms a week after inoculation.

Kulkarni and Kulkarni (1994) while studying the most susceptible growth stage of groundnut to *S. rolfsii*, maximum mortality was recorded in 15 days old plants and the least mortality in 105 days old plants. Potential death and estimated field yield losses of 10% or more in the southern-eastern USA was observed by stem and pod rot of groundnut caused by *S. rolfsii* (Shokes and Gorbet, 1998). Reduction in seed germination and plant growth in soybean by *S. rolfsii* was reported by Blum and Rodriguez (2004).

Palaiah and Adiver (2004) conducted virulence studies of twelve isolates of *S. rolfsii* collected from the stem rot infected groundnut samples in Karnataka. Based on the virulence studies, the isolates were categorized into highly virulent and moderately virulent. Highly virulent isolates showed seedling mortality in the range of 42-56 per cent, whereas the moderately virulent isolates showed seedling mortality of 25 to 37 per cent.

Awasthi *et al.* (2010) investigated the pathogenicity of different isolates of *S. rolfsii* Sacc. on groundnut and observed marked variation in the virulence of different isolates. All the isolates were found to be pathogenic towards groundnut but extent of pathogenicity and disease severity differed in some isolates. Isolate 10 showed highest disease severity of 54.4 per cent which was superior as compared to all other isolates whereas, Isolate 2 and Isolate 8 showed least disease severity of 40.8 and 40.9 per cent respectively.

Prasad *et al.* (2010) studied the pathogenic variability among twenty isolates of *S. rolfsii* on groundnut variety, TGCS888 and indicated that the isolates CSr1, CSr2, CSr9, CSr10, KSr15, KSr17, KSr19 were virulent with regard to disease severity resulting in different degrees of per cent disease incidence ranging from 80 to 100 per cent.

2.2 EFFECT OF INOCULUM DENSITIES OF *S. rolfsii* ON INCIDENCE OF STEM ROT DISEASE

Kilpatrick and Merkle (1967) reported the effect of different levels of *S. rolfsii* inoculum on the foot rot of wheat and found that, 0.5 and 1.0 per cent inoculum was superior to 3, 5 and 10 per cent level.

Nargund (1981) considered two per cent inoculum level as sufficient to cause considerable infection on foot rot of wheat, even though hundred per cent disease incidence was noticed at six per cent and above inoculum level.

Palakshappa *et al.* (1987) observed considerable foot rot infection, when betelvine were inoculated with two and three per cent inoculum and recorded 100 per cent infection at four per cent and above inoculum levels.

Harlapur (1988) reported that, two per cent inoculum was essential for infection. But, maximum infection (100%) was noticed at inoculum level of more than four per cent in foot rot disease of wheat.

Mc Clain and Scharpf (1989) reported a positive correlation between seedling mortality of coniferous species and inoculum density, whereas plant height and weight of the surviving seedlings were negatively correlated with sclerotial density of *Macrophomina phaseolina*.

Uma Singh and Thapliyal (1998) reported inoculum density levels of 2.5 to 10 g kg^{-1} soil significantly increased the emergence of seed and seedling rot of soybean caused by *S. rolfsii* which was ranged from 36.70 to 90 per cent.

Azhar *et al.* (2006) observed positive correlation between disease severity and inoculum concentrations (3-20 g/560 g of soil) in collar rot of chickpea incited by *S. rolfsii*, where seedling mortality increased with an increase in inoculum load.

2.3 EFFECT OF SOIL TEMPERATURE, pH AND MOISTURE ON THE VIABILITY OF *S. rolfsii*

2.3.1 Soil Temperature

Satyabrata Maiti and Chitreshwar Sen (1988) reported that higher temperatures (25-40°C) reduced the survivability of sclerotia of *S. rolfsii* with rising temperature and increasing time.

The effect of soil temperature and moisture on eruptive germination and viability of sclerotia of *Sclerotinia minor* and *S. sclerotiorum* in field soil

was examined by Matheron and Porchas (2005) and found that the proportion of sclerotia of both pathogens that germinated in wet soil (≥ -0.02 MPa) tended to decrease as soil temperature increased from 15 to 40°C, with no germination of sclerotia of *S. minor* and *S. sclerotiorum* detected after 1 and 2 weeks respectively, at 40°C.

Gour and Sharma (2010) reported that maximum growth (86.67 mm) of the *S. rolfsii* causing stem rot of ground nut occurred at 30^0 C followed by 35^0 C (76.67 mm) and the lowest mycelial growth at 15^0 C (19-67 mm).

Zape and Gade (2013) found that the *S. rolfsii* showed rapid mycelia growth at 30°C and maximum sclerotial production at 25°C.

Maximum mycelial growth of *S. rolfsii,* was observed at 30°C, while the excellent degree of sclerotia production was observed at 30 and 35°C temperature (Shridha Chaurasia, 2013).

The effect of various temperatures, viz., 15, 20, 25, 30, 35, 40 and 45°C, on the production of organic acid by *S. rolfsi* was studied by Shridha Chaurasia, (2014) and found that when *S. rolfsii* was incubated at very low temperature i.e., 15°C, a little amount of organic acid was detected. The accumulation of organic acid was increased gradually with increase in temperature up to 30°C. She also reported that, further increase in temperature above 30°C have no effect on the production of organic acid and concluded that 30°C temperature was found to be the best and optimum for the production of organic acid by *S. rolfsii.*

2.3.2 Soil pH

Gour and Sharma (2010) reported that *S. rolfsii* grew on a wide range of pH from 4 to 9 but the maximum growth of the fungus was recorded on the medium having pH value of 6.0 (87.00 mm) followed by pH 5.0 (76.67 mm), lowest mycelial growth was obtained at pH 9.0 (28.67 mm) and pH 8.0 (40.33

mm). He concluded that optimum pH for best growth of *S. rolfsii* lies between pH 6 to 7.

Shridha Chaurasia (2013) reported that, pH 5.0 was optimum for mycelial growth, while pH 4.0 to 7.0 were found to be most favourable for the production of sclerotia of *S. rolfsii* and the maximum radial growth of *S. rolfsii* was observed at pH 6.5 followed by pH 6.0 and 7.0 and for formation of sclerotial bodies, it was at pH 7.0.

Shridha Chaurasia (2014) reported that pH 5.0 was found to be the best and optimum for the production of organic acid by *S. rolfsii.*

2.3.3 Soil Moisture

Flados (1958) found the reduction in growth of *S. rolfsii* with the increase in soil moisture and also observed that, the organisms can grow from an inoculum source through air dry soils.

Curl (1961) reported abundant population of *S. rolfsii* at low levels of irrigation than at high levels.

Reddy and Patil Kulkarni (1972) and Ramarao and Raju (1980) conducted a series of laboratory experiments with *S. rolfsii* isolated from wheat and recorded the highest mortality of seedlings at 25 per cent moisture holding capacity of soil and least per cent disease incidence at 40 per cent soil moisture.

Sclerotia of *S. rolfsii* survived well at moisture contents up to 75 per cent WHC but at 100 per cent the population declined rapidly and none were recovered after 60 days. The contents of the sclerotia were found to lyse without germination leaving hollow rinds (Satyabrata Maiti and Chitreshwar Sen, 1988).

Katti *et al*. (1983) reported that, the survival of *S. rolfsii* was highest at soil moisture levels between 30 and 50 per cent.

Palakshappa (1986) studied the effect of different soil moisture levels on foot rot of betelvine caused by *S. rolfsii* and reported that, the fungus survived better at low soil moisture than at higher levels. The survival ability was highest between 20 and 40 per cent soil moisture. However, higher saprophytic activity of the fungus was observed at 40 per cent moisture level and the least was at 60 and 70 per cent soil moisture where the saprophytic activity of the fungus was found to be very less.

2.4 EFFECT OF BIOAGENTS, FUNGICIDES AND HERBICIDES ON THE MYCELIAL GROWTH OF *S. rolfsii*

2.4.1 Bioagents

Elad *et al*. (1983) studied mycoparasitism of *Trichoderma* spp. on *S. rolfsii* and *Rhizoctonia solani* by using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) and these ultra structural studies have clearly shown the degraded walls of sclerotial cells**.**

Trichoderma has been reported as effective in controlling damping-off of beans caused by *S. rolfsii* in the green house (Henis, 1984).

Wokocha *et al.* (1986) noticed that, a native strain of *Pseudomonas fluorescens*, migula restricted the mycelial growth of *S. rolfsii* on peanut.

Ganesan and Gnanamanickam (1987) found that, the native station of *P. fluorescens* restricted the growth of *S. rolfsii* causing stem rot of groundnut.

Muthamilan and Jeyarajan (1992) reported 67.4 per cent reduction of sclerotial production in *S. rolfsii* in the presence of *T. viride* and also noticed that mature sclerotia from each dual culture plate were smaller than the control plate.

Iqbal *et al.* (1995) identified that the micro-organisms viz., *Trichoderma harzianum, Trichoderma koningii Ouden, Trichoderma viridae, Gliocladium virens Miller, Aspergillus canditus Link, Paecilomyces lilacinus* (Thom) Samson and *Bacillus spp*. showed antagonism significantly and inhibited the mycelial growth of *S. rolfsii*, of these *T. harzianum, T. koningii* and *T. viride* overlapped the pathogen and suppressed growth by 63.6 per cent, 54.9 per cent and 51.9 per cent respectively.

Virupaksha *et al.* (1997) reported the antagonistic activity of *T. harzianum* and *T. viride* against *S. rolfsii* and found to be effective in inhibiting the mycelial growth and reducing production of sclerotial bodies irrespective of inoculation periods.

Rajya Lakshmi (2002) observed 37.5 to 41.3 per cent reduction in growth of *S. rolfsii* and also significant reduction in sclerotial production was observed *in vitro* by *T. viride* and *T. harzianum*.

Chandrasekhar *et al.* (2005) reported that the antagonists *T. harzianum* and *T. viride* overgrew and completely suppressed the growth of *S. rolfsii*. The least zone of inhibition was produced by *A. flavus* while *P. fluorescens* produced the maximum inhibition zone of 11 mm. Pot culture experiments indicated that seed treatment and soil drenching with antagonists increased the per cent survival of treated seedlings. Seed treatment was more effective than soil application. The maximum per cent survival of 89.5 was recorded by treating the seeds with *T. viride* followed by *P. fluorescens* with 86.4% survival. Soil application with *T. viride* (77.9%) and *P. fluorescens* (69.5%) enhanced survival of seedlings in both treatments. Pre emergence mortality was nil in soil and seed treatments with *Trichoderma sp*. and *P. fluorescens*.

Varadharajan *et al.* (2006) evaluated three isolates of *T. viride*, one isolate each of *T. harzianum* and *P. fluorescens* in dual culture and found inhibitory to the growth of *S. rolfsii*, the causal agent of stem rot of groundnut.

Hoyos *et al*. (2008) investigated *in vitro* analysis of the antagonistic capacity of *T. viride* isolates under greenhouse conditions, four isolates were highly effective against *S. rolfsii* in bean nursery plants (>90% of disease reduction) and two isolates were effective against *R. solani* in cotton nursery plants (58 and 61% of disease reduction).

Mundhe *et al*. (2009) reported that all the bio agents tested were found effective in reducing the growth of the *S. rolfsii,* causing foot rot of finger millet. Maximum inhibition of *S. rolfsii* was observed with *T. harzianum* (P) followed by *T. harzianum* (JCR), *P. fluorescens* and *Bacillus subtilis*.

Rekha *et al*. (2012) studied the efficacy of different *Trichoderma* isolates against *S. rolfsii* causing stem rot of groundnut. Among them, Tri-8 (*T. piluliferum*) showed maximum per cent inhibition of mycelial growth (71.02%) of *S. rolfsii* while the isolate Tri-33 had shown minimum per cent inhibition of mycelial growth (43.30%). The isolates Tri- 23 (69.26%), Tri-13 (68.98%), Tri-27 (67.20%), Tri- 16 (66.76%), Tri-19 (66.02%), Tri- 41 (65.83%), Tri-15 (65.37%) and Tri-44 (64.63%) were also found to be effective in inhibiting the mycelial growth of the pathogen.

Chanutsa *et al.* (2014) reported 100 per cent inhibition in growth of *S. rolfsii* with culture filtrate of *P. fluorescence.*

Salvi *et al.* (2017) screened three bio-agents *in vitro* namely *Trichoderma viride, T. harzianum* and *P. fluorescens* and found that *T. viride* showed maximum growth inhibition (83.33%) against *S. rolfsii.*

2.4.2 Fungicides

Siddaramaiah and Hegde (1983) reported that seed treatment with Bavistin [Carbendazim] or Captan at 2.5 g kg^{-1} seed is effective in protecting against seed rot and seedling blight of safflower.

Jhonson and Subramanyam (2000) found that carbendazim, chlorothalonil and copper oxychloride were least effective against *S. rolfsii*.

Seed treatment with propiconazole and hexaconazole were superior in checking stem rot of groundnut caused by *S. rolfsii* and increasing the shoot and root length (Charde *et al*., 2002).

Seed treatment of soybean with hexaconazole and propiconazole inhibited *S. rolfsii* and these fungicides were found to be absorbed by roots and translocated to shoot and leaf (Tajane *et al*., 2002).

Prabhu and Hiremath (2003) and Arunasri *et al.* (2011) reported that the triazoles viz., hexaconazole, propiconazole, difenconazole were highly inhibitory to the growth of *S. rolfsii.*

Tiwari and Ashok Singh (2004) reported that, fungicides like carboxin, epoxiconazole, hexaconazole, propiconazole and triadimefon were found highly effective against *R. solani* and *S. rolfsii*, and can be formulated as seed dresser either with thiram or mancozeb to control both collar rot and root rot as well as seed mycoflora effectively.

Sheoraj *et al.* (2005) studied the efficacy of several fungicides in controlling *S. rolfsii* causing collar rot of lentil *in vitro* and found that mancozeb, thiram and carboxin showed 100 per cent control against the pathogen.

Yaqub and Shahzad (2006) tested fungicides *viz*., Benomyl, Mancozeb, Thiovit, Dithane M-45, Carbendazim and Topsin-M against *S. rolfsii in vitro* and reported that at low concentrations no fungicide inhibited the growth of *S. rolfsii.* However, Dithane M-45 significantly reduced the growth at high concentration.

Toorray *et al.* (2007) reported complete inhibition of growth of *S. rolfsii* by captan, thiram, mancozeb, hinosan and antracol whereas, kavach showed partial inhibition at low concentration. Carbendazim did not show

much inhibition at all concentrations. In *in vivo* captan, kavach and thiram showed reduction in pre emergence mortality.

Jhonson *et al.* (2008) evaluated *in vitro* efficacy of five fungicides hexaconazole, propiconazole, mancozeb, chlorpyriphos and quinalphos against *S. rolfsii* and reported that hexaconazole at a concentration of 1000, 1500 and 2000 ppm and propiconazole at a concentration of 500, 750 and 1000 ppm completely inhibited the growth of *S. rolfsii*.

Perez *et al*. (2009) evaluated the *in vitro* reaction of fungicides to *S. rolfsii* and showed that tebuconazole inhibited mycelial growth and sclerotial production.

Rakholiya (2010) reported complete growth inhibition of *S. rolfsii* with propiconazole and mancozeb, while wettable sulphur, copper oxychloride and carbendazim were found least effective against *S. rolfsii in vitro*.

Manu *et al.* (2012) found that three triazole compounds viz., hexaconazole, propiconazole and difenconazole had significant inhibitory effect at all concentrations on *S. rolfsii*. Carbendazim, tricyclazole and thiophanate methyl did not show any inhibition on the mycelial growth of *S. rolfsii* at any of the concentrations tested.

Bhuiyan *et al.* (2012) tested different fungicides for their efficacy against the radial colony growth of *S. rolfsii*. The complete inhibition was obtained with Provax-200 at all the selected concentrations. Complete inhibition also obtained at the highest concentration of Tilt. The highest concentration of Rovral 50WP inhibited 93.88% radial growth and significantly superior to Dithane M-45 at the highest concentration. Bavistin and Ridomil were found to be significantly lower when used against the test pathogen.

In vitro evaluation of new fungicide mixtures carbendazim + mancozeb and hexaconazole + zineb against *S. rolfsii* revealed that these mixtures could control the test pathogen even at 250 ppm concentration (Kumar *et al*., 2014).

Rakholiya (2015) screened different systemic, contact and combination of fungicides *in vitro* condition against *S. rolfsii*. Among systemic fungicides carboxin, hexaconazole and propiconazole showed 100 per cent growth inhibition at all concentrations. While fosetyl-Al, thiophanate methyl and carbendazim were found least effective at 500 ppm. Among non systemic fungicides, mancozeb showed 100 per cent growth inhibition of *S. rolfsii* at all concentrations, while sulphur, zineb and copper oxychloride were found least effective even at higher concentrations. Carbendazim 50 WP + mancozeb 75 WP (1:2 manual) gave cent per cent inhibition of fungus at 500 ppm. Cymoxanil 8% + mancozeb 64% and carbendazim 50 WP + thiram 75 WP (1:2 manual) were also gave cent per cent growth inhibition of the fungus at 1000, 1500 and 2000 ppm concentrations.

Rangarani *et al.* (2017) tested different fungicides against *S. rolfsii.* Among systemic fungicides, propiconazole and tricyclazole recorded 100 per cent growth inhibitions at all concentrations. Thiophanate methyl and carbendazim was found least effective. Among non systemic fungicides, mancozeb and thiram were found 100 per cent growth inhibition at all concentrations. In combination of fungicides carbendazim 12% WP + mancozeb 63% WP minimum growth inhibition was recorded at 500 ppm (47.40%) followed by 1000 ppm (87.03%).

Salvi *et al.* (2017) tested fungicides *viz*., difenoconazole, hexaconazole, propiconazole, thiophenate methyl at 0.1 per cent, hexaconazole and propiconazole at 0.05 per cent and mancozeb at 0.25 per cent concentration. All fungicides were shown inhibitory effects against growth of mycelium and sclerotia formation of *S. rolfsii*.

2.4.3 Herbicides

Different herbicides incorporated into PDA at 50, 100 and 500 µg ml-1 reduced mycelial growth and sclerotial formation of *S. rolfsii*, *M. phaseolina* and *F. oxysporum* isolated from the soybean cv JS-72-44 *in vitro* (Vyas *et al.*, 1986).

2, 4-D and fluchloralin drastically inhibited the growth of *S. rolfsii* and *R. bataticola* (Tripathi *et al*., 1988).

Lal and Nagarajan (1988) evaluated different herbicides i.e., alachlor, basalin and trifluralin against *S. rolfsii* causing collar rot disease of tobacco at 125, 250, 500, 1000 and 2000 ppm concentrations. The per cent growth inhibition was 67.3 – 88.14 upto 500 ppm concentration. While cent per cent inhibition was noticed at higher concentrations (1000 and 2000 ppm).

The effect of Dicuran MA on the colony growth of five soil borne fungal pathogens of chickpea (*Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, *S. rolfsii*, *R. solani* and *Fusarium oxysporum* was tested under *in vitro* conditions and the results showed that the treatment decreased the growth of all the pathogens tested, but to different extents. *S. rolfsii* was completely inhibited and *M. phaseolina* showed low sensitivity and the growth of other fungi was inhibited by 50 - 62.4 per cent (Iqbal *et al*., 1994).

Pastro and March (1999) evaluated the effects of different herbicides on the production and viability of *S. rolfsii in vitro.* Trifluralin and pendimethalin notably reduced the production of viable sclerotia.

Madhuri and Narayan Reddy (2013) evaluated different herbicides for their efficacy on *S. rolfsii* by poisoned food technique and found that oxyflourfen, alachlor, quizalofop ethyl and 2, 4-D sodium salt completely inhibited the growth of *S. rolfsii.*

Madhuri and Sagar (2016) reported that quizalofop ethyl showed cent per cent inhibition followed by pendimethalin (92.22%), imazethapyr (68.88%) and oxyflourfen (51.85%) against *S. rolfsii in vitro.*

Rangarani *et al.* (2017) evaluated different herbicides against *S. rolfsii.* and reported that pendimethalin, quizalofop ethyl shown hundred per cent inhibition of the pathogen. While in 2, 4-D Na salt 65.6 per cent growth inhibition of *S. rolfsii* was observed.

Rakholiya and Jadeja (2017) screened several herbicides *in vitro* to find out effective herbicides for the control of *S. rolfsii*. Among herbicides, quizalofop ethyl and metasulfuron exhibited cent per cent growth inhibition at all the concentrations. While fluchloralin and pendimethalin were found cent per cent inhibition at 2000 ppm, oxyfluorfen (92.22%) and glyphosate (75.74%) were also effective growth inhibited at 2000 ppm.

2.5 EFFECT OF BIOAGENTS, FUNGICIDES AND HERBICIDES AGAINST SCLEROTIAL VIABILITY OF *S. rolfsii*

2.5.1 Bioagents

D'ambra and Ferrata (1984) observed the reduction of mycelial growth, sclerotial formation, sclerotial germination and number of sclerotia of *S. rolfsii* when inoculated with different inoculum concentration of *T. harzianum*.

Ganesan and Gnanamanickam (1987) found that sclerotia of *S. rolfsii* showed 10-20 per cent loss of germination after immersion in *P. fluorescence* suspension for one hour and about 50-60 per cent after one week.

Vasantha Devi *et al*. (1989) studied the effect of strains of fluorescent and non fluorescent bacteria that were isolated from rice rhizospheres of Southern India and showed antagonism towards *R. solani*, for biological control of rice sheath blight. The results showed that efficient strains of

P. fluorescence and non fluorescent strain NF2 caused reductions in sclerotial viability and sclerotium germination declined with prolonged incubation periods, they also inhibited the mycelial growth of *R. solani*.

Khattabi *et al.* (2001) tested the effect of four isolates of *T. harzianum* against the sclerotial viability of *S. rolfsii* and found that all four isolates of *T. harzianum* reduced the sclerotial viability.

Pande and Chaube (2003) reported that pretreatment of *P. fluorescens* isolates to sclerotia of *R. solani* to different lengths of time affected sclerotial viability *in vitro.* Pre- treatment of sclerotia resulted in 3.3 to 100% inhibition in their germination after these were immersed in bacterial cell suspension of *P. fluorescens* isolates for 1 min. to 4 weeks.

Khattabi *et al.* (2004) screened seventy isolates of *Trichoderma* spp. against the viability of sclerotia of *S. rolfsii* in natural and sterilized soil and found that almost all *Trichoderma* isolates reduced the viability of sclerotia both in natural and sterilized soil, but greater reduction of viability is observed in sterilized soil.

Mostapha Niknejad Kazempour (2004) studied the effect of *P. fluorescens* against *R. solani* and reported that all isolates inhibited the mycelial growth, antagonistic isolates reduced the germination and cause lysis of sclerotia.

Srinivasulu *et al.* (2005) observed *in vitro* antagonism of three *Trichoderma spp.* against *S. rolfsii* and reported that sclerotial bodies of *S. rolfsii* gave rise to respective *Trichoderma spp*. mycelia when subjected to germination tests after 10 days of parasitisation and above.

2.5.2 Fungicides

Hawthorne and Jarvis (1973) tested different fungicides for activity against mycelial growth, germination of ascospores and sclerotia, and production of stipes of *Sclerotinia minor* and found that three fungicides, benomyl, thiophanate, and thiophanate-methyl greatly reduced the germination of sclerotia to form mycelium.

Anil Kumar *et al.* (1979) investigated the effect of several fungicides on the survival of sclerotia of *S. rolfsii* and found that Sicarol, Vitavax and Brestan were highly effective in reducing sclerotial viability.

Chakraborty and Bhowmik (1985) showed that thiram was best in limiting mycelial growth and inhibiting sclerotial germination followed by ceresan wet, benlate, captan.

Khattabi *et al.* (2001) tested the effect of different fungicides against the sclerotial viability of *S. rolfsii* and found that benomyl showed the greatest reduction, producing 53.3 per cent of non-viable sclerotia at a concentration of 50 mg a.i./m^2 and 68.3 per cent at 250 mg a.i./m^2.

2.5.3 Herbicides

Singh and Dwivedi (1990) evaluated herbicide nitrofen and the insecticide nuvacron against the sclerotial viability of *S. rolfsii* and reported that both nitrofen and nuvacron greatly reduced the germination of sclerotia.

Pathak *et al*. (1996) observed the effect of four herbicides (butachlor, thiobencarb, 2, 4-D and paraquat) on the viability of buried sclerotia of *R. solani*. He found that paraquat was the most potent in reducing the viability of sclerotia, mycelial growth and production of sclerotia followed by thiobencarb, butachlor and 2, 4-D.

Pastro and March (1999) evaluated the effects of acetochlor, imazethapyr, metachlor, pendimethalin, trifluralin and mixture acetochlor and imazethapyr on the production and viability of *S. rolfsii in vitro* and found trifluralin and pendimethalin were the most efficient compounds because they notably reduced the production of viable sclerotia.

Bhoraniya *et al*. (2002) reported the inhibition of sclerotial germination of *S. rolfsii* by different pesticides viz., metalachlor, fluchloralin, alachlor, pendimethalin, 2,4-D sodium salt, mancozeb, captan, copper oxychloride, tridemorph and carboxin through soil plate technique. The effectiveness of carboxin (98.99 per cent) followed by tridemorph (97.89 per cent) and fluchloralin (94.02 per cent). However, they were statistically at par (P=0.05), metalachlor was also found effective in inhibiting the sclerotial germination (62.78 per cent).

2.6 INTEGRATION OF FUNGICIDE AND BIOCONTROL FOR THE PLANT DISEASE MANAGEMENT

No single method can effectively control plant diseases, integration of two or more methods is advocated. Of the several practices, integration of chemical and biological means gained importance. However, it is necessary to assess the compatibility of potential antagonists before integrating with the biocontrol agent.

2.6.1 Compatibility of Potential Antagonists with Fungicides and Herbicides *in vitro*

Shanmugam and Varma (1999) reported that mancozeb had low rate of inhibition against *T. harzianum* and was compatible with the the fungus.

Metalachlor (50% EC) completely inhibited the growth of *T. viride* even at 100 ppm concentration under in vitro condition (Ramarethinam *et al*., 2001).

Vijayaraghavan and Abraham (2004) tested *in vitro* compatibility of *T. harzianum*, *T. viride* and *T. longibrachiatum* with several fungicides and found that mancozeb was compatible with all the three antagonists at 0.2, 0.3 and 0.4 per cent concentrations.

Gupta *et al.* (2005) reported that carbendazim was incompatible with *T. viride* TV2 isolate but with carboxin compatible for integrated treatment.

Combined application of fungicidal tolerant *Pseudomonas aeruginosa* GSE-18-R and thiram significantly improved the control of pre-emergence rotting of groundnut seeds in the *Aspergillus niger* infested potting mixture (Kishore *et al.,* 2005).

Kishore *et al.* (2005) reported that the *P. aeruginosa* isolates GSE-18 and GSE-19 antagonistic to *Phaeosiariopsis personata* causing late leaf spot of groundnut were tolerant to recommended field application rate of chlorathalonil.

Gampala and Pinnnamaneni (2010) reported that *T. viride* was more compatible with fertilizers and pesticides. Hence *T. viride* can be safely used with chemical fertilizers that give major nutrients for any crop.

Ranavay *et al.* (2011) studied efficacy of fungicides against *Trichoderma spp*. He selected different fungicides to study the tolerance by *T. viride* and stated that the copper oxychloride was least toxic followed by propineb to the growth and sporulation of *Trichoderma*, indicating that these fungicides may be applied safely as seed treatment along with bioagent *T. viride*. Carbendazim alone and in mixture proved highly toxic and not advocated for seed or soil application along with *T. viride*.

Gaikwad *et al.* (2011) studied the efficacy of fungicides against *T. viride* and found that *T. viride* was compatible with wettable sulphur and mancozeb at all concentrations and incompatible with fungicides carbendazim, carboxin (at all concentrations), captan (only at higher concentration) and aureofungin found to be partially inhibitory to *T. viride*.

Tapwal *et al.* (2012) studied compatibility of *T. viride* with different fungicides and reported that captan, copper oxychloride were recorded compatible to some extent with *T. viride* at all concentrations (50, 100, 200, 300 ppm).

Deepika *et al.* (2014) tested the effect of some commonly used fungicides, insecticides and herbicides on the mycelia growth of *T. harzianum.* Among fungicides, captan, thiram, chlorothalonil and copperhydroxide were found compatible with the test antagonist up to 100 µg a.i./ml, while mancozeb up to 250 µg a.i./ml, as these did not adversely affect the growth of test antagonist. Benomyl, thiophanate methyl, bayleton and ipridione were found incompatible with the test antagonist even at 25 µg a.i./ml. Among insecticides monocrotophos, dichlorvos, prophenophos and triazophos were found compatible up to 250 µl a.i./ml, while deltamethrin and quinalphos up to 100 µl a.i./ml were compatible.

Vasundara *et al.* (2015) checked the compatibility of insecticides, fungicides and their combinations on *T. viride* and the results showed that mancozeb (3000 ppm), imidacloprid (2000 ppm) and combination of mancozeb (3000 ppm) + imidacloprid (2000 ppm) showed high compatibility with *T. viride* by showing 7, 11 and 11 per cent growth inhibition respectively.

Louis *et al.* (2016) evaluated the compatibility of *P. fluorescens* with different fungicides commonly used in management of banana diseases and the results of the study proved that fungicides *viz*., propiconazole, hexaconazole, tebuconazole, difenconazole, azoxystrobin, carbendazim and famoxadone + cymoxanil compatible at all tested concentrations. Kresoxim

methyl was less compatible and mancozeb, copper oxy chloride and copper hydroxide are not compatible with *P. fluorescens*.

Compatibility of bioagents with fungicides reveled that all the three species viz. *T. viride, T. harzianum* and *T. hamatum* were compatible with the chemical azoxystobin (Vineela *et al*., 2017).

2.7 INTEGRATED DISEASE MANAGEMENT OF *S. rolfsii*

Asghari and Mayee (1991) reported that, application of *T. harzianum* inoculum and soil drenching with 0.2 per cent carbendazim reduced the stem rot of groundnut caused by 44-60 per cent and increased the pod yields by 17 to 47 per cent.

Muthamilan and Jeyarajan (1996) found in their glass house studies that integration of seed treatment with *T. harzianum*, Rhizobium + *T. harzianum* inoculum added to soil 6 days after sowing plus carbendizam (0.1%) soil drenching on 30 DAS was more effective in reducing root-rot of groundnut caused by *S. rolfsii*.

Rama Yalla Reddy (2002) found that integrated use of *T. viride, P. fluorescens*, neem cake and thiram for seed treatment of groundnut improved seed yield and controlled soil microflora *viz*., *A. niger, Alternaria spp, Curvularia sp, Fusarium spp, Penicillium sp, R. stolonifer, R. solani, S. rolfsii and Verticillium sp.*

Arunasri (2003) reported that seedling root dip in thiram 0.1 per cent + seedling root dip in *Trichoderma* suspension (T_1) + seedling root dip in *Pseudomonas* spp. (B_1) reduced the *S. rolfsii* incidence in crossandra to about 6.66 per cent compared to control (73.66%).

Toorray *et al.* (2007) reported that *Trichoderma* when integrated with captan, thiram and mancozeb resulted insignificant reduction in *S. rolfsii* collor rot in chickpea.

Jhonson and Reddy (2008) reported that integration of *P. fluorescence* and in combination with tryptophan and Farm Yard Manure (FYM), has reduced the stem rot incidence and the highest yields were recorded against *S. rolfsii.*

Hemalatha *et al.* (2009) tested a mancozeb tolerant native potential *Trichoderma* spp. and *P. fluorescens* for their ability to reduce root rot of sugar beet and found that combined application of both biocontrol agents and mancozeb @ 500 ppm was superior in reducing the disease incidence to an extent of 10 per cent as compared to control (81%).

Bindu Madhavi and Bhattiprolu (2011) found that integration of different treatments including seedling dip with carbendazim + mancozeb, addition of vermicompost, drenching with fungicide and application of *T. harzianum* (7%) were found to be effective in management of dry root rot of chilli caused by *S. rolfsii* in comparison with individual treatments.

Kuldhar and Suryawanshi (2017) observed highest seed germination (98.33%) with the treatment Thiram + Carbendazim + *T. harzianum* + *P. fluorescens* + NSC + *A. indica* extract. Significantly highest reduction in pre-emergence (97.61 %), post-emergence (95.77 %) and average (96.69 %) mortality were recorded with treatment of Thiram + Carbendazim + *T. harzianum* + *P. fluorescens* + NSC + *A. indica* extract.

Chapter ~ III

Material and Methods

Chapter – III

MATERIAL AND METHODS

The general laboratory techniques adopted in the dissertation are same as proposed by Dhingra and Sinclair (1995), Rangaswami and Mahadevan (1999), Nene and Thapliyal (1993) and Aneja (1993). Mention has been made of other special procedures / techniques used during the course of investigation(s).

3.1 LOCATION OF WORK

The present experiment(s) were carried out in the Department of Plant Pathology, Sri Venkateswara Agricultural College, Tirupati, Chittoor district, Andhra Pradesh.

3.2 GLASSWARE, CHEMICALS AND EQUIPMENT

3.2.1 Glassware

Glassware made of Corning or Borosil with standard measurement were used throughout the study. The glassware used were Petri plates (90 mm diameter), conical flasks (100, 250, 500 and 1000 ml), test tubes, measuring cylinders (10, 50 and 100 ml), beakers (150 and 250 ml), pipettes (0.1, 1.0, 2.0, 5.0 and 10 ml) etc.

3.2.2 Cleaning of Glassware

The glassware was first washed with a detergent followed by thorough cleaning with tap water before placing them in cleaning solution for 24 hours, rinsed 3-4 times with distilled water and air dried.

Cleaning solution

Potassium dichromate ($K_2Cr_2O_7$)	:	60 gm
Concentrated sulphuric acid (H_2SO_4)	:	60 ml
Distilled water	:	1000 ml

3.2.3 Pots

Plastic pots of size 22.5 cm × 19 cm (5 kg of soil capacity) containing sandy loam soil were used for raising groundnut seedlings in present studies.

3.2.4 Equipments

Compound microscope (10x, 40x magnifications) was used for observing the fungi. Hot air oven and autoclave were used for sterilization of glassware and media respectively. BOD Incubators were used for incubating test materials at 28 ± 1°C. The cultures were stored at 4°C in a refrigerator. Weighing was done on a single pan electronic balance with a sensitivity of 0.001 g. Other tools include camel brush, inoculation needle, pots etc.

3.3 CULTURE MEDIA

Test pathogen *S. rolfsii* and antagonist *T. viride* cultures were maintained on Potato Dextrose Agar, (PDA). For the maintenance of bacterial culture, Nutrient Agar (NA) and Nutrient broth (NB) media were used. Cultures were temporarily stored at 4°C in refrigerator.

Composition of medium:

a. Potato Dextrose Agar (PDA)

Peeled potato slices	:	200 g
Dextrose	:	20 g
Agar- agar	:	20 g
Distilled water	:	1000 ml
pH	:	6.5

Peeled and sliced potato tubers (200 g) were boiled in 500 ml of distilled water. Potato extract was filtered with muslin cloth into another container and mixed with 20 g of dextrose. Twenty grams of agar-agar was melted separately in 500 ml of distilled water and mixed with potato dextrose broth. Final volume of the medium was adjusted to one liter by adding distilled water pH of the medium was adjusted to 6.5 by using either 1N HCL or NaOH.

b. Potato Dextrose Broth

Peeled potato	:	200 g
Dextrose	:	20 g
Distilled water	:	1000 ml
pH	:	7.0

Potato extract was prepared by boiling 200 g of peeled potato pieces in 500 ml of distilled water. To which 500 ml dextrose solution was added. Final volume was made up to 1000 ml. The pH was adjusted to 6.5.

c. Nutrient Agar medium (NA)

Peptone	:	5 g
Beef extract	:	3 g
Agar- agar	:	17 g
Distilled water	:	1000 ml
pH	:	7.0

Required quantity of all the ingredients were added to 1000 ml of distilled water in a beaker and then boiled on water bath till agar gets melted. pH was adjusted to 7.0.

d. Nutrient Broth (NB)

Peptone	:	5 g
Beef extract	:	3 g
Distilled water	:	1000 ml
pH	:	7.0

Five grams of peptone and 3 g of beef extract was added to 1000 ml of distilled water in a beaker and then boiled the content on water bath. pH was adjusted to 7.0.

3.4 STERILIZATION

Glassware used for present investigation were kept in sterilization tins or wrapped in aluminum foil and were sterilized in hot air oven at 160°C for 90 minutes. Surface of laminar air flow chamber was sterilized by wiping with cotton swab dipped in alcohol. Inoculation loop and cork borer was sterilized by dipping in alcohol and heating to red hot. The culture media and distilled water were sterilized in an autoclave at 15 psi. for 15 minutes.

3.5 CROP, VARIETY, PATHOGEN AND ANTAGONISTS

Stem rot susceptible variety of groundnut Narayani was used in present study. The test pathogen *S. rolfsii* was isolated from the stem rot diseased groundnut plants, tested for pathogenicity, pure cultured by single hyphal tip method and used for the present study, Fungal and bacterial antagonists i.e *T. viride* and *P. fluorescens* for my current research was collected from the department of plant pathology of S. V. Agricultural college, Tirupati.

3.5.1 Identification of the Pathogen

The pathogen was identified based on its mycelial and sclerotial characters described by Barnett and Hunter, (1972).

3.6 PROVING PATHOGENICITY TEST OF THE PATHOGEN, *S. rolfsii*

Pathogenicity test was conducted using groundnut cultivar Narayani. Eight plants were raised on sterilized soil in earthen pots and Koch's postulates were proved by adopting soil infestation method.

3.6.1 Mass Multiplication of *S. rolfsii*

The test pathogen *S. rolfsii* was multiplied on sorghum grains. Sorghum grains were pre-soaked in 2 per cent sucrose solution overnight, boiled in fresh water for 30 minutes and drained. They were transferred into 500 ml flasks @ 200 g and autoclaved for 15 lb p.s.i (121^{o}C) for 20 minutes. The flasks were allowed to cool at room temperature and inoculated with 5 mm discs of 3 to 4 day old culture of *S. rolfsii* grown on PDA. Five discs per flask were added and the flasks were incubated for two weeks at 28 ± 2^{o}C.

3.6.2 Soil Inoculation Method

Inoculum grown on sorghum grains was added to the top 6-7 cm layer of sterilized soil in 9 inch diameter earthen pots at the rate of 30 g kg^{-1} soil and mixed thoroughly and moistened with water. The pots were incubated for 2 days by covering with polythene sheets. Pots without pathogen inoculum were maintained as control. Seeds of groundnut cultivar Narayani were sown in pots at the rate of 8 seeds per pot. Pots were observed regularly for the germination of seeds and appearance of symptoms on plants.

3.7 EVALUATION OF INOCULUM DENSITY OF *S. rolfsii* ON DISEASE SEVERITY

Sterilized soil of one kg was filled in pots. Later mass multiplied *S. rolfsii* on sorghum grains was mixed with soil @ 10 g (1% inoculum), 20 g (2% inoculum), 30 g (3% inoculum), 40 g (4% inoculum) and 50 g (5% inoculum) kg^{-1} soil. Inoculums was mixed in top 2" depth of soil. Water was sprinkled to maintain moisture. The seeds of groundnut were sown after two days of pathogen inoculation @ 8 seeds per pot and an uninoculated control was maintained. The pots were observed for seed germination and per cent seed germination was recorded. After 15 days of sowing root length, shoot length, Per cent Disease Incidence (PDI) and Per cent Disease Severity were recorded. Each treatment was replicated three times.

$$\text{Per cent germination} = \frac{\text{Number of seeds germinated in each treatment}}{\text{Total number of seeds planted}} \times 100$$

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of seeds germinated}} \times 100$$

3.8 INFLUENCE OF SOIL TEMPERATURE, pH AND MOISTURE ON THE VIABILITY OF SCLEROTIA OF *S. rolfsii*

3.8.1 Soil temperature

Sandy loam soil of pH 6 was collected, dried and autoclaved for 15 lb p.s.i (121^{o}C) for 20 minutes. Dried, sterilized soil of 50 g is filled in 250 ml plastic cups and in each cup 10 sclerotia were added. Cups were placed in water bath set at 5 different temperatures i.e., 20^{o}C, 25^{o}C, 30^{o}C, 35^{o}C, 40^{o}C. Soil temperatures were regularly checked by using soil thermometer. After 10 days the sclerotia were retrieved and placed on PDA for testing their viability. Three replications were maintained. Number of sclerotial germinated were recorded and germination percentage was calculated using the following formula,

Per cent germination of sclerotia =

$$\frac{\text{Number of sclerotia germinated}}{\text{Total number of sclerotia added in cup}} \times 100$$

3.8.2 Soil H-ion Concentration (pH)

Soils with seven different pH i.e, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 was collected from different fields having desirable pH levels, then the soil was sterilized in an autoclave and later dried soil of 50 g with seven different pH are added in seven different 250 ml cups. In each cup 10 sclerotia were mixed with the soil. Cups are maintained at room temperature. After 10 days the sclerotia were retrieved and placed on PDA for testing their viability. Three replications are maintained. Number of sclerotia germinated were recorded and germination percentage was calculated using the formula given in 3.8.1.

3.8.3 Soil Moisture

Sandy loam soil of pH 6 was collected, dried and autoclaved for 15 lb p.s.i (121^{o}C) for 20 minutes. Fifty grams of soil was filled in 250 ml cups and in each cup 10 sclerotia were added. Four soil moisture regimes i.e. 40, 60, 80 and 100 per cent were maintained at constant temperature of 35^{o}C. The Soil moisture content was determined using the gravimetric method on an oven-dry basis. The method includes saturation of soil sample followed by removal of available soil moisture by oven drying (100°C – 110°C) until the weight remains constant. After removing from oven, samples were cooled slowly to room temperature and weighed again. The difference in weight was amount of moisture in the soil. The available SMC in the soil was calculated by the following formula:

Soil moisture content (%) =

$$\frac{\text{Saturated soil weight - Oven dry soil weight}}{\text{Oven dry soil weight}} \times 100$$

The four levels of SMC (40%, 60%, 80% and 100%) was adjusted by maintaining the constant weight by regular weighing and replacing the moisture deficit in each cup by watering. Deionized water was used for maintaining the soil moisture content (SMC) in each treatment. After 10 days the sclerotia were retrieved and placed on PDA for testing their viability. Three replications are maintained. Number of sclerotia germinated were recorded and germination percentage was calculated using the following given in 3.8.1.

3.9 EVALUATION OF EFFICACY OF BIOAGENTS, FUNGICIDES AND HERBICIDES AGAINST MYCELIAL GROWTH OF *S. rolfsii*

3.9.1 *Trichoderma viride* Isolates

Three isolates of *T. viride* available at Plant Pathology lab, S.V Agricultural College, Tirupati were evaluated *in vitro* against *S. rolfsii* by dual culture method (Dennis and Webster, 1971). Twenty ml of sterilized PDA was poured in to Petri plate of 9 cm diameter aseptically. Mycelial discs measuring 6 mm diameter from four day old cultures of both fungal antagonist and the test pathogen were inoculated 7 cm apart (leaving 1 cm from periphery). Four replications were maintained for each treatment. Radial growth of the mycelium was recorded and per cent inhibition over control was calculated. The data were analysed statistically using Completely Randomized Design (CRD).

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

where,

I = Per cent reduction in growth of test pathogen.

C = Radial growth (mm) in monocultured check.

T = Radial growth (mm) in dual cultured plates.

3.9.2 *Pseudomonas fluorescens* Isolates

Three isolates of *P. fluorescens* available at Plant Pathology lab, SV Agricultural College, Tirupati were evaluated *in vitro* against *S. rolfsii* by dual culture method (Dennis and Webster, 1971). *S. rolfsii* was inoculated at the center of PDA plate. Test bacterial cultures were streaked individually on both the sides of the *S. rolfsii* at 2.5 cm distance leaving 2.0 cm periphery. Plates inoculated with *S. rolfsii* alone were maintained as checks. Inoculated plates were incubated at 28 ± 2°C. Four replications were maintained for each treatment. Observations were recorded as zone of inhibition up to four days when *S. rolfsii* completely occupied the plate in monoculture check. Per cent inhibition of mycelia growth over control was calculated using the formula given in 3.9.1. The data were analyzed statistically using CRD design.

3.9.3 Fungicides and Herbicides

*In vitro e*fficacy of ten fungicides and three herbicides were evaluated against the *S. ralfsii,* incitant of stem rot of groundnut. The details of fungicides and herbicides, their recommended dosage and the concentrations tested in the present study were given in Table 3.1.

Poisoned food technique was employed to evaluate the efficacy of these chemicals. Stock solution of 30,000 mg a.i. ml^{-1} was prepared from each fungicide. From the stock solution required quantity was pipetted out and added to required quantity of PDA medium in conical flasks to obtain final test concentration. Twenty ml of fungicidal/ herbicidal stock solution was amended to the medium in each pre-sterilized Petri plate. Small disc (0.5cm diameter) was cut with sterilized cork borer from 15 day old culture of *S. rolfsii* under aseptic conditions and placed at the centre of each poisoned plate. The experiment was laid out in CRD with four replications per treatment. Control was maintained by placing fungal discs in plates containing untreated (non poisoned) medium. The inoculated Petri plates were incubated at 28 ± 2°C in BOD incubator.

Table 3.1. Details of fungicides and herbicides employed in the study

Chemical	Active ingredient	Recommended dose (%)	Concentration tested (ppm)
FUNGICIDE			
Carbendazim	50 WP	0.2	500,1000,1500,2000
Pyraclostrobin	20 EG	0.2	500,1000,1500,2000
Azaxystrobin	23 SC	0.2	250,500,750,1000
Hexaconazole	5 EC	0.2	500,1000,1500,2000
Tebuconazole	25.9 EC	0.1	500,1000,1500,2000
Validamycin	3L	0.1	250,500,750,1000
Carboxin	Carboxin 37.5 + Thiram 37.5 SD	0.2	500,1000,1500,2000
Clorothalonil	75 WP	0.3	1000,1500,2000,2500
Mancozeb	75 WP	0.3	1000,1500,2000,2500
Copper oxychloride	50 WP	0.25	1000,1500,2000,2500
HERBICIDE			
Pendimethalin	30 EC		1000, 500, 250, 100
Imazethapyr	10 SL		1000, 500, 250, 100
Quizalofop ethyl	5 EC		1000, 500, 250, 100

The data on radial growth of the fungal mycelium was recorded 4 days after inoculation. Per cent inhibition of mycelial growth of test pathogen over control was calculated by using the formula mentioned above. The data were analysed statistically using CRD.

3.10 EVALUATION OF EFFICACY OF BIOAGENTS, FUNGICIDES AND HERBICIDES ON SCLEROTIAL GERMINATION OF *S. rolfsii*

3.10.1 *T. viride* (Tr-DM)

Dried and sterilized red loamy soil is taken into plastic cups and ten sclerotia of *S. rolfsii* was mixed with the soil. This is a unit representing a replication of a treatment. The culture suspension of effective *T. viride* isolate was used for the study. Spore suspension was prepared with different spore concentrations of 1×10^4, 1×10^5, 1×10^6, 1×10^7 and 1×10^8 and 20 ml of spore suspension was added to the plastic cup containing sclerotia. The soil in the cups was moistened up to saturation and incubated for 10 d. After 10 days, sclerotia were retrieved and placed on PDA medium in Petri plates and incubated at 28 ± 2°C. Three replications were maintained. Control was maintained with distilled water. Number of sclerotia germinated was recorded and per cent inhibition of sclerotial germination was recorded using the formula,

Per cent inhbition =

$$\frac{\text{Total number of sclerotia - number of germinated sclerotia}}{\text{Total number of sclerotia}} \times 100$$

3.10.2 *P. fluorescens* (Pf-DM)

Dried and sterilized red loamy soil was used in this experiment. 10 g of soil was taken into plastic cups and ten sclerotia of the pathogen were mixed with the soil. This is a unit representing a replication of a treatment. The bacterial suspension of *P. fluorescens* from 10^{-4} to 10^{-8} CFU/ml was added to the plastic cup containing sclerotia, moistened upto saturation and incubated for 10 days. After 10 days the sclerotia were retrieved and placed on PDA medium in Petri plates and incubated at 28 ± 2°C for testing their viability. Three replications were maintained. Control was maintained with distilled water. Number of sclerotia germinated was recorded and per cent inhibition of sclerotial germination was recorded using the formula given in 3.10.1.

3.10.3 Fungicides and Herbicides

Dry soil of red loamy soil was used in this experiment. 10 g of soil was taken into plastic cups and ten sclerotia of *S. rolfsii* were mixed with the soil. This is a unit representing a replication of a treatment. All the fungicides and herbicides presented in the Table 3.1 was tested at recommended dose. The required dose of fungicide and herbicide solution were prepared and added to the plastic cup containing sclerotia. The soil in the cups was moistened upto saturation and incubated for 10 days at 28 ± 2°C. Control was maintained with distilled water. After 10 days the sclerotia were retrieved and placed on PDA medium and incubated at 28 ± 2°C for testing their viability. Number of sclerotia germinated was recorded and per cent inhibition of sclerotial germination was recorded using the formula given in 3.10.1.

3.11 TESTING THE COMPATIBILITY OF POTENTIAL BIOCONTROL AGENT WITH EFFECTIVE FUNGICIDES, HERBICIDE AND THEIR COMBINATIONS *In Vitro*

Potential biocontrol agent identified from the experimentation was tested for compatibility with the effective fungicides, herbicides and their combinations. *T. viride* isolate (Tr-DM) was tested for its compatibility by poisoned food technique (Nene and Thapliyal, 1993). The selected effective fungicide and herbicide were tested at a concentration of general field recommended dose using CRD design.

For each treatment, 30 ml of double strength potato dextrose agar (PDA) medium was taken in 100 ml conical flask and autoclaved. Specified chemical concentration was dissolved in 30 ml distilled water was added to the medium at lukewarm temperature and mixed thoroughly. The poisoned medium was equally distributed in three Petri plates, which were treated as three replications and allowed to solidify. The test pathogen *T. viride*

(Tr-DM) was cut into 5mm discs from the periphery of actively growing colony with sterilized cork borer and transferred to the centre of each plate containing poisoned medium. Control was maintained by placing *T. viride* discs in plates containing untreated (non poisoned) medium. The inoculated Petri plates were incubated at 28 ± 2°C in BOD incubator. The radial growth of bioagent in the treatments was measured when growth in the control plate attained maximum and per cent inhibition of mycelia growth was calculated using the formula,

$$I = \frac{C - T}{C} X100$$

where,

I = Per cent reduction in growth of pathogen

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment.

3.12 INTEGRATED MANAGEMENT OF STEM ROT OF GROUNDNUT

Experiment was conducted in plastic pots of size 22.5 cm × 19 cm in the green house of the Plant Pathology department of S V Agricultural College, Tirupati. Narayani variety was used for the present investigation. In each pot, red loamy soil mixed with FYM in the ratio of 1:2 was used. Mass multiplied *S. rolfsii* added to each pot @ 10 gm kg^{-1} soil except in T_8 (control). Four replications were maintained for each treatment. The experiment was conducted with the following treatments,

Treatments:

Table 3.2. List of treatments employed for pot culture study

T_1	Soil drenching with effective herbicide
T_2	Soil drenching effective fungicide
T_3	Soil application with effective bioagent
T_4	$T_1 + T_2$
T_5	$T_2 + T_3$
T_6	$T_1 + T_2 + T_3$
T_7	Sick soil
T_8	Control

Eight groundnut seeds (Narayani variety) were sown in each pot and observations are recorded at 30 DAS. Observations were recorded on per cent germination of seeds at 10 days after sowing, disease incidence/plant mortality, root length, shoot length, dry weight of the plants. Dry weight of the plant was determined by oven drying of fresh plant samples (90^0 C for 48 hrs) and later weighed by using electric balance. Untreated seeds were sown in pathogen uninoculated pots to compare germination percentage and disease incidence. Per cent mortality and per cent germination was calculated using the formula,

$$\text{Mortality (\%)} = \frac{\text{Total number of plants died}}{\text{Total number of seeds sown}} \times 100$$

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

The data was analysed statistically using C.R.D. and the results were presented.

Chapter ~ IV

Results & Discussion

Chapter – IV

RESULTS AND DISCUSSION

Sclerotium rolfsii is an important soil borne pathogen and incitant of stem rot of groundnut causing huge economic losses directly to farming communities, indirectly to our country. In view of economic importance of this pathogen, present investigation on "**Studies on the *Sclerotium rolfsii* Sacc. incitant of stem rot of groundnut (*Arachis hypogaea* L.)"** was carried out in the P.G. laboratory at Department of Plant Pathology, S.V. Agricultural College, Tirupati, during 2017-18.

4.1 ISOLATION AND PURE CULTURE OF *S. rolfsii*

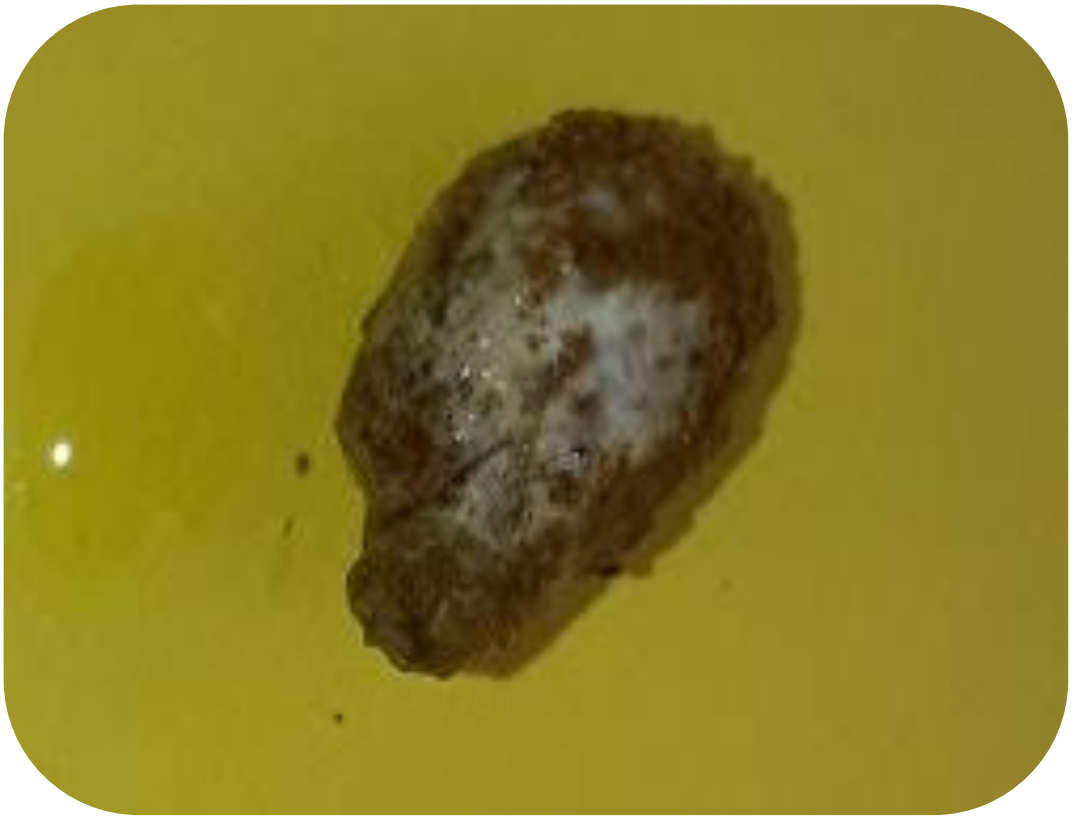
The causal organism was isolated from the infected portion of the stem on potato dextrose agar (PDA). Based on the cultural and morphological characters the fungus was identified as *S. rolfsii* and further purified through sub culturing on PDA by single hyphal tip method at the department of plant pathology, S.V. Agricultural college, Tirupati.

4.2 PATHOGENICITY TEST

Before conducting the experiment, the isolate collected from the stem rot affected fields of Sri Venkateswara Agricultural College, Tirupati is subjected to pathogenicity test on groundnut by soil infestation method in glass house of department of Plant Pathology. Soil was sterilized in an autoclave at 15 lbps, 121°C for 15 min and two sets of pots were maintained one with pathogen inoculated soil and the other with healthy soil in earthen pots. Healthy groundnut seeds were sown in both treatments. Groundnut seed germination was very poor in pathogen inoculated soil compared to control where cent per cent germination was recorded. After 16 DAS, ungerminated seeds from pathogen inoculated soil, surface sterilized in 0.1 per cent sodium hypochlorite solution followed by rinsing thrice with

Healthy uninoculated soil

Inoculated (Sick) soil



Ungerminated groundnut seed sown in sick plot with mycelial growth of *S. rolfsii*

Plate 4.1. Groundnut seed germination and growth of plants affected due to *S. rolfsii* after 15 days of sowing

Plate 4.2a. ***S. rolfsii*** **from ungerminated groundnut seed plated on PDA after 3 days**

Plate 4.2b. ***S. rolfsii*** **from ungerminated groundnut seed plated on PDA after 30 days**

distilled water and were inoculated on PDA medium. After four days growth of white pluffy mycelium was observed (Plate 4.2a). After 30 days brown, small, round sclerotia were formed in the Petri plate (Plate 4.2b). This was similar to the original pathogen confirming pathogenicity test.

4.3 EFFECT OF DIFFERENT INOCULUM DENSITIES OF *S. rolfsii* ON SEED GERMINATION, SEEDLING GROWTH PARAMETERS AND DISEASE INCIDENCE

Studies on the effect of inoculum densities of *S. rolfsii* on the incidence of stem rot in groundnut revealed that the germination percentage of groundnut seeds was highest at one per cent inoculum density (45.83%) i.e., 52.17 per cent reduction over control. The least germination was recorded at 5 per cent inoculum density (16.66%), i.e., 86.21 per cent decrease over control. The results are presented in the Table 4.1. Germination of groundnut seeds gradually decreased with increase in inoculum densities from 1 to 5 per cent and was significantly different from each other. The germination percentages recorded at different inoculum densities were in the following order.

Control > 1% > 2% > 3% > 4% > 5%.

The highest root length was recorded at one percent inoculum density (12.37 cm) and the least at 5 percent inoculum density (7.50 cm). Root length of groundnut plant gradually decreased with increase of inoculum densities from 1 to 5 per cent (Plate 4.4). The root length at 1 per cent inoculum density was reduced to 17.69 per cent compared to control (15.03 cm) and the difference was significant. The difference in root length was significantly different from each other at 1 and 2 per cent inoculum density. The root length decreased with increase in the inoculums density however, the reduction in the root length was not significant at different densities from 2 per cent to 5 per cent. The root length recorded at different inoculums densities were in the following order.

Control > 1% > 2% > 3% > 4% > 5%

Shoot length was highest at one percent inoculum density (15.16 cm) i.e., 21.85 per cent reduction over control (19.40 cm). Least shoot length was recorded at 5 percent inoculum density (8.33 cm) which is 57.61 per cent reduction over control. Shoot length of groundnut plant gradually decreased with increase of inoculum densities from 1 to 5 per cent (Plate 4.4). The reduction in shoot length observed at one percent inoculums density is significant compared to control however, the difference in shoot length recorded from 1 per cent to 4 per cent inoculum densities was not significant. The reduction in the shoot length was significant between 4 per cent to 5 per cent inoculum densities. The shoot length recorded at different inoculums densities were in the following order.

Control > 1% > 2% > 3% > 4% > 5%

From the above experiment, it is clear that even at one percent inoculum density the germination percentage of groundnut seeds was affected by more than 50 per cent. Hence throughout the experimentation one percent inoculums was applied and also sick soil was maintained by adding one percent inoculum.

Disease symptoms were not observed in any of the treatments at 14 days after sowing. However development of sclerotial bodies were observed at 4 and 5 per cent inoculum (Plate 4.5). Mc Clain and Scharpf (1989) reported a positive correlation between seedling mortality and inoculum density in coniferous species, whereas plant height and weight of the surviving seedlings were negatively correlated with sclerotial density of *Macrophomina phaseolina*. Azhar *et al.* (2006) reported that seedling mortality increased with increase in inoculum levels in collar rot of chickpea. Harlapur (1988) noticed maximum infection (100%) in inoculum level of more than four per cent in foot rot disease of wheat.

Table 4.1. Effect of different inoculum densities of *S. rolfsii on* seed germination, plant growth parameters and stem rot disease incidence in groundnut

Inoculum density (%)	Germination (%)	Per cent inhibition over control	Root length (cm)	Per cent inhibition over control	Shoot length (cm)	Per cent inhibition over control	Symptoms observed
1	45.83 (42.57**)	52.17	12.37	17.69	15.16	21.85	No symptoms
2	37.50 (37.57)	60.86	10.73	28.60	14.20	26.80	No symptoms
3	33.33 (35.16)	65.21	9.77	34.99	13.33	31.28	No symptoms
4	29.17 (32.57)	69.56	8.33	44.57	11.33	41.59	Sclerotia were observed at the base of stem
5	16.66 (23.79)	82.61	7.50	50.09	8.33	57.61	Blackening of stem portion and formation of sclerotia at blackened potion
Control	95.83 (83.08)		15.03		19.40		
C.D.	12.41		1.24		2.33		
SE(m)	3.98		0.39		0.75		
SE(d)	5.63		0.56		1.06		
C.V.	16.25		6.48		9.54		

** Figures in parentheses are angular transformed values

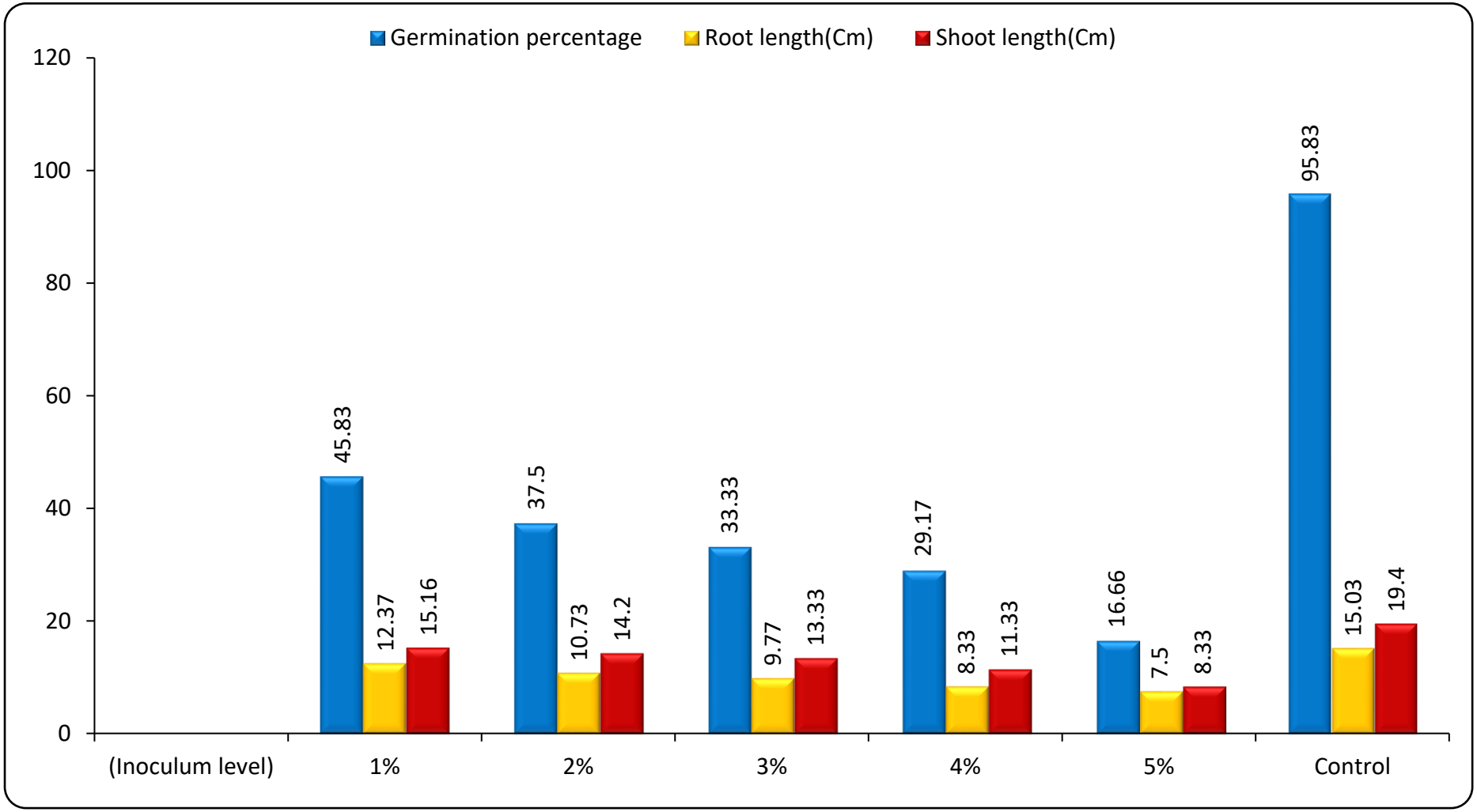


Fig. 4.1. Effect of different inoculum densities of *S. rolfsii* on seed germination, plant growth parameters of groundnut

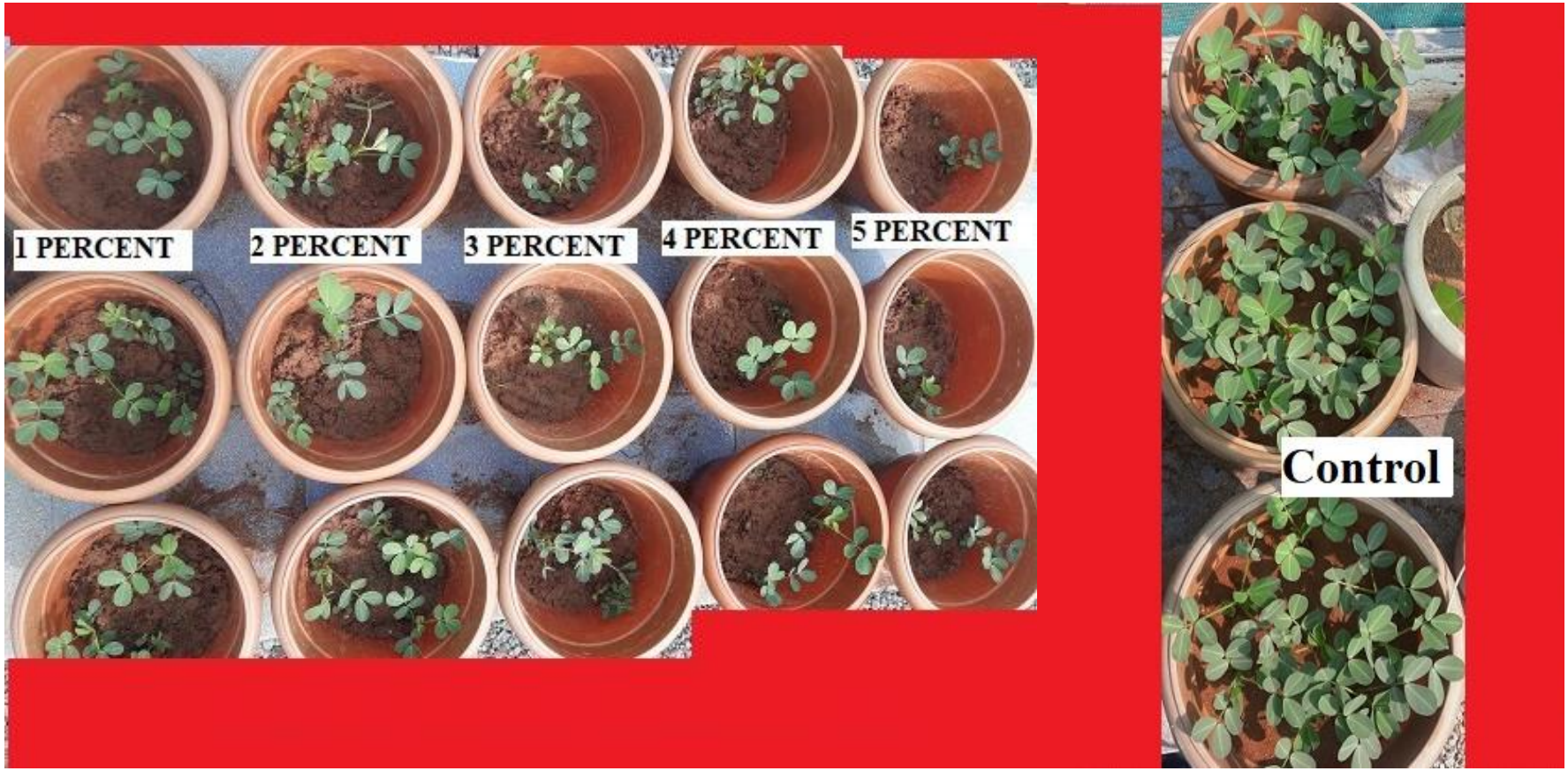


Plate 4.3. Effect of different inoculum densities of *S. rolfsii* on seed germination and growth of groundnut plants

Plate 4.4. Differences in root and shoot length of groundnut plants at different inoculum densities.

4% inoculum **5% inoculum**

Plate 4.5. Sclerotial development at 4 and 5 per cent inoculum density

4.4 INFLUENCE OF SOIL TEMPERATURE, pH AND MOISTURE ON THE VIABILITY OF SCLEROTIA OF *S. rolfsii*

4.4.1 Soil Temperature

Study on the influence of different soil temperatures on the viability of sclerotia revealed that the germination of sclerotia was not affected at 30°C (100%) and at par with control i.e., at room temperature (27°C). Germination of sclerotia was highly affected (76.66%) i.e., 23.34 per cent reduction over control at lowest temperature (20°C) of study. Least reduction in germination of sclerotia (3.34%) was observed at 35°C followed by 25°C (10.0%) (Plate 4.6) and at par with each other. The results are presented in the Table 4.2 and Fig. 4.2.

Effect of temperature on sclerotial germination percentage was recorded in the following order:

Control = 30°C > 35°C > 25°C > 40°C > 20°C.

Remandi *et al.* (2010) conducted experiment to study the effect of temperature against *S. rolfsii in vitro* and they found that the optimum growth of mycelium at 30-35°C. Gour and Sharma (2010) reported that maximum growth of *S. rolfsii* occurred at 30°C followed by 35°C and the lowest mycelial growth at 15°C. Zape and Gade (2013) found that *S. rolfsii* showed rapid mycelial growth at 30°C and maximum sclerotial production at 25°C. Satyabrata Maiti and Chitreshwar Sen (1988) reported that higher temperatures (25-40°C) reduced the survivability of sclerotia of *S. rolfsii* with rising temperature for prolonged period of time.

4.4.2 Soil H-ion Concentration (pH)

The study of different soil pH levels on the viability of sclerotia revealed that germination of sclerotia was not affected (100%) at pH 7.5 (100%) and at par with control i.e., pH 7.2 of the native soil. This clearly

Table 4.2. Influence of soil temperature on the viability of sclerotia of *S. rolfsii.*

Temperature (ºC)	Sclerotia inoculated (No)	Sclerotia germinated (No)	Sclerotial germination (%)	Per cent inhibition over control
20	30	23	76.66 (61.19**)	23.34
25	30	27	90.00 (71.53)	10.00
30	30	30	100.00 (90.00)	0.00
35	30	29	96.66 (83.84)	3.34
40	30	25	83.33 (66.11)	16.67
Control (27°C=Room temperature)	30	30	100.00 (90.00)	
C.D.	–	0.75	9.00	
SE(m)	–	0.25	2.89	
SE(d)	–	0.36	4.08	
C.V.	–	4.77	6.49	

** Figures in parentheses are angular transformed values

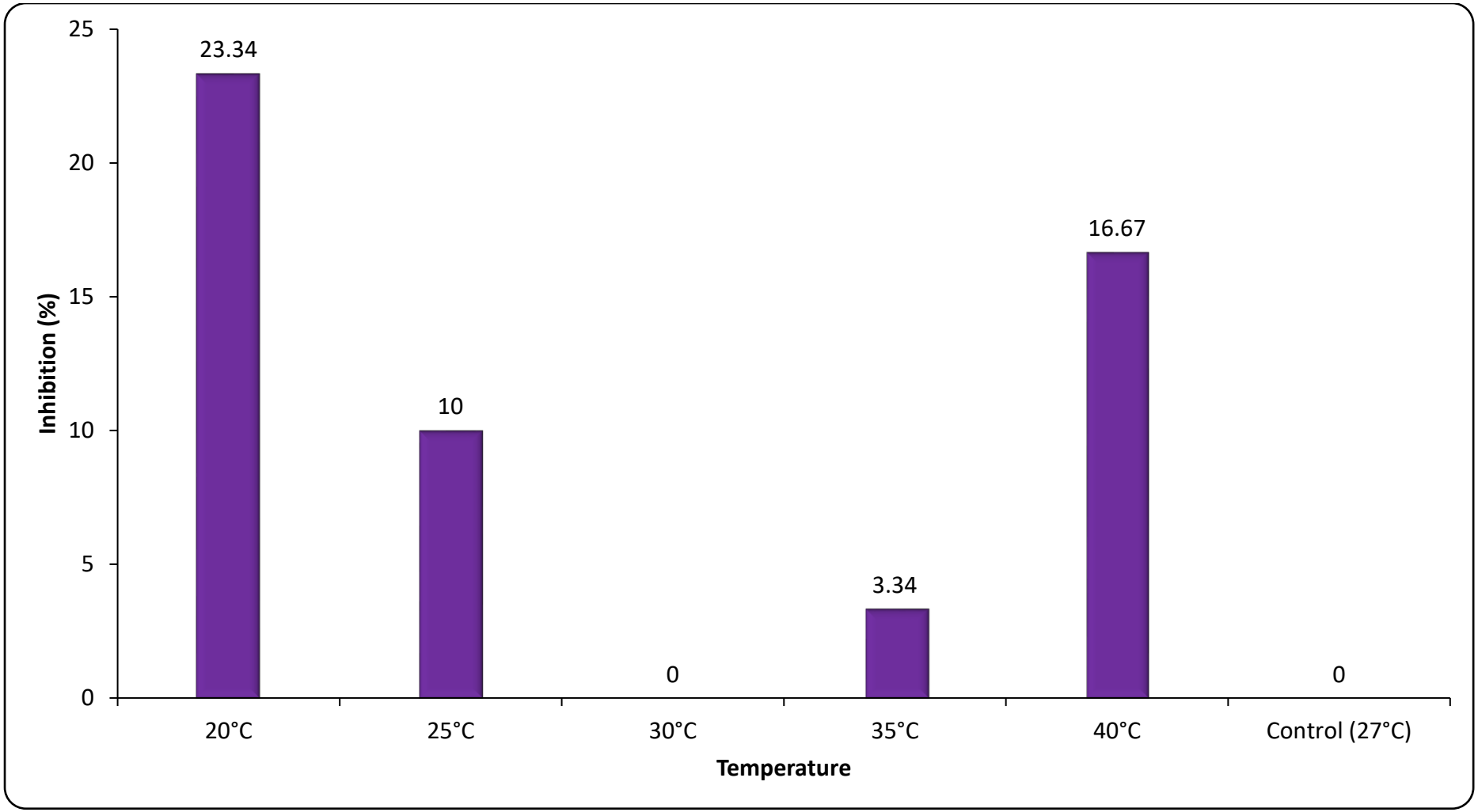


Fig. 4.2. Influence of soil temperature on the viability of sclerotia of *S. rolfsii* after 10 days

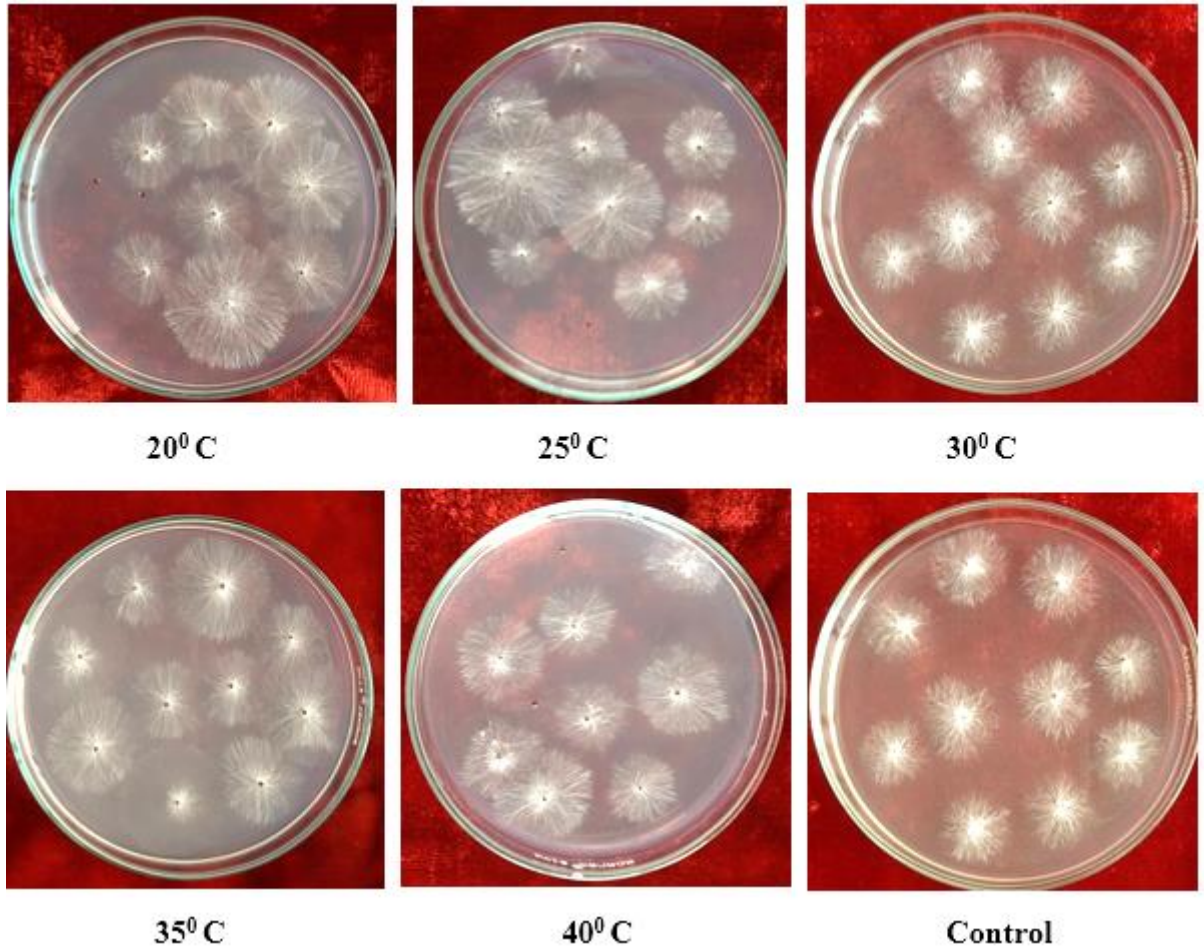


Plate 4.6. Influence of soil temperature on the viability of sclerotia of *S. rolfsii* after 10 days

indicates that slightly basic pH is highly congenial for the survival of the sclerotia. Slight reduction in germination percentage (3.34%) was observed at neutral pH· and the difference is not significant. Reduction in the survival of sclerotia was observed with reduction in the pH i.e., 90.00 per cent at 6.5 and 86.60 per cent at 6.0 and the difference between them is 3.40 per cent. Highest reduction in germination percentage of sclerotia (23.34%) was recorded at pH 9.0 and the difference in sclerotial germination at 8.5 and 9.0 pH is non-significant (Plate 4.7). The results are presented in the Table 4.3 and Fig 4.3.

Effect of pH on sclerotial germination was observed in the following order:

Control (pH 7.2) = pH 7.5 = 6.5 > 7.0 > 8.0 > 6.0 > 8.5 > 9.0.

Shridha Chaurasia (2013) observed 5.0 as optimum pH for mycelial growth, while pH 4.0 to 7.0 were found to be most favourable for the production of sclerotia of *S. rolfsii.* Gour and Sharma (2010) reported that *S. rolfsii* grew on a wide range of pH from 4 to 9 but the maximum growth of the fungus was recorded on the medium having pH value as 6.0 and lowest mycelial growth was obtained at pH 9.0 and pH 8.0. The present study is in agreement with the earlier studies.

4.4.3 Soil moisture

Maximum sclerotial germination percentage (100%) was observed at 40 per cent soil moisture level and was at par with control (50% moisture). Increase in the soil moisture resulted in reduction of sclerotial germination percentage was observed. Highest inhibition of sclerotial germination (23.33%) was observed at 100 per cent soil moisture and is significantly different with all the soil moisture levels (Plate 4.8). The reduction in sclerotial germination at 40, 60 and 80 per cent soil moisture levels is non significant compared to control. The results are presented in the Table 4.4 and Fig. 4.4.

Table 4.3. Influence of soil pH on the viability of sclerotia of *S. rolfsii*

H-ion concentration (pH)	Sclerotia inoculated (No)	Sclerotia germinated (No)	Sclerotial germination (%)	Per cent inhibition over control
6.0	30	26	86.60 (68.82**)	13.40
6.5	30	27	90.00 (71.53)	10.00
7.0	30	29	96.66 (83.84)	3.34
7.5	30	30	100.00 (90.00)	0.00
8.0	30	27	90.00 (71.53)	10.00
8.5	30	25	83.33 (66.11)	16.67
9.0	30	23	76.66 (61.19)	23.34
Control (Native soil =7.2)	30	30	100.00 (90.00)	
C.D.	–	0.65	8.10	
SE(m)	–	0.22	2.68	
SE(d)	–	0.33	3.79	
C.V.	–	4.14	5.97	

** Figures in parentheses are angular transformed values

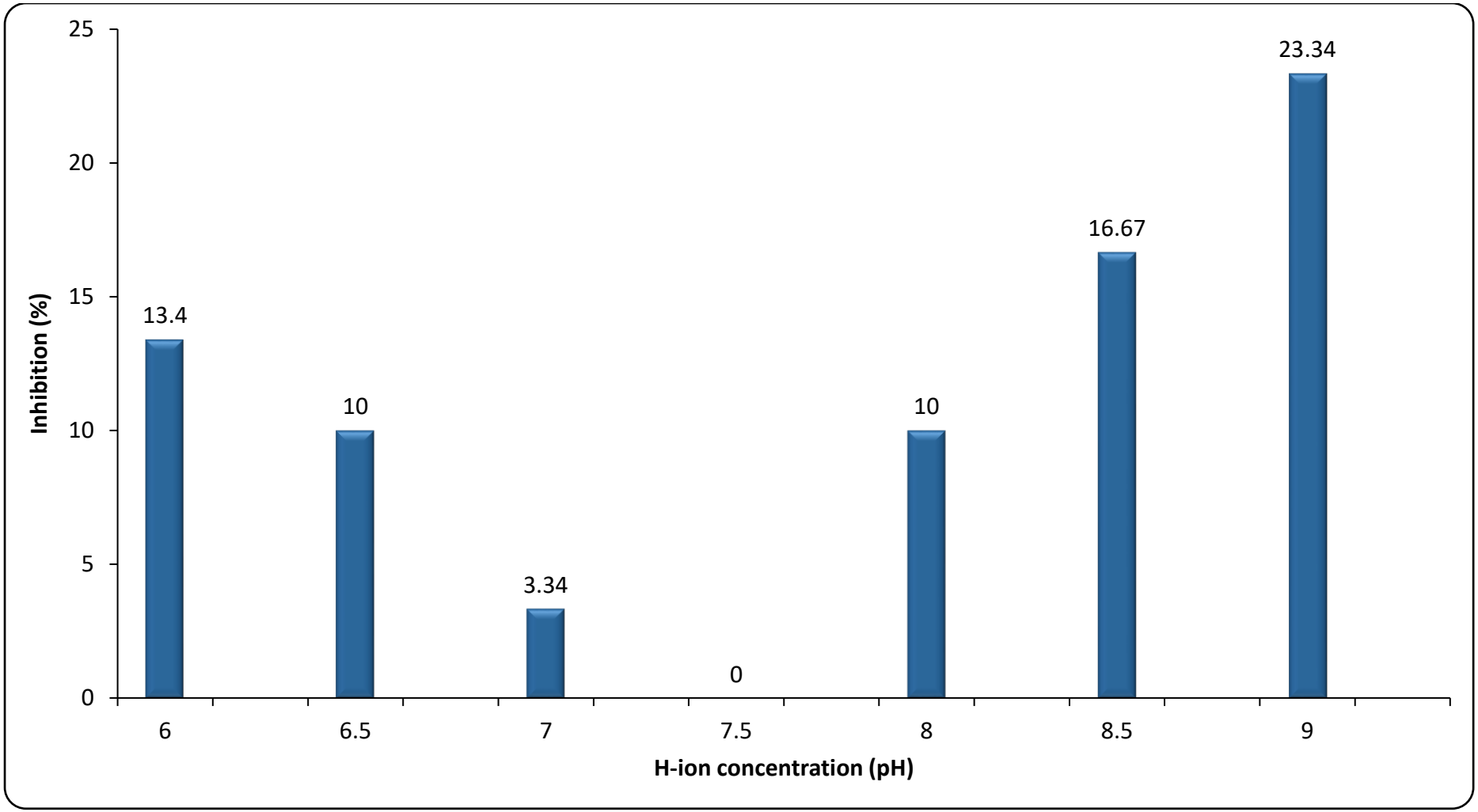
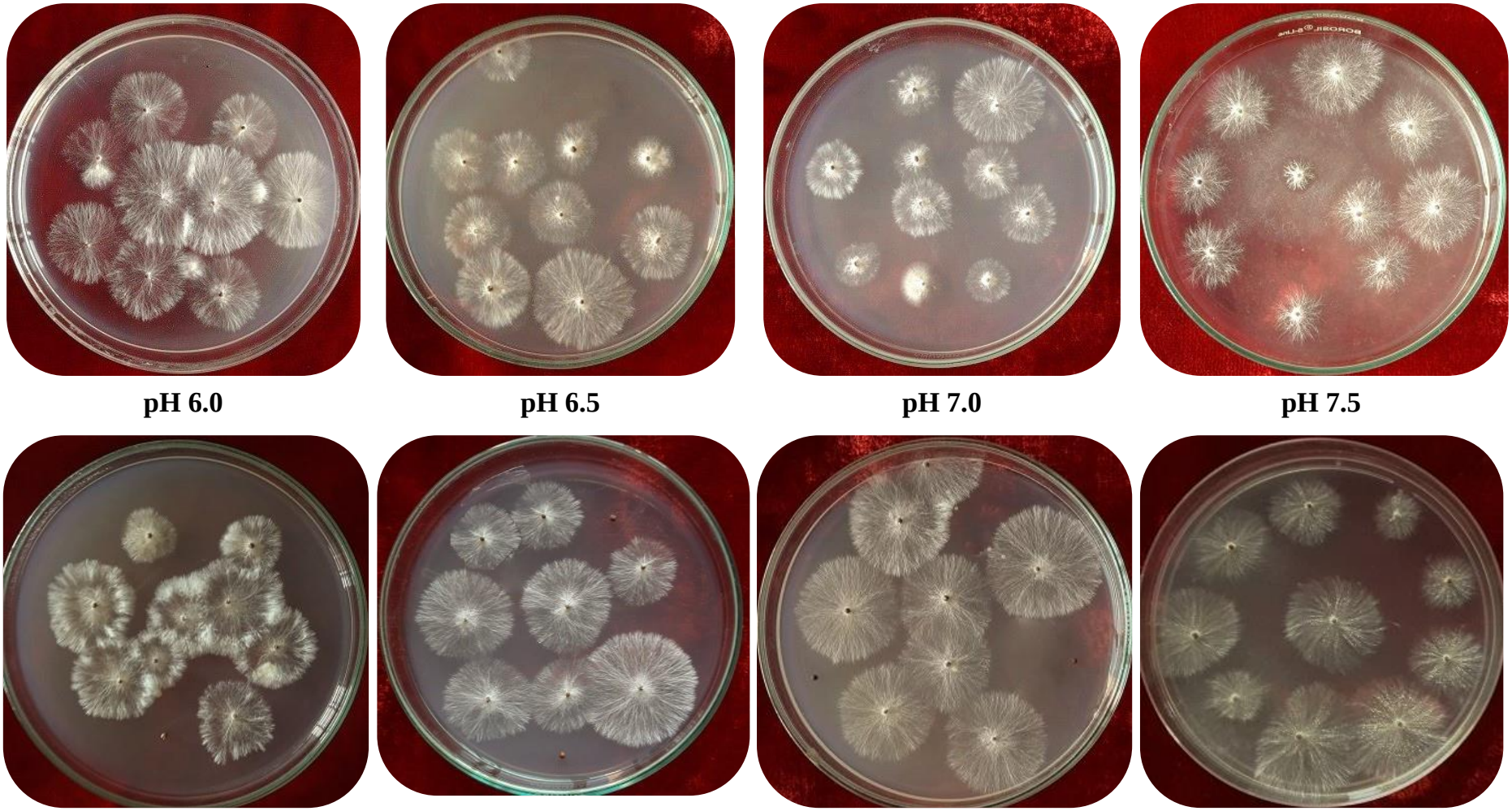


Fig. 4.3. Influence of soil pH on the viability of sclerotia of *S. rolfsii* after 10 days



pH 6.0 **pH 6.5** **pH 7.0** **pH 7.5**

pH 8.0 **pH 8.5** **pH 9.0** **Control**

Plate 4.7. Influence of soil pH on the viability of sclerotia of *S. rolfsii* after 10 days

Effect of soil moisture on sclerotial germination was observed in the following order:

Control = 40% > 60% > 80% > 100%.

Sclerotia of *S. rolfsii* survived well at moisture contents up to 75 per cent WHC but at 100 per cent the none were recovered after 60 days and the contents of the sclerotia were found to lyse without germination leaving hollow rinds (Satyabrata Maiti and Chitreshwar Sen, 1988). Katti *et al*. (1983) reported that, the survival of *S. rolfsii* was highest at soil moisture levels between 30 and 50 per cent. Palakshappa (1986) reported that *S. rolfsii* causing foot rot of betelvine survived better at low soil moisture i.e., between 20 and 40 per cent than at higher levels (>40%). The present study also indicated that low soil moisture is suitable for sclerotial germination and also increase in moisture per cent reduced sclerotial germination.

4.5 EFFICACY OF DIFFERENT BIOAGENTS, FUNGICIDES AND HERBICIDES ON MYCELIAL GROWTH OF *S. rolfsii.*

4.5.1 Bioagents

Effect of different isolates of *Trichoderma viride* and *Pseudomonas fluorescens* were tested on the mycelial growth of *S.rolfsii*, incitant of stem rot of groundnut and the results were presented in Table 4.5.

Significant differences were observed among the three isolates of *T. viride* in inhibiting the mycelial growth of *S. rolfsii.* Maximum per cent inhibition (87.19%) of mycelial growth was observed in Tr-DM isolate followed by Tr-SNG isolate (79.13%). Tr-RGP isolate proved to be least affective (75.93%) among the three isolates (Plate 4.9).

Among the three isolates of *P. fluorescens*, maximum per cent inhibition (28.25%) of mycelial growth of *S. rolfsii* was observed with Pf-DM isolate followed by Pf-SNG isolate (9.40%). Pf-RGP isolate did not show any affect in inhibiting the mycelial growth of *S. rolfsii* (Plate 4.10).

Table 4.4. Influence of soil moisture on the viability of sclerotia of *S. rolfsii*

Moisture (%)	Sclerotia inoculated (No)	Sclerotia germinated (No.)	Sclerotial germination (%)	Per cent inhibition over control
40	30	30	100.00 (90.00**)	0.00
60	30	28	93.33 (77.69)	6.67
80	30	27	90.00 (71.53)	10.00
100	30	23	76.67 (61.19)	23.33
Control (50% moisture)	30	30	100.00 (90.00)	
C.D.	–	0.45	9.33	
SE(m)	–	0.15	2.92	
SE(d)	–	0.29	4.13	
C.V.	–	2.83	6.48	

** Figures in parentheses are angular transformed values

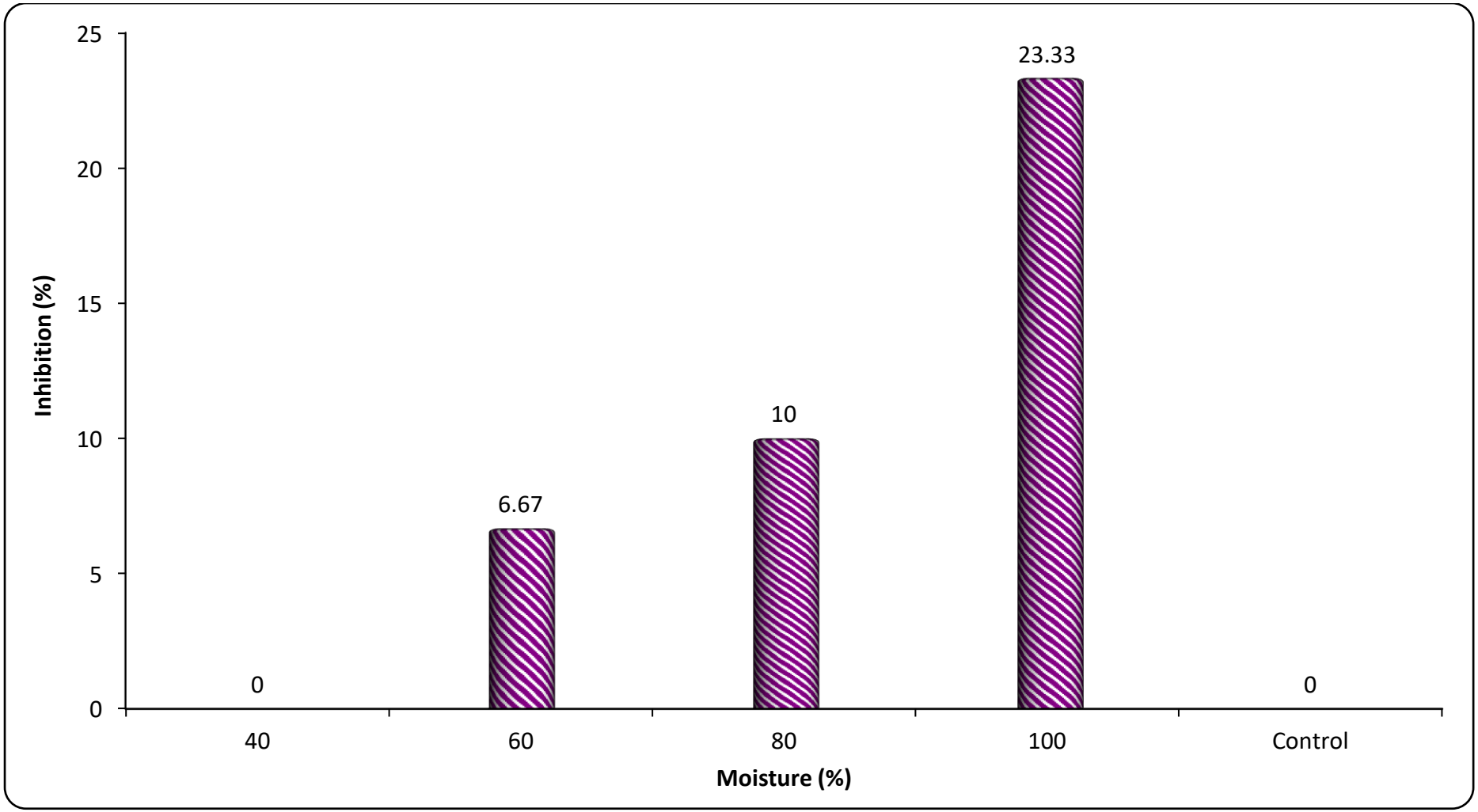


Fig. 4.4. Influence of soil moisture on the viability of sclerotia of *S. rolfsii* after 10 days

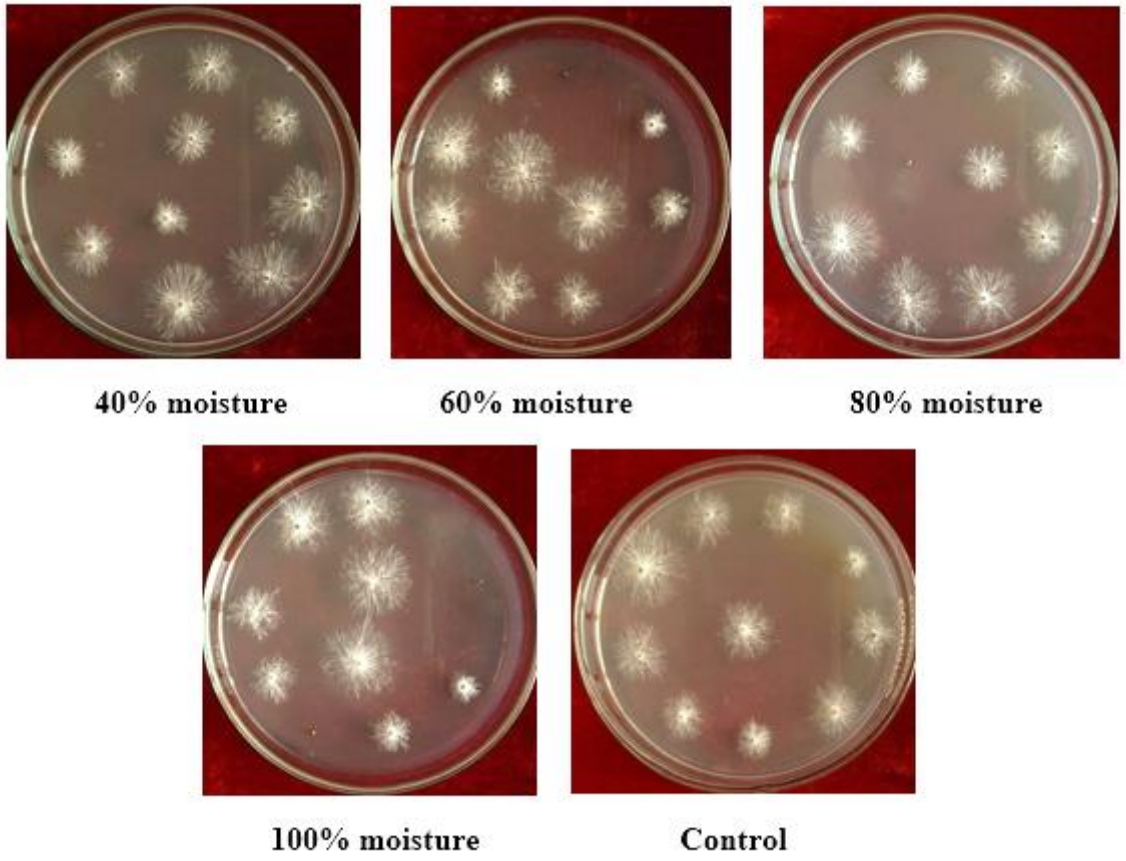


Plate 4.8. Influence of soil moisture on the viability of sclerotia of *S. rolfsii* after 10 days

Among the three *T. viride* fungal bio agents and three bacterial *P. fluorescens* bio agents, fungal bio agents proved to be significantly effective in reducing mycelial growth of *S. rolfsii* compared to bacterial bio agents.

Antagonistic activity of *T. harzianum* and *T. viride* against *S. rolfsii* was found to be effective in inhibiting both the mycelial growth and reducing production of sclerotial bodies (Virupaksha *et al.*, 1997 and Rajya Lakshmi 2002). Salvi *et al.* (2017) screened three bio-agents *in vitro* namely *T. viride, T. harzianum* and *P. fluorescens* and found that *T. viride* showed maximum growth inhibition (83.33%) against *S. rolfsii.* Ganesan and Gnanamanickam (1987) and Wokocha *et al.* (1986) reported that, the native strain of *P. fluorescens* restricted the growth of *S. rolfsii* causing stem rot of groundnut. Chanutsa *et al.* (2014) reported 100 per cent inhibition in growth of *S. rolfsii* with culture filtrate of *P. fluorescens.* The present study is in agreement with the earlier studies in this regard.

4.5.2 Fungicides

Mycelial growth of *S. rolfsii* varied in its sensitivity to the fungicides tested. It was most sensitive to systemic fungicides tebuconazole, azoxystrobin, carboxin, hexaconazole and non systemic fungicide mancozeb and showed 100 per cent inhibition at all the concentrations tested. The fungus was least sensitive to the systemic fungicide carbendazim at all the concentrations tested and the difference in the mycelial growth was significant. Copper oxychloride was least effective in inhibiting the mycelial growth (35.7 mm) at 100 ppm and the sensitivity has increased with increase in the concentration i.e., 20.73 per cent inhibition at 1000 ppm and 48.10 per cent inhibition growth at 2500 ppm. Another non systemic fungicide chlorothalonil was highly effective (100%) in inhibiting the mycelia growth at concentrations of 1500 ppm and above, while the inhibition was 54.76 per cent at 1000 ppm. The efficacy of validamycin is high (80.70%) in

Table 4.5. Efficacy of different isolates of *T. viride* and *P. fluorescens* on mycelial growth of *S. rolfsii* after 4 days incubation.

Bioagent	Mycelial growth (cm)	Inhibition (%)
T. viride		
Tr-SNG	1.57	79.13 (62.83**)
Tr-DM	1.02	87.18 (69.01)
Tr-RGP	1.92	75.93 (60.60)
P. fluorescens		
Pf-SNG	4.07	9.40 (17.75)
Pf-DM	3.22	28.25 (32.09)
Pf-RGP	4.50	0.00 (0.00)
Control	4.50	
C.D.		1.84
SE(m)		0.62
SE(d)		0.88
C.V.		3.59

** Figures in parentheses are angular transformed values

Tr-DM : Damaramadugu isolate

Tr-SNG : Sangam isolate

Tr-RGP : Rangampet isolate

Pf-DM : Damaramadugu isolate

Pf-SNG : Sangam isolate

Pf-RGP : Rangampet isolate

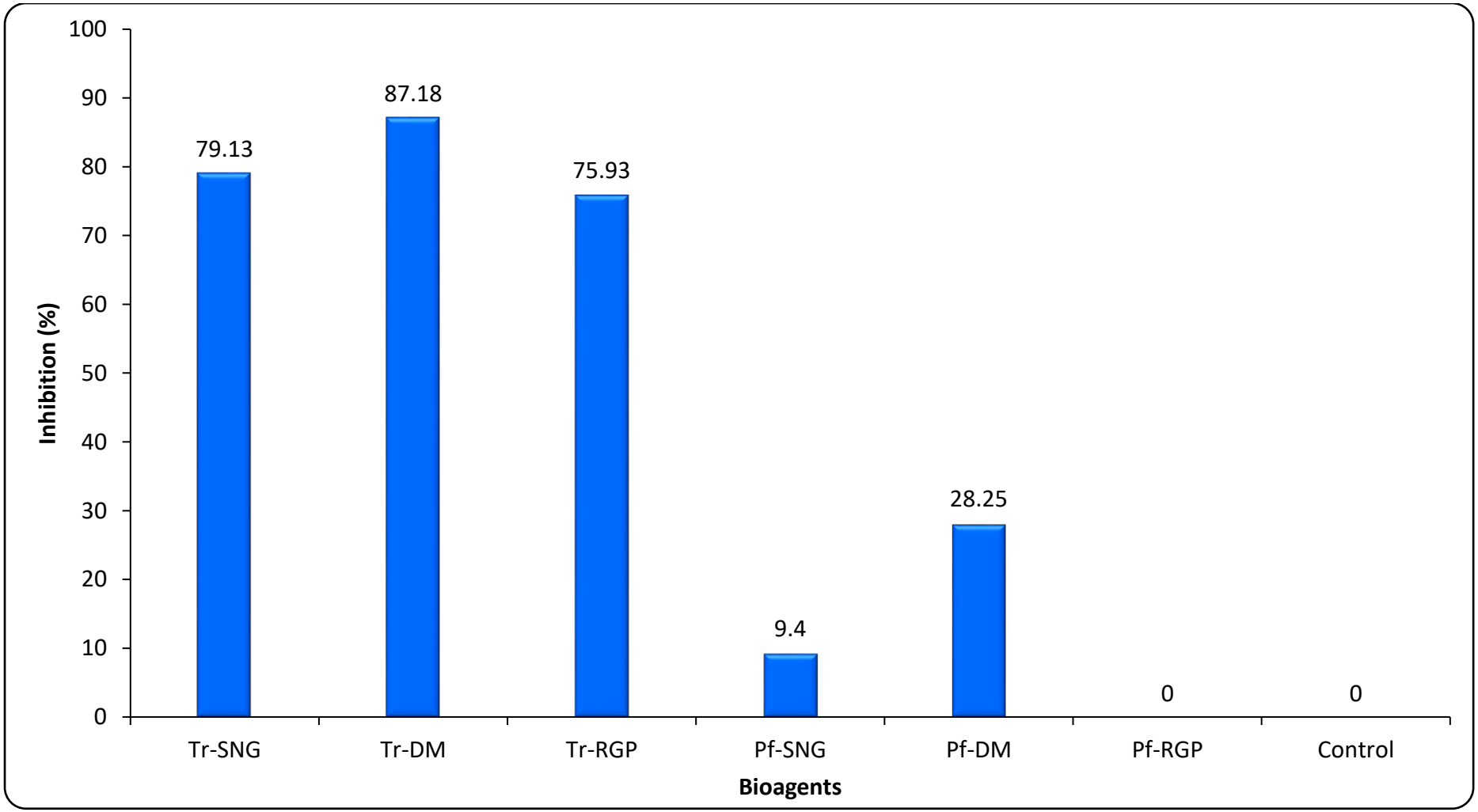


Fig. 4.5. Efficacy of different isolates of *T. viride* and *P. fluorescens* on mycelial growth of *S. rolfsii* after 4 days incubation

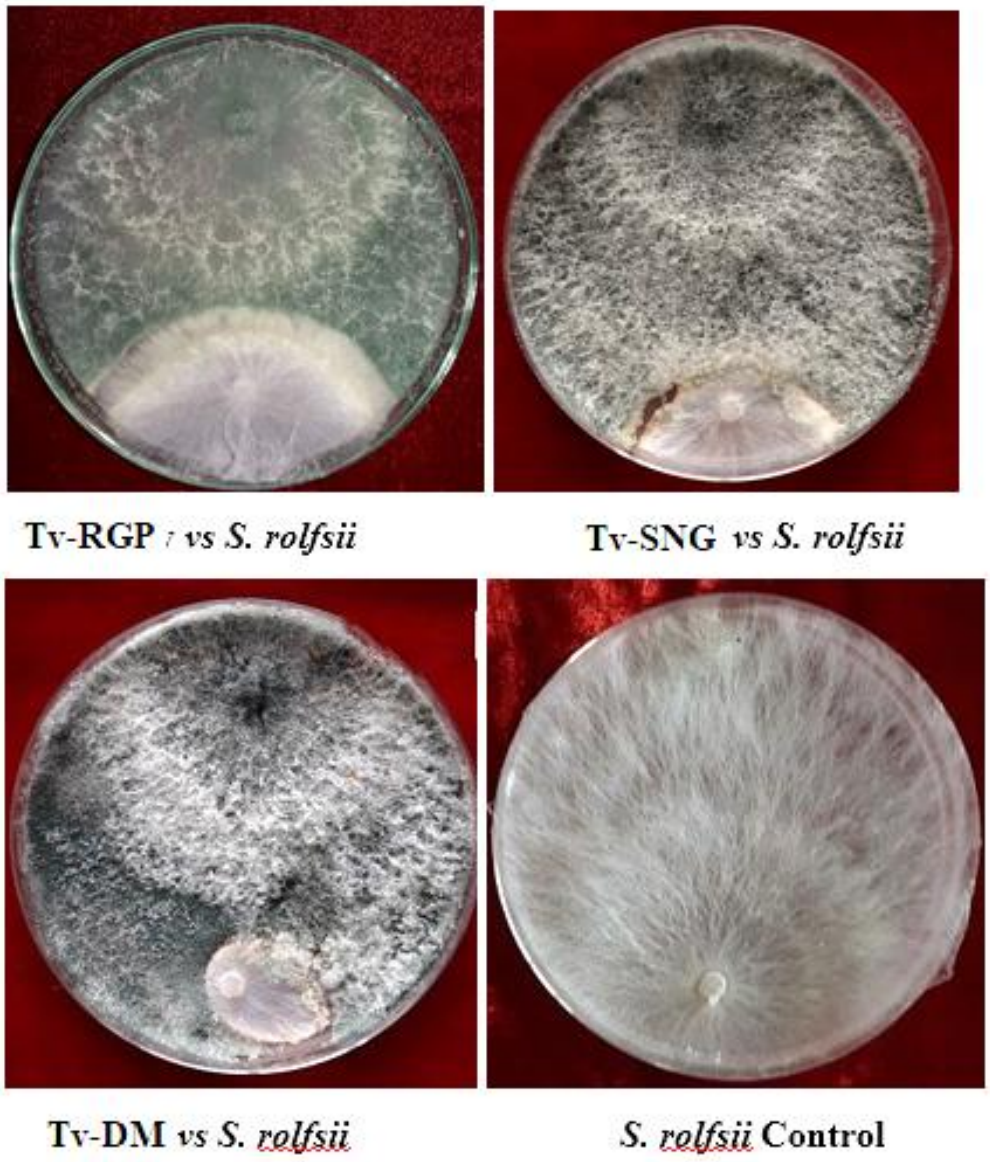
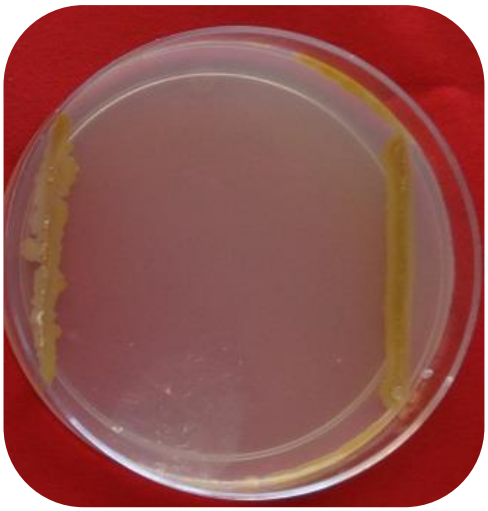


Plate 4.9. Efficacy of different isolates of *T. viride* on mycelial growth of *S. rolfsii* after 4 days incubation

***S. rolfsii* control**

Pf-DM *vs S. rolfsii*



Pf-DM control

Plate 4.10. Efficacy of effective isolate of *P. fluorescens* on mycelial growth of *S. rolfsii* after 4 days incubation

inhibiting the mycelial growth at 1000 ppm and the sensitivity decreased with decrease in concentration and the differences are significant (Plate 4.11 and Plate 4.12). Among all the fungicides tested, carbendazim proved to be less effective in inhibiting the mycelial growth of *S. rolfsii* followed by copper oxychloride and validamycin. The results were presented in the Table 4.6 and Fig. 4.6, 4.7a, 4.7b.

The efficacy of fungicides in inhibiting the mycelia growth is as follows:

Tebuconazole = azoxystrobin = carboxin = mancozeb = hexaconazole > chlorothalonil > pyraclostrobin > validamycin > copper oxychloride > carbendazim.

Yaqub and Shahzad (2006) reported that at lower concentration, fungicides were not effective in inhibiting the growth of *S. rolfsii* but at higher concentration mancozeb significantly reduced the growth. The efficacy of hexaconazole and propiconazole in cent per cent inhibition of mycelial growth of *S. rolfsii* was reported by Jhonson *et al.* (2008) and Rakholiya (2010). Prabhu and Hiremath (2003) and Arunasri *et al.* (2011) reported that the triazoles viz., hexaconazole, propiconazole, difenconazole were highly inhibitory to the growth of *S. rolfsii.* Perez *et al*. (2009) evaluated different fungicides against *S. rolfsii* and the results showed that tebuconazole inhibited mycelial growth and sclerotia production. Similar results were observed in the present studies where triazoles, strobilurins and carboxin were effective in reducing mycelial growth under *in vitro* studies.

4.5.3 Herbicides

Among the herbicides tested, *S. rolfsii* was highly sensitive to quizalofop ethyl compared to others, showed 100 per cent inhibition at 1000 ppm of quizalofop ethyl and the sensitivity decreased with decrease in concentration. Least sensitivity was observed with imazethapyr at 100 ppm

Table 4.6. Effect of fungicides on the mycelial growth of *S. rolfsii* after 4 days of incubation.

Fungicide	Trade name	Concentration (ppm)	Mycelial growth (cm)	Per cent inhibition over control (%)
Copperoxychloride (50% WP)	**Dhanucop**	**1000**	3.57	20.73 (27.06**)
		1500	3.10	31.10 (33.88)
		2000	2.77	38.47 (38.31)
		2500	2.33	48.10 (43.89)
Mancozeb (75% WP)	**Dithane M-45**	**1000**	0.00	100.00 (90.00)
		1500	0.00	100.00 (90.00)
		2000	0.00	100.00 (90.00)
		2500	0.00	100.00 (90.00)
Chlorothalonil (75% WP)	**Daconil**	**1000**	2.03	54.76 (47.71)
		1500	0.00	100.00 (90.00)
		2000	0.00	100.00 (90.00)
		2500	0.00	100.00 (90.00)
Hexaconazole (5% EC)	**Trigger**	**500**	0.00	100.00 (90.00)
		1000	0.00	100.00 (90.00)
		1500	0.00	100.00 (90.00)
		2000	0.00	100.00 (90.00)
Carboxin (Carboxin 37.5% + Thiram 37.5%)	**Vitavax**	**500**	0.00	100.00 (90.00)
		1000	0.00	100.00 (90.00)
		1500	0.00	100.00 (90.00)
		2000	0.00	100.00 (90.00)

Cont…

Table 4.6. Cont…

Fungicide	Trade name	Concentration (ppm)	Mycelial growth (cm)	Per cent inhibition over control (%)
Tebuconazole (25.9% EC)	**Torque**	**500**	0.00	100.00 (90.00)
		1000	0.00	100.00 (90.00)
		1500	0.00	100.00 (90.00)
		2000	0.00	100.00 (90.00)
Pyraclostrobin (20% EG)	**Insignia**	**500**	1.43	68.07 (55.57)
		1000	1.03	76.97 (61.30)
		1500	0.47	89.57 (71.15)
		2000	0.00	100.00 (71.15)
Carbendazim (50 WP)	**Dhanustin**	**500**	4.43	1.46 (5.68)
		1000	4.13	8.10 (16.40)
		1500	3.97	11.83 (20.09)
		2000	3.87	14.03 (21.93)
Azoxystrobin (23% SC)	**Onestar**	**250**	0.00	100.00 (90.00)
		500	0.00	100.00 (90.00)
		750	0.00	100.00 (90.00)
		1000	0.00	100.00 (90.00)
Validamycin (3% L)	**Sheathmar**	**250**	1.97	56.27 (48.58)
		500	1.73	61.47 (51.61)
		750	1.30	71.03 (57.45)
		1000	0.86	80.70 (63.94)
Control			4.50	0.00 (0.00)
			C.D.	1.967
			SE(m)	0.698
			SE(d)	0.987
			C.V.	1.755

** Figures in parentheses are angular transformed values

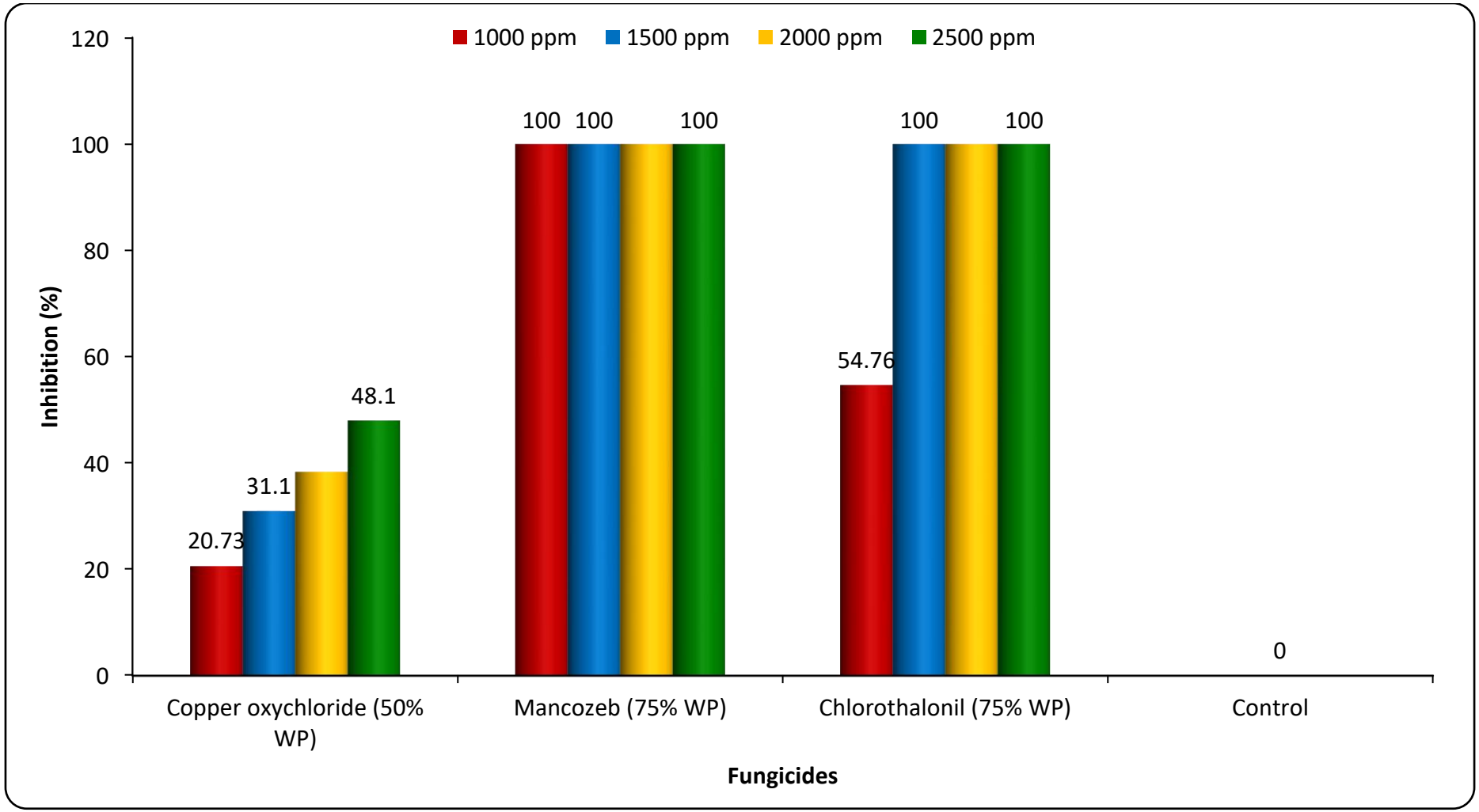


Fig. 4.6. Per cent inhibition of *S. rolfsii* by different non systemic fungicides

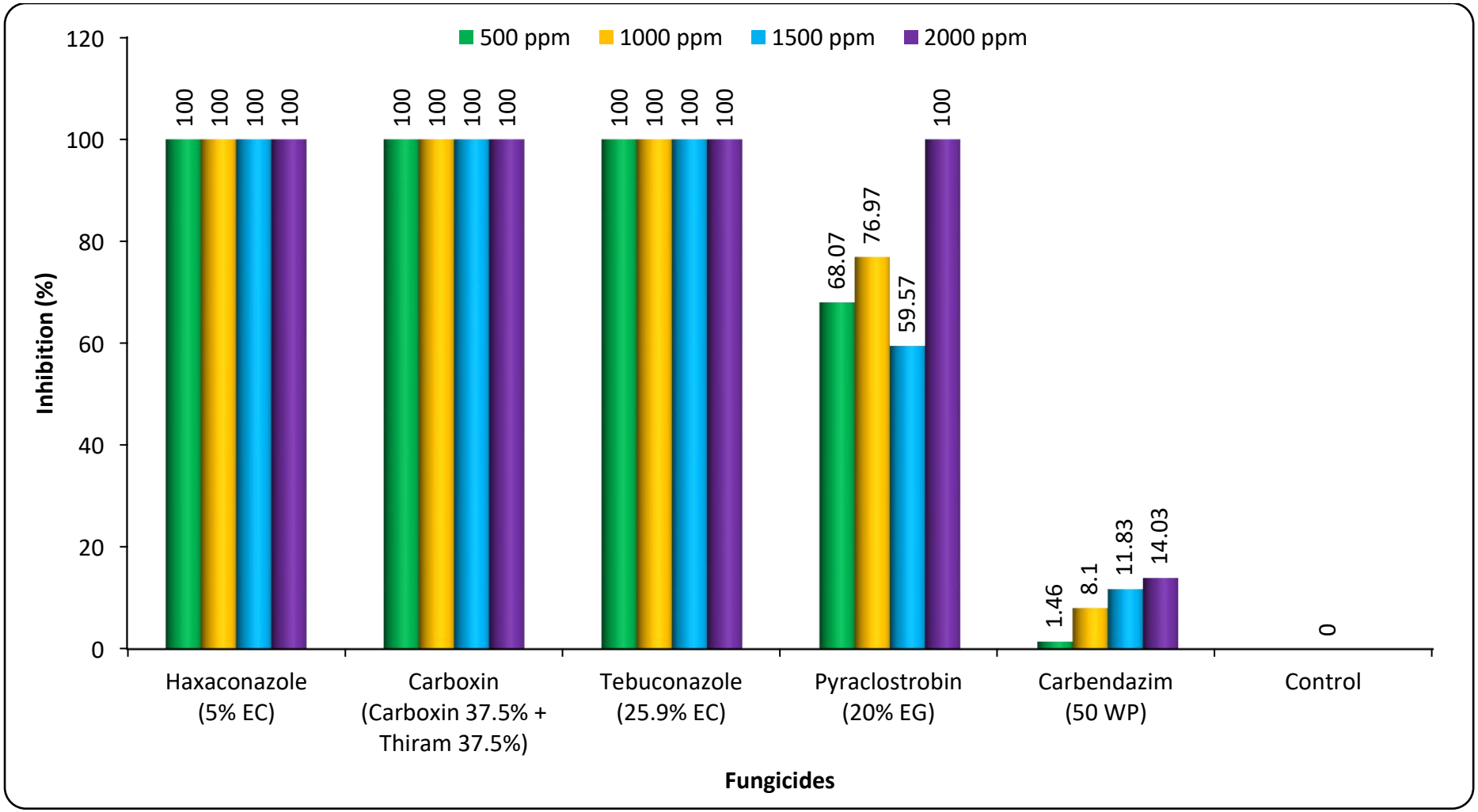


Fig. 4.7a. Per cent inhibition of *S. rolfsii* by different systemic fungicides

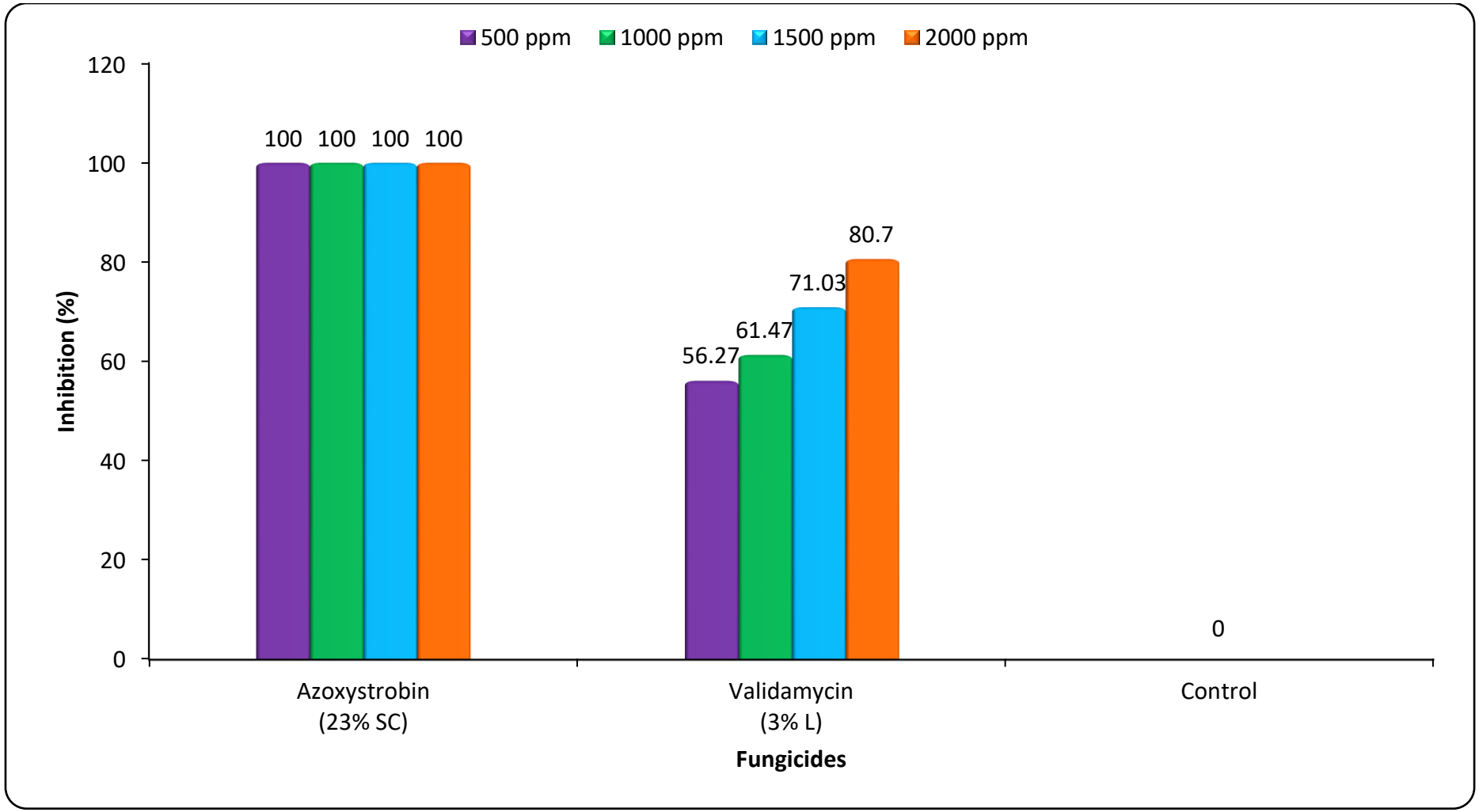


Fig. 4.7b. Per cent inhibition of *S. rolfsii* by different systemic fungicides

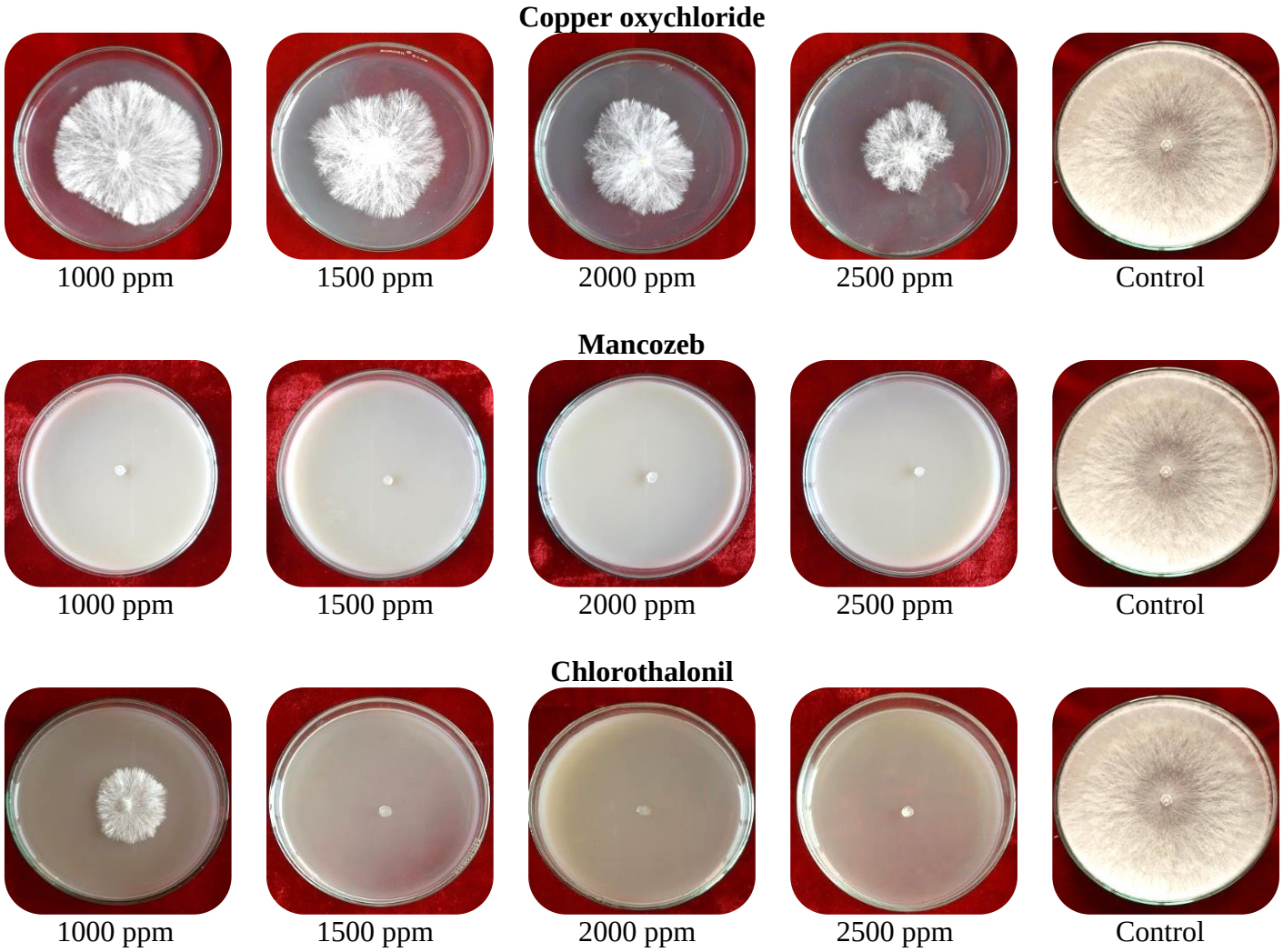
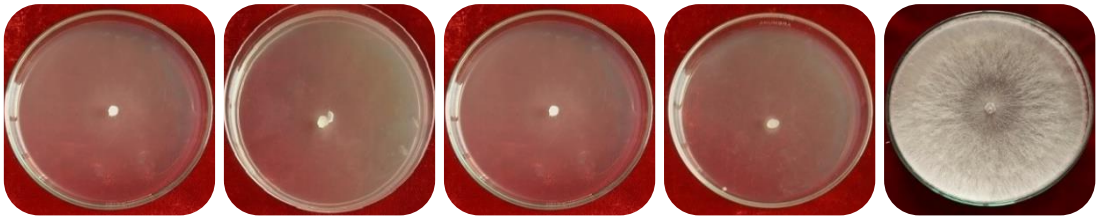


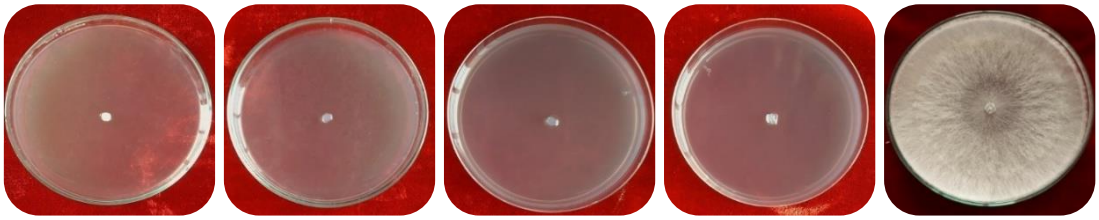
Plate 4.11. Effect of different non systemic fungicides against mycelial growth of *S. rolfsii* after 4 days of incubation

Hexaconazole



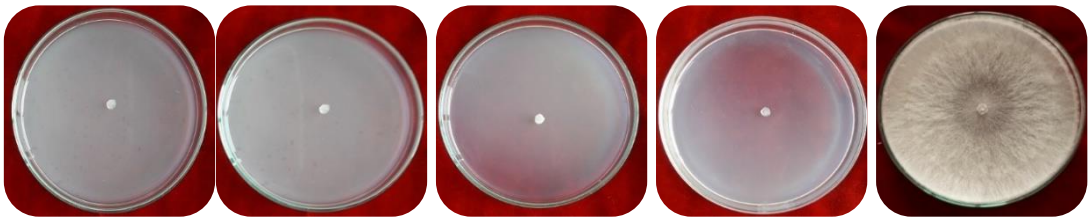
500 ppm 1000 ppm 1500 ppm 2000 ppm Control

Carboxin



500 ppm 1000 ppm 1500 ppm 2000 ppm Control

Tebuconazole



500 ppm 1000 ppm 1500 ppm 2000 ppm Control

Pyraclostrobin

500 ppm 1000 ppm 1500 ppm 2000 ppm Control

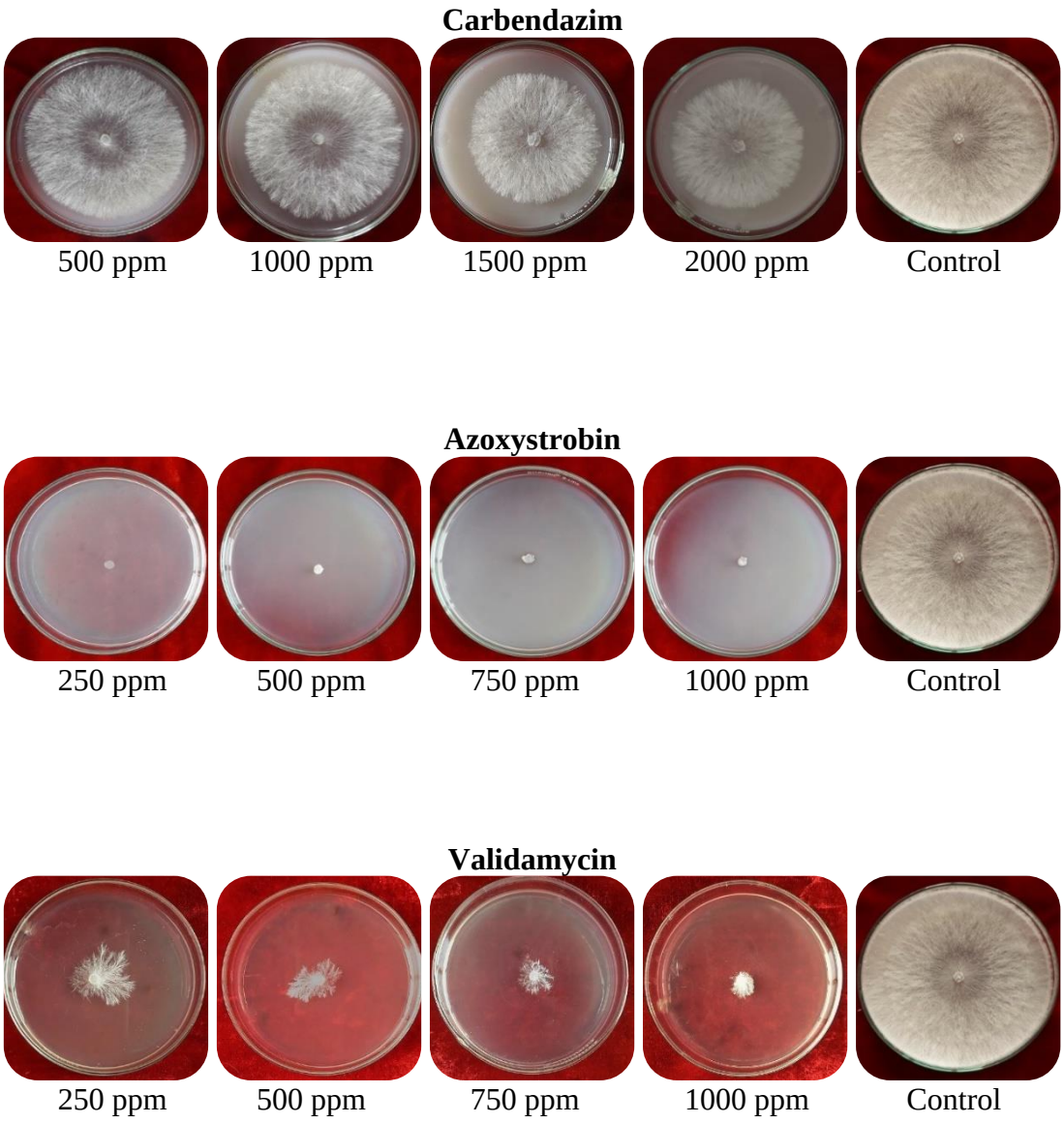


Plate 4.12. Effect of different systemic fungicides against mycelial growth of *S. rolfsii* after 4 days of incubation

and showed 21.46 per cent inhibition over control. The sensitivity of *S. rolfsii* to imazethapyr and pendimethalin has increased with increase in the concentration i.e., 58.46 per cent and 96.96 per cent respectively at 1000 ppm concentration (Plate 4.13). The results were presented in the Table 4.7 and Fig. 4.8.

The efficacy of herbicides in inhibiting the mycelia growth in descending order was as follows:

Quizalofop ethyl > pendimethalin > imazethapyr > control.

Lal and Nagarajan (1988) evaluated different herbicides i.e., alachlor, basalin and trifluralin against *S. rolfsii* at various concentrations and reported that all herbicides reduced mycelial growth of *S. rolfsii,* while cent per cent inhibition was noticed at higher concentrations (1000 and 2000 ppm). Madhuri and Narayan Reddy (2013) reported that oxyflourfen, alachlor, quizalofop ethyl and 2, 4-D sodium salt completely inhibited the growth of *S. rolfsii.* The efficacy of quizalofop ethyl, pendimethalin, imazethapyr and oxyflourfen on *S. rolfsii* was reported by Madhuri and Sagar (2016). Rangarani *et al.* (2017) evaluated three herbicides against *S. rolfsii* and reported that pendimethalin and quizalofop ethyl was highly effective. As the usage of herbicides is gaining importance due to increased labour costs, the present study is important as management of weeds plays a key role in suppressing stem rot. Besides, herbicides themselves were reported to show inhibitory effect on *S. rolfsii* and the present study is in agreement with the reports earlier carried out.

Table 4.7. Effect of herbicides on the mycelia growth of *S. rolfsii* after 4 days of incubation.

	Trade name	Mycelium growth (cm)				Per cent inhibition over control			
		100 ppm	**250 ppm**	**500 ppm**	**1000 ppm**	**100 ppm**	**250 ppm**	**500 ppm**	**1000 ppm**
Pendemethalin (30% EC)	Dhanutop	2.00	1.67	0.86	0.13	55.53 (48.16**)	58.46 (49.92)	80.70 (63.94)	96.96 (80.07)
Quizalofopethyl 5% EC)	Targa super	0.60	0.26	0.06	0.00	85.10 (67.54)	94.03 (75.88)	98.46 (84.16)	100.00 (90.00)
Imazythpyr (10% SL)	Guard	2.86	2.66	2.03	1.76	21.46 (27.58)	25.86 (30.54)	48.83 (44.31)	58.46 (49.85)
Control		4.5				0.0			
		C.D.				4.66			
		SE(m)				1.59			
		SE(d)				2.25			
		C.V.				5.04			

**Figures in parentheses are angular transformed values

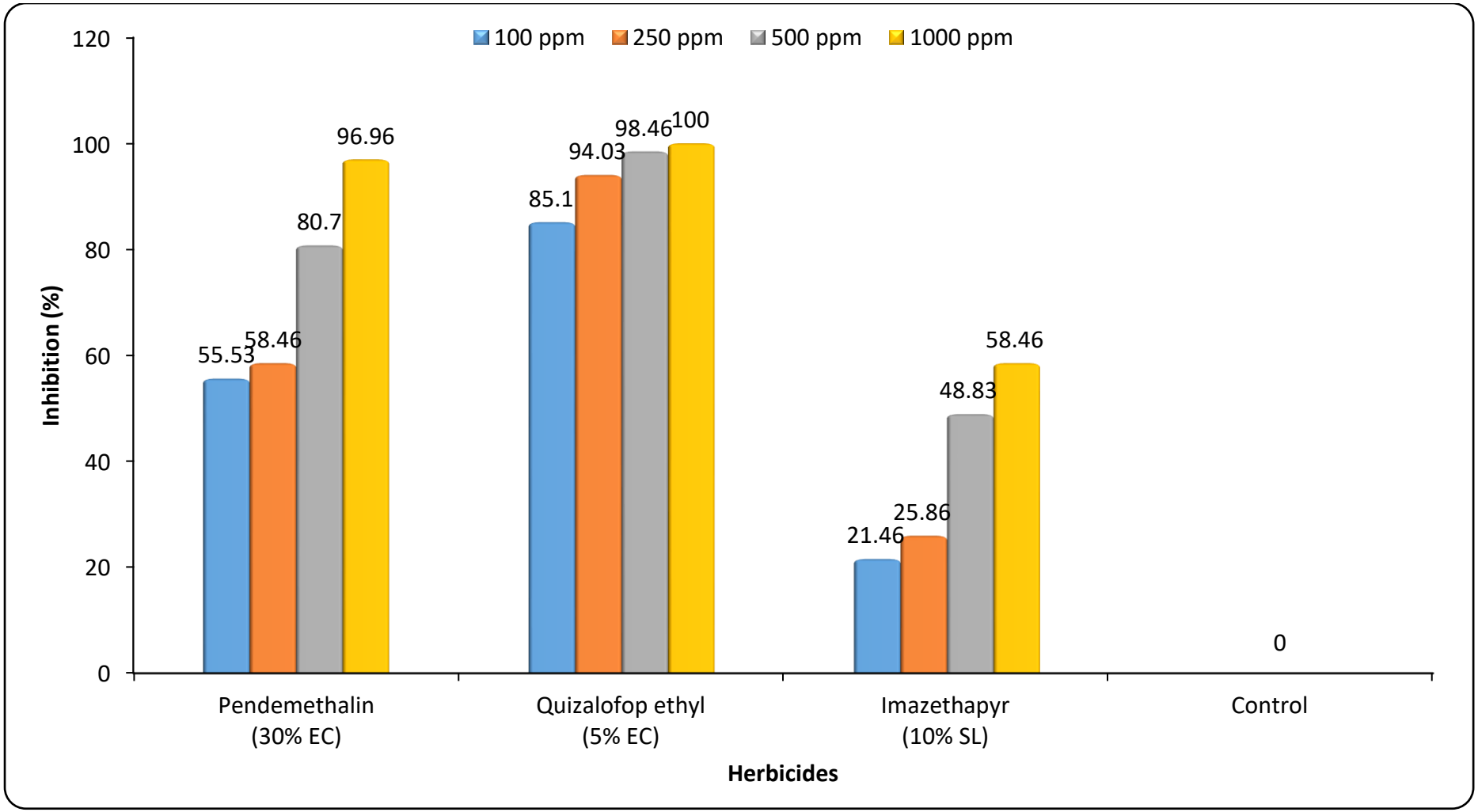


Fig. 4.8. Per cent inhibition of *S. rolfsii* by different herbicides

Pendimethalin

100 ppm 250 ppm 500 ppm 1000 ppm Control

Quizalofop ethyl

100 ppm 250 ppm 500 ppm 1000 ppm Control

Imazethapyr

100 ppm 250 ppm 500 ppm 1000 ppm Control

Plate 4.13. Effect of herbicides on the mycelia growth of *S. rolfsii* after 4 days of incubation

4.6 EFFICACY OF BIOAGENTS, FUNGICIDES AND HERBICIDES ON SCLEROTIAL GERMINATION OF *S. rolfsii*

4.6.1 Bioagents

a) *T. viride*

Effect of *T. viride* effective isolate Tr-DM at different spore concentrations on sclerotial germination of *S. rolfsii* revealed that spore concentration at 1 x 10^8 was highly effective in inhibiting the sclerotial germination (33.33%) i.e., 66.66 per cent inhibition over control and significantly different from others. The effect of the Tr-DM isolate increased with increase in spore concentration i.e., 56.66 per cent sclerotial germination at 1 × 10^4 followed by 46.66 per cent at 1 × 10^5 spore concentration and were significantly different from each other. Significant difference was not observed between 1 × 10^6 and 1 × 10^7 spore concentration on sclerotial germination i.e., 43.33 per cent and 40.00 per cent respectively (Plate 4.14). The results were presented in Table 4.8 and Fig. 4.9.

D'ambra and Ferrata (1984) observed the reduction of mycelial growth, sclerotial formation, sclerotial germination and number of sclerotia of *S. rolfsii* when inoculated with different inoculum concentration of *T. harzianum*. Khattabi *et al.* (2001) tested the effect of four isolates of *T. harzianum* against the sclerotial viability of *S. rolfsii* and found that all four isolates of *T. harzianum* reduced the sclerotial viability. The present study is in agreement with the earlier studies.

b) *P. fluorescens*

Sclerotial germination of *S. rolfsii* was highly inhibited at 10^{-4} and 10^{-5} CFU of Pf-DM isolate of *P. fluorescens* (66.66%) and least at 10^{-8} CFU (36.66%) and was significantly different from other treatments. With increase in bacterial concentration, sclerotial germination was affected resulting in reduction of sclerotial germination percentage (Plate 4.15). The

Table 4.8. Efficacy of *T. viride* effective isolate (Tr-DM) on sclerotial germination

Spore concentration	Sclerotial germination (%)	Inhibition (%)
1×10^4	56.66 (48.82**)	43.33 (41.13)
1×10^5	46.66 (43.06)	53.33 (46.90)
1×10^6	43.33 (41.13)	56.66 (48.82)
1×10^7	40.00 (39.21)	60.00 (50.74)
1×10^8	33.33 (35.20)	66.66 (54.76)
Control	100.00 (90.00)	
C.D.	4.94	5.54
SE(m)	1.58	1.73
SE(d)	2.24	2.45
C.V.	5.54	6.21

** Figures in parentheses are angular transformed values

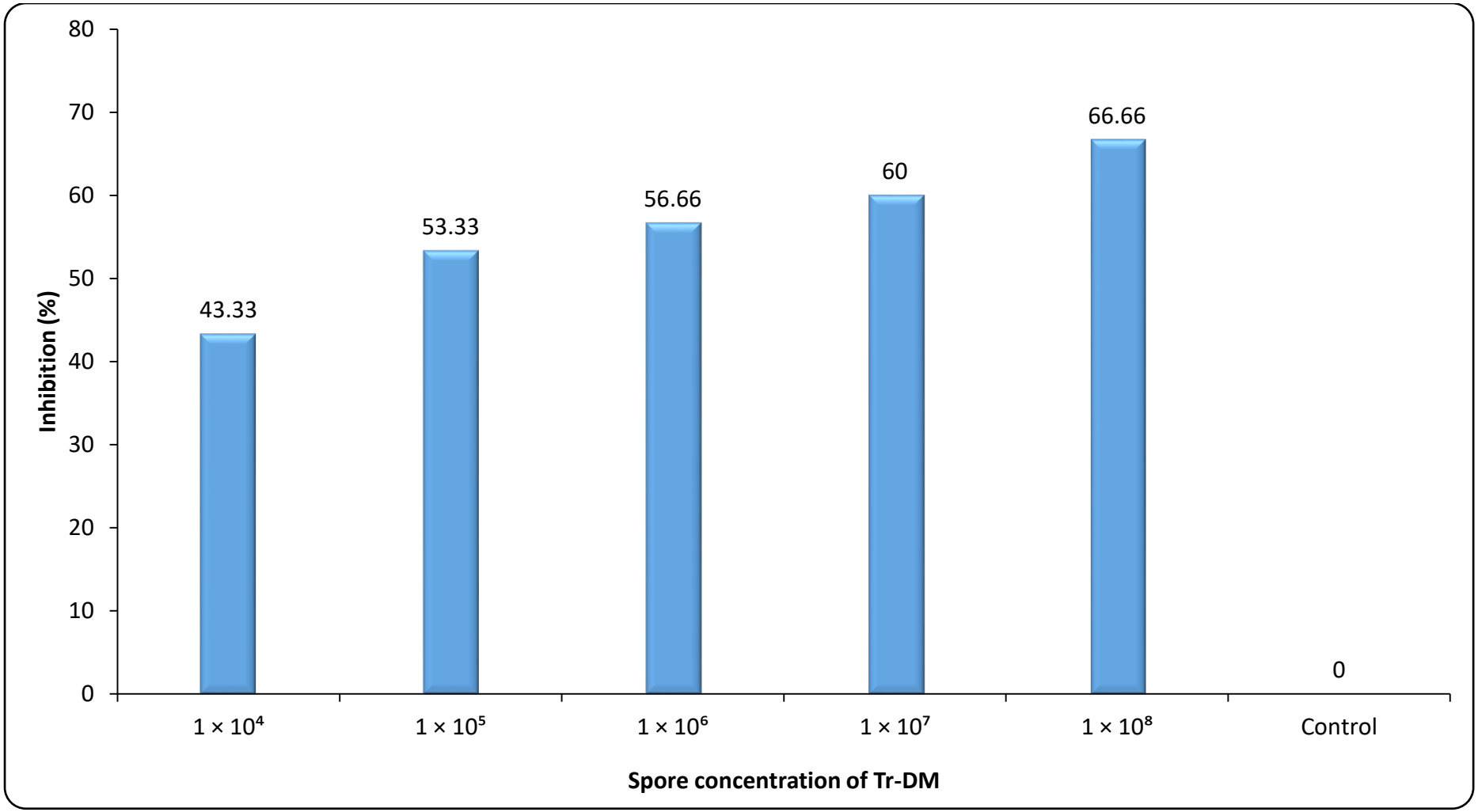


Fig. 4.9. Efficacy of *T. viride* effective isolate (Tr-DM) on sclerotial germination

1×10^4 spore suspension 1×10^5 spore suspension 1×10^6 spore suspension

1×10^7 spore suspension 1×10^8 spore suspension

Plate 4.14. Efficacy of *T. viride* effective isolate (Tr-DM) on sclerotial germination of *S. rolfsii*

Table 4.9. Efficacy of *P. fluorescens* effective isolate (Pf-DM) on sclerotial germination of *S. rolfsii*

Pf-DM	Sclerotial germination (%)	Inhibition (%)
10^{-4} CFU	33.33 (35.20**)	66.66 (54.76)
10^{-5} CFU	33.33 (35.20)	66.66 (54.76)
10^{-6} CFU	53.33 (46.90)	46.66 (43.06)
10^{-7} CFU	53.33 (46.90)	46.66 (43.06)
10^{-8} CFU	63.33 (52.75)	36.66 (37.21)
Control	100.00 (90.00)	0.00 (0.00)
C.D.	5.61	5.61
SE(m)	1.80	1.80
SE(d)	2.54	2.54
C.V.	6.09	8.03

** Figures in parentheses are angular transformed values

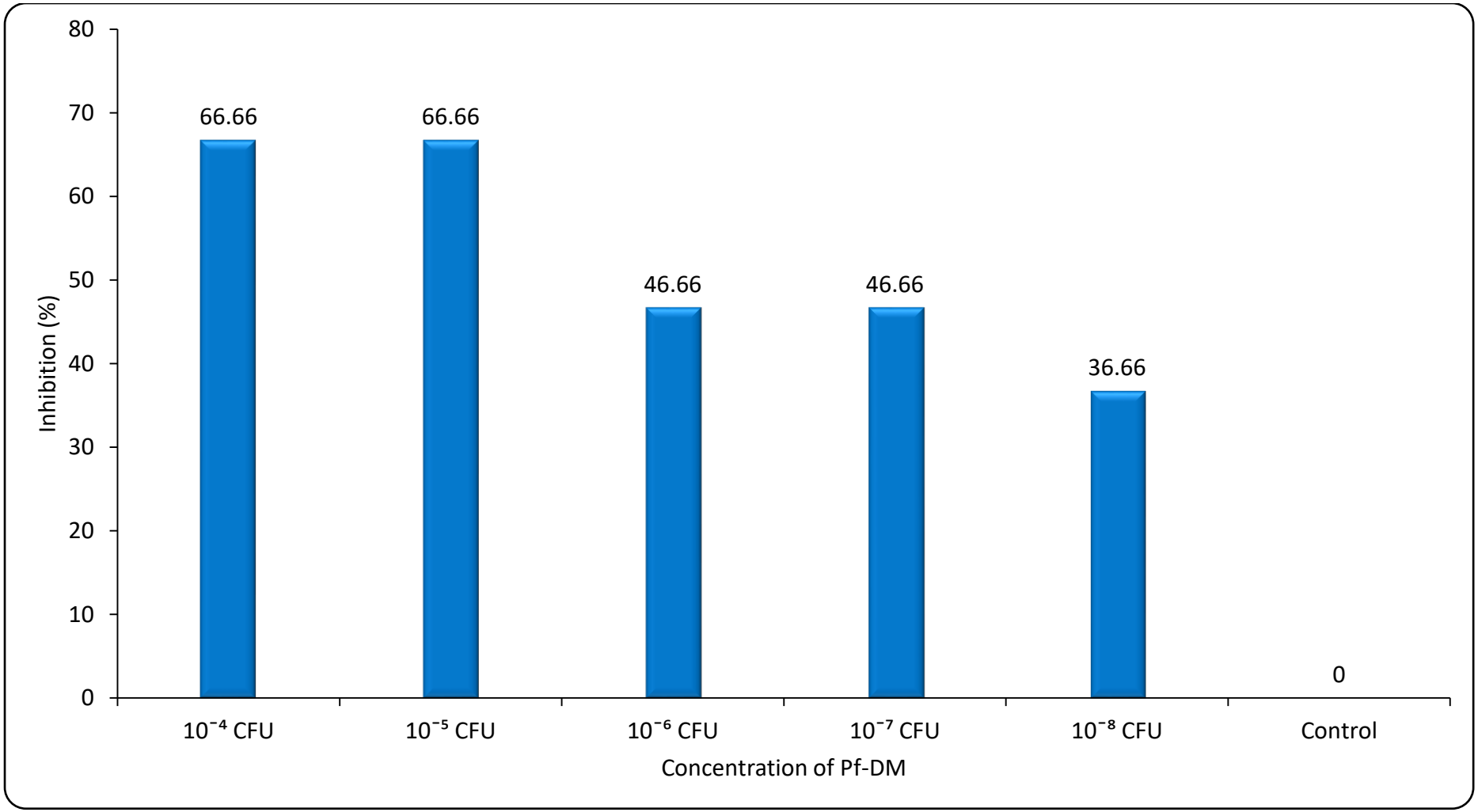


Fig. 4.10. Efficacy of *P. fluorescens* effective isolate (Pf-DM) on sclerotial germination of *S. rolfsii*

10^{-4} CFU 10^{-5} CFU 10^{-6} CFU 10^{-7} CFU 10^{-8} CFU

Plate 4.15. Efficacy of *P. fluorescens* effective isolate (Pf-DM) on sclerotial germination of *S. rolfsii*

effect on sclerotial germination was at par at bacterial concentration of 10^{-6} and 10^{-7} CFU and significantly different from others. The results were presented in Table 4.9 and Fig. 4.10.

Vasantha Devi *et al*. (1989) reported that inhibition in sclerotial germination declined with prolonged incubation periods with bacteria. Pande and Chaube (2003) reported that pre treatment of *P. fluorescens* isolates to sclerotia of *R. solani* to different lengths of time affected sclerotial viability *in vitro.* Pre- treatment of sclerotia resulted in 3.3 to 100 per cent inhibition in their germination after these were immersed in bacterial cell suspension of *P. fluorescens* isolates for 1 min. to 4 weeks. Mostapha Niknejad Kazempour (2004) studied the effect of *P. fluorescens* against *R. solani* and reported that all the isolates inhibited the mycelial growth, antagonistic isolates reduced the germination and cause lysis of sclerotia.

4.6.2 Fungicides

Among the different fungicides tested mancozeb, tebuconazole and hexaconazole were highly effective and completely inhibited the germination of sclerotia. Validamycin was least effective and recorded sclerotial germination of 83.33 per cent followed by carbendazim (76.66%) and copper oxychloride (76.66%) and were at par with each other and significantly different from validamycin. Chlorothalonil inhibited sclerotial germination up to 30 per cent (70.0%) and is significantly different from other fungicides. Strobilurin fungicides also found to be highly effective after triazoles and mancozeb. Sclerotial germination was more effected by pyraclostrobin (13.33%) compared to azoxystrobin (16.66%), however the difference is non significant. Carboxin in combination with thiram was also highly effective and completely inhibited the germination of sclerotia (Plate 4.16). The results were presented in the Table 4.10 and Fig. 4.11.

Table 4.10. Effect of fungicides on sclerotial germination of *S. rolfsii*

Fungicides	Trade name	Concentration (%)	Sclerotial germination (%)	Per cent inhibition over control
Carbendazim (50 WP)	Dhanustin	0.2	76.66 (61.19**)	23.33 (28.76)
Pyraclostrobin (20% EG)	Insignia	0.2	13.33 (21.13)	86.66 (68.82)
Copperoxychloride (50% WP)	Dhanucop	0.25	76.66 (61.19)	23.33 (28.76)
Mancozeb (75% WP)	Dithane M-45	0.3	0.00 (0.00)	100.00 (90.00)
Tebuconazole (25.9% EC)	Torque	0.1	0.00 (0.00)	100.00 (90.00)
Hexaconazole (5% EC)	Trigger	0.2	0.00 (0.00)	100.00 (90.00)
Azoxystrobin (23% SC)	One star	0.2%	16.66 (23.84)	83.33 (66.11)
Chlorothalonil (75% WP)	Daconil	0.3%	70.00 (56.76)	30.00 (33.19)
Validamycin (3% L)	Sheathamar	0.1%	83.33 (66.11)	16.66 (23.84)
Carboxin (Carboxin 37.5% WP+ Thiram 37.5% WP)	Vitavax	0.2%	0.00 (0.00)	100.00 (90.00)
Control			100.00 (90.00)	0.00 (0.00)
C.D.			5.02	5.02
SE(m)			1.70	1.70
SE(d)			2.40	2.40
C.V.			8.52	5.29

** Figures in parentheses are angular transformed values

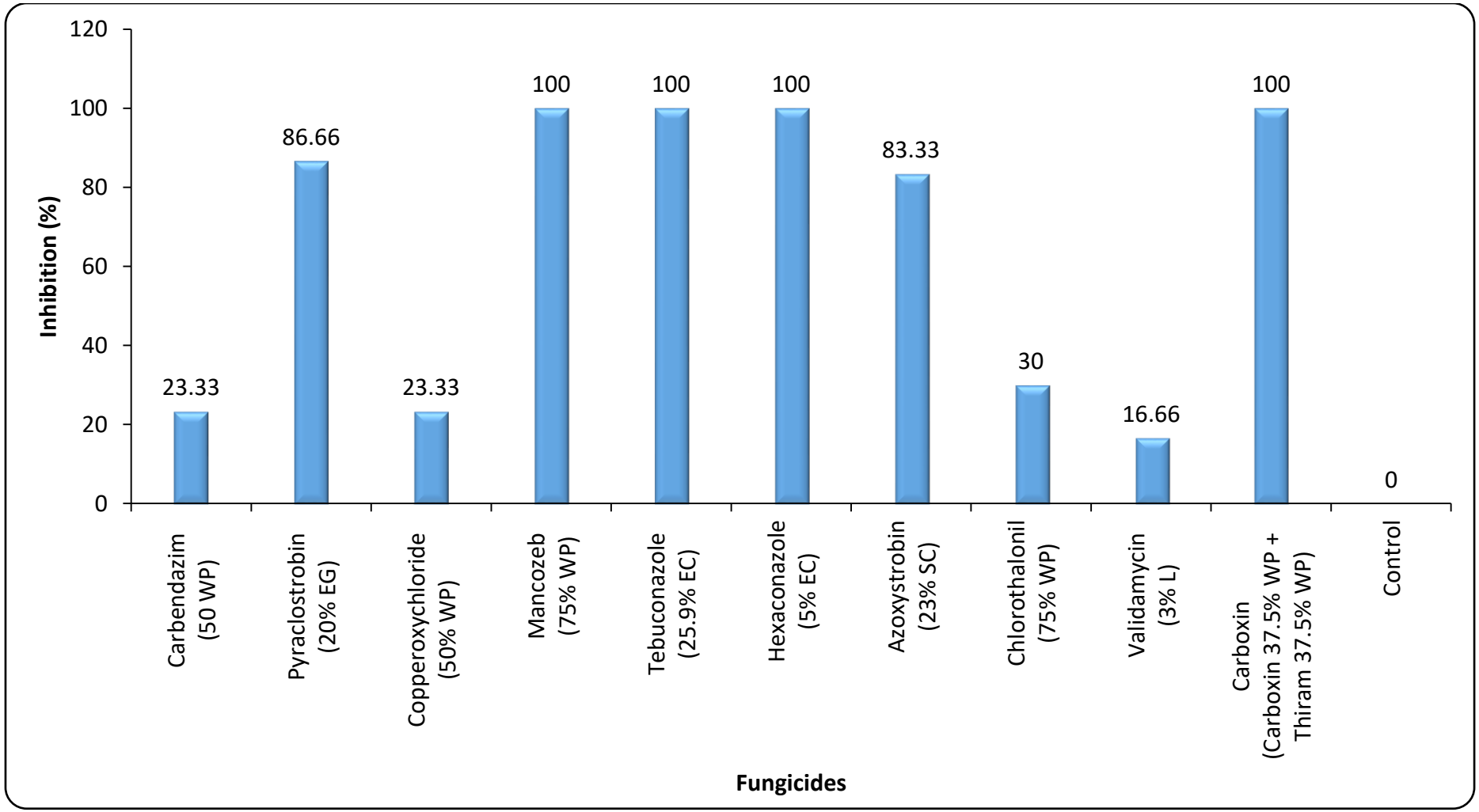


Fig. 4.11. Effect of fungicides on sclerotial germination of *S. rolfsii*

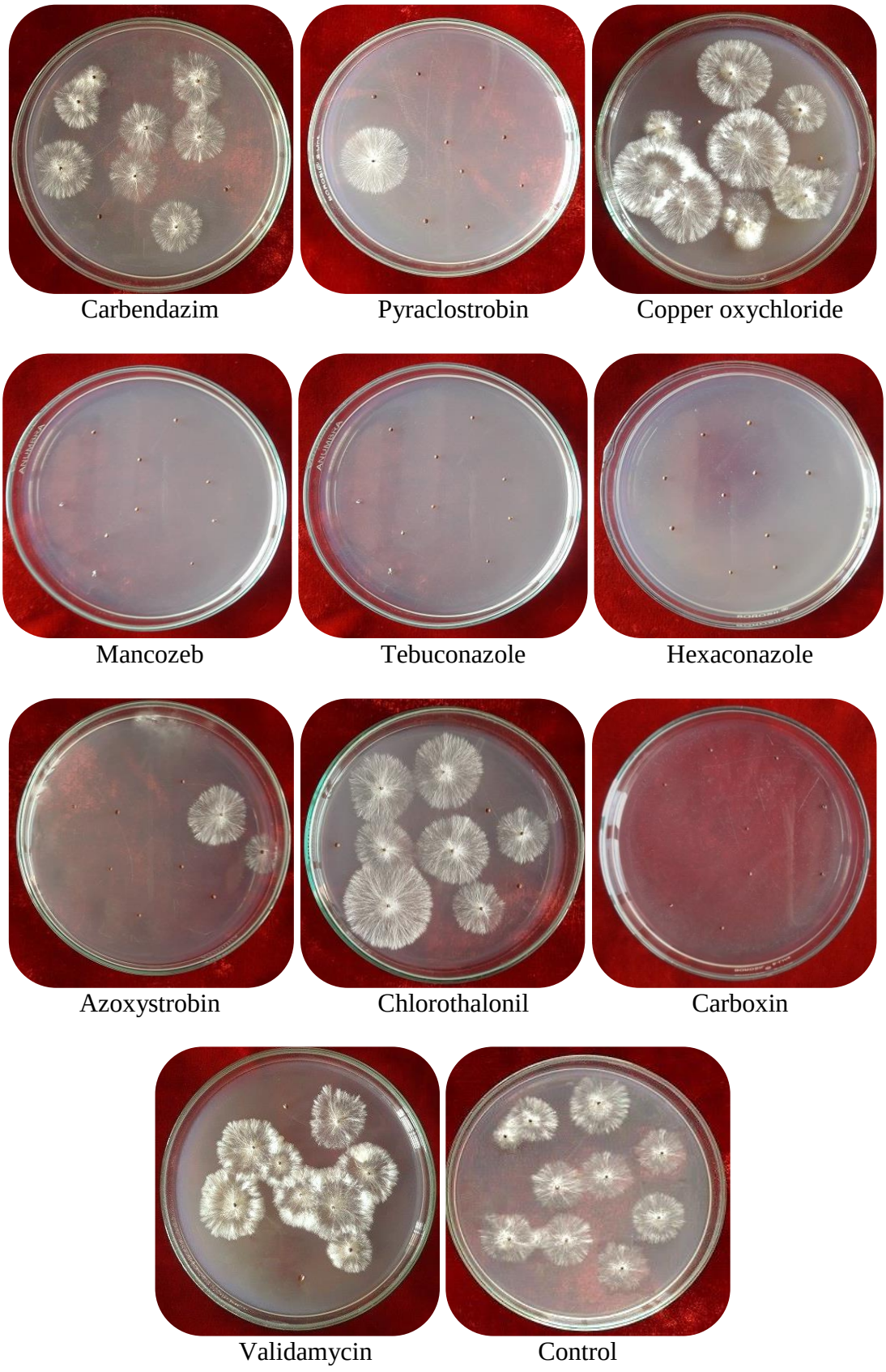


Plate 4.16. Effect of fungicides on sclerotial germination of *S. rolfsii*

Efficacy of fungicides in inhibiting the sclerotial germination was in the following order:

mancozeb = tebuconazole = hexaconazole = carboxin > pyraclostrobin > azoxystrobin > chlorothalonil > carbendazim = copper oxychloride > validamycin > control.

Anil Kumar *et al.* (1979) investigated the effect of seven fungicides on the survival of sclerotia of *S. rolfsii* and found that sicarol, vitavax and brestan were highly effective in reducing sclerotial viability. Chakraborty and Bhowmik (1985) showed that thiram was best in limiting mycelial growth and inhibiting sclerotial germination followed by ceresan wet, benlate, captan. Khattabi *et al.* (2001) found that, benomyl was highly effective in reducing sclerotial viability producing 53.3 per cent of non-viable sclerotia at a concentration of 50 mg a.i./m^2 and 68.3 per cent at 250 mg a.i./m^2.

4.6.3 Herbicides

Among the three herbicides tested quizalofop ethyl was highly effective in inhibiting the germination of sclerotia and recorded 36.66 per cent followed by pendimethalin (73.33%) and were significantly different from each other. Imazethapyr did not show any effect in inhibiting the sclerotial germination (Plate 4.17). The results were presented in the Table 4.11 and Fig. 4.12.

Efficiency of herbicides in inhibiting the scerotial germination was in the following order:

Quizalofop ethyl > pendimethalin > imazethapyr = control

Singh and Dwivedi (1990) reported that nitrofen and nuvacron greatly reduced the germination of sclerotia. Pathak *et al*. (1996) observed the viability of buried sclerotia of *R. solani,* and found that paraquat was the

Table 4.11. Effect of herbicides on sclerotial germination of *S. rolfsii*

Herbicide	Trade name	Concentration (%)	Sclerotial germination (%)	Per cent inhibition over control
Imazethapyr (10% SL)	Guard	0.15	100.00 (90.00**)	0.00 (0.00)
Quizalfop ethyl (5% EC)	Targa super	0.2	36.66 (37.21)	63.33 (52.75)
Pendimethalin (30% EC)	Dhanutop	0.65	73.33 (58.98)	26.66 (30.98)
Control			100.00 (90.00)	0.00 (0.00)
C.D.			4.94	4.94
SE(m)			1.49	1.49
SE(d)			2.11	2.11
C.V.			3.74	12.36

** Figures in parentheses are angular transformed values

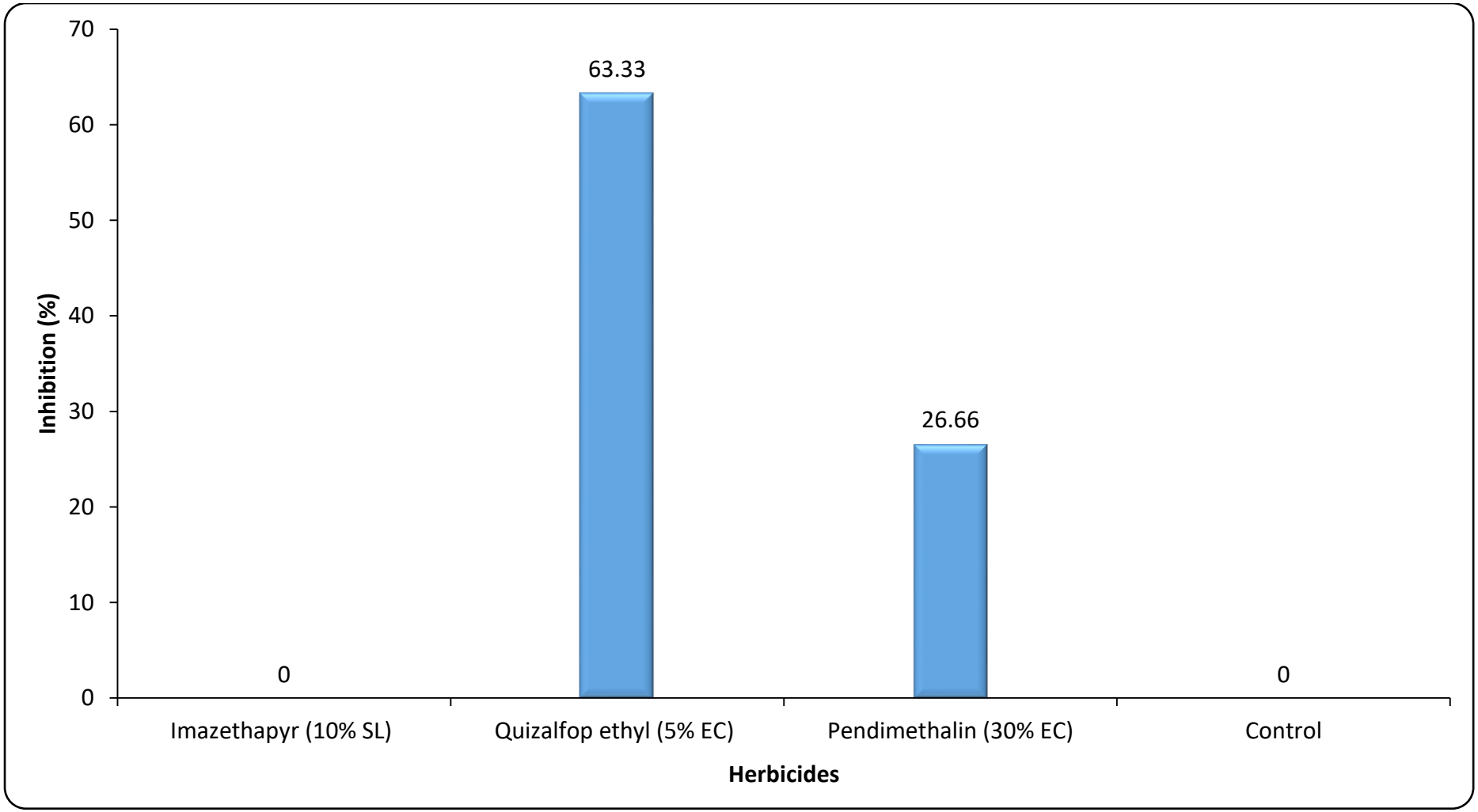


Fig. 4.12. Effect of herbicides on sclerotial germination of *S. rolfsii*

Pendimethalin Quizalofop ethyl Imazethapyr

Control

Plate 4.17. Effect of herbicides on sclerotial germination of *S. rolfsii*

most potent in reducing the mycelial growth and production of sclerotia followed by thiobencarb, butachlor and 2, 4-D. Pastro and March (1999) evaluated the effects of herbicides on the production and viability of *S. rolfsii in vitro* and found trifluralin and pendimethalin were the most efficient compounds because they notably reduced the production of viable sclerotia.

4.7 TESTING THE COMPATIBILITY OF POTENTIAL BIOCONTROL AGENT WITH EFFECTIVE FUNGICIDES, HERBICIDE AND THEIR COMBINATIONS *IN VITRO*

Study conducted to know the compatibility among effective fungicides and herbicides on efficient biocontrol isolate (Tr-DM) of *T. viride* has revealed that among fungicides, mancozeb showed compatibility and did not effect the mycelial growth of *T. viride* and at par with control. Tebuconazole completely inhibited the growth of *T. viride* followed by carboxin + thiram (0.97 cm). After mancozeb, hexaconazole was found to be compatible and recorded mycelial growth of 1.80 cm followed by azoxystrobin (1.27 cm) and was significantly different from each other.

Quizalofop ethyl is recorded mycelial growth of 1.83 cm. Mancozeb even though found to be highly compatible but in combination with quizalofopethyl inhibited the mycelial growth of *T. viride* (1.40 cm). Tebuconazole alone and also in combination with quizalofop ethyl completely inhibited the mycelial growth. Azoxystrobin and hexaconazole in combination with quizalofop ethyl were more effective when compared with fungicides alone and the difference was significant (85.13) (Plate 4.18). The results were presented in the Table 4.12 and Fig. 4.13.

Efficiency of fungicides in inhibiting the mycelial growth was in the following order:

Tebuconazole > carboxin + thiram > azoxystrobin > hexaconazole > mancozeb

Table 4.12. Efficacy of effective fungicides, herbicide and their combinations on Tr-DM isolate of *T. viride* after 4 days of inoculation

Fungicide / herbicide	Concentration (%)	Mycelial growth of Tr-DM isolate of *T. viride* (cm)	Per cent inhibition over control
Tebuconazole (25.9% EC)	0.1	0.00	100.00 (90.00**)
Azoxystrobin (23% SC)	0.2	1.27	71.80 (57.91)
Hexaconazole (5% EC)	0.2	1.80	60.00 (50.74)
Mancozeb (75% WP)	0.3	4.50	0.00 (0.00)
Carboxin (Carboxin 37.5% + Thiram 37.5%)	0.2	0.97	78.47 (62.33)
Quizalophopethyl (5% EC)	0.2	1.83	59.23 (50.30)
Tebuconazole (25.9% EC) + Quizalophopethyl (5% EC)	0.1 + 0.2	0.00	100.00 (90.00)

Cont…

Table 4.12. Cont…

Fungicide / herbicide	**Concentration (%)**	**Mycelial growth of Tr-DM isolate of *T. viride* (cm)**	**Per cent inhibition over control**
Azoxystrobin (23% SC) + Quizalophopethyl (5% EC)	0.2 + 0.2	0.87	80.70 (63.94)
Hexaconazole (5% EC) + Quizalophopethyl (5% EC)	0.2 + 0.2	0.67	85.13 (67.33)
Mancozeb (75% WP) +Quizalophopethyl (5% EC)	0.3 + 0.2	1.40	68.80 (56.02)
Carboxin (Carboxin 37.5% WP + Thiram 37.5% WP) + Quizalophopethyl (5% EC)	0.2 + 0.2	1.03	76.33 (60.87)
Control		4.50	0.00 (0.00)
C.D.		0.12	1.73
SE(m)		0.04	0.59
SE(d)		0.06	0.8
C.V.		4.50	1.89

** Figures in parentheses are angular transformed values

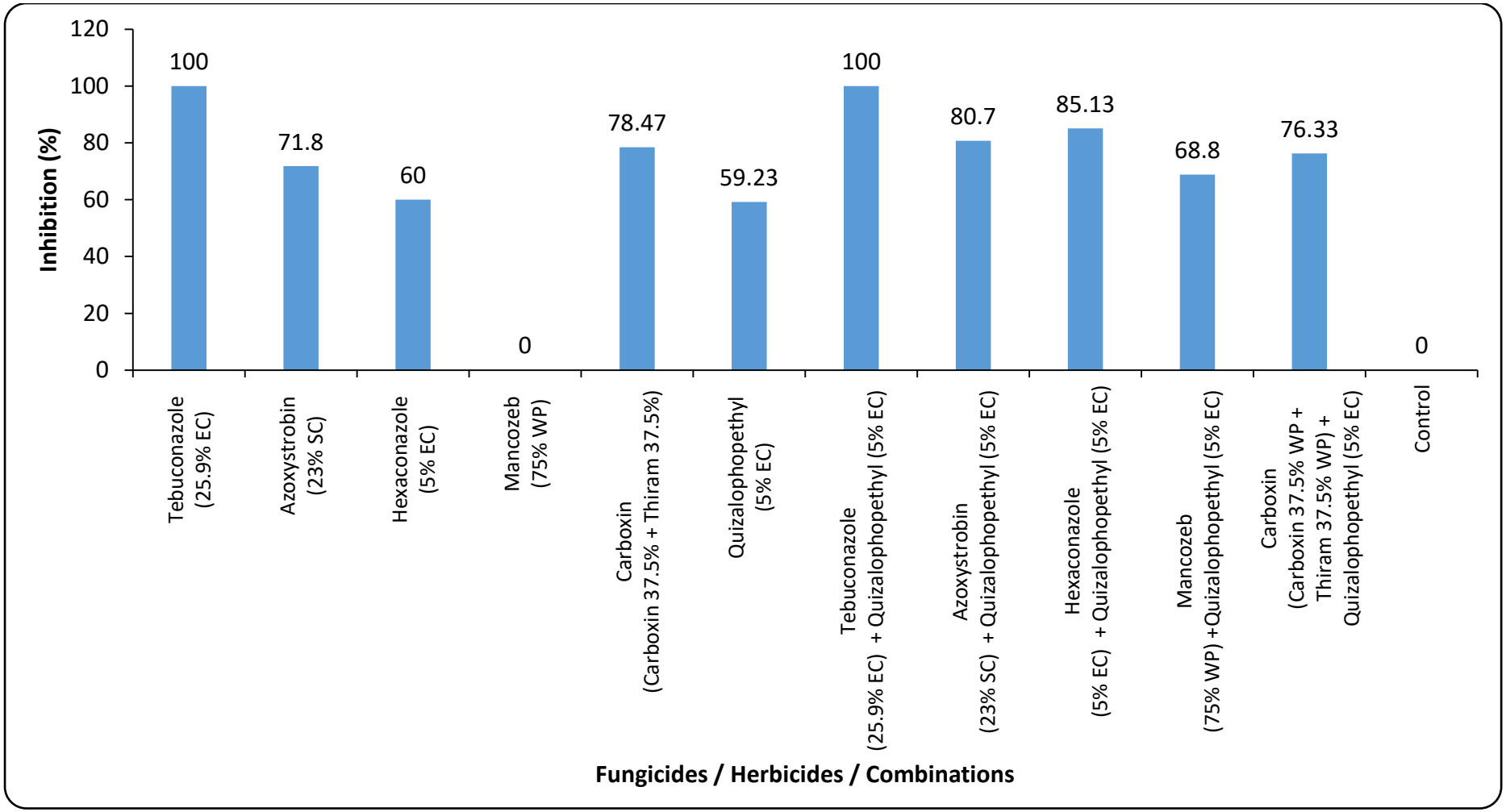


Fig 4.13. Efficacy of effective fungicides, herbicide and their combinations on Tr-DM isolate of *T. viride* after 4 days of inoculation

Plate 4.18. Efficacy of effective fungicides, herbicide and their combinations on Tr-DM isolate of *T. viride* after 4 days of inoculation

Efficiency of combination of fungicides and herbicide on in inhibiting the mycelial growth was in the following order:

Tebuconazole + quizalofop ethyl > hexaconazole + quizalofop ethyl > azoxystrobin + quizalofop ethyl > carboxin + thiram + quizalofop ethyl > mancozeb + quizalofop ethyl.

Vijayaraghavan and Abraham (2004) tested *in vitro* compatibility of *T. harzianum*, *T. viride* and *T. longibrachiatum* with nine fungicides and found that mancozeb was compatible with all the three antagonists. Tapwal *et al.* (2012) studied compatibility of *T. viride* with five fungicides and reported that among all captan, copper oxychloride were compatible to some extent with *T. viride* at all concentrations. Deepika *et al.* (2014) tested the effect of some commonly used nine fungicides, six insecticides and seven herbicides on the mycelia growth of *T. harzianum*. Among fungicides, mancozeb was compatible even at high concentration (250 µg a.i./ml). Among herbicides, benomyl, thiophanate methyl, bayleton and iprodione were found incompatible with the test antagonist even at low concentration (25 µg a.i./ml).Compatibility of bioagents with fungicides reveled that all the three species viz. *T. viride*, *T. harzianum* and *T. hamatum* were compatible with the chemical azoxystobin (Vineela *et al.*, 2017).

4.8 INTEGRATED DISEASE MANAGEMENT STEM ROT OF GROUNDNUT

Pot culture experiment was conducted with the effective bioagent and compatable fungicide (Mancozeb 75WP), effective herbicide (Quizalofop ethyl 5EC) and effective isolate Tr-DM of *T. viride* and their combinations for the management of stem rot of groundnut. After 30 days, data was taken and presented in the Table 4.13 and Fig. 4.14, 4.15.

The details of treatments were as follows;

T_1 : Soil drenching with quizalofopethyl @ 0.3 ml a.i / kg soil

T_2 : Soil drenching with mancozeb @ 0.3% a.i

T_3 : Soil application with *T. viride* Tr-DM isolate @ 10g/kg soil

T_4 : $T_1 + T_2$

T_5 : $T_2 + T_3$

T_6 : $T_1 + T_2 + T_3$

T_7 : Sick soil

T_8 : Control

Studies revealed that the groundnut seeds have germinated well and recorded germination percentage of 91.67 at T_6 (soil application of mancozeb @0.3% a.i + quizalofop ethyl 0.3% a.i + Tr-DM isolate of *T. viride* @ 10g/kg soil) and significantly superior over T_7 (Sick soil) (33.33%) and significantly inferior over control. Among all the treatments least germination was recorded at T_7 (sick soil) i.e., 33.33 per cent followed by T_3 (Soil application with *T. viride* Tr-DM isolate @ 10g/ kg soil) (58.33%) and were significantly different from each other. T_2 (Soil drenching with mancozeb @ 0.3% a.i) alone and in combination with herbicide quizalofop ethyl @ 0.3% a.i were equally effective in improving the germination of groundnut seeds compared to control and the difference is not significant. Application of Biocontrol agent alone (Soil application with *T. viride* Tr-DM isolate @ 10g/ kg soil) and in combination with fungicide (T_5- Soil application with *T. viride* Tr-DM isolate @ 10g/ kg soil + mancozeb @0.3% a.i) also showed their effectiveness in controlling the stem rot disease and improving the seed germination but the difference between them was not significant.

Effectiveness of treatments in controlling the disease and on seed germination were in the following order:

T_8 (control) >T_6 (quizalofopethyl + mancozeb + DM-Tr) > T_4 (quizalofopethyl + mancozeb) > T_2 (mancozeb) > T_5 (mancozeb + DM-Tr) > T_1 (quizalofopethyl) > T_3 (DM-Tr) > T_7 (sick plot).

Root length of groundnut was recorded high at T_6 (22.33 cm) i.e., i.e., significantly superior over T_7 (Sick soil) (14.33 cm) and significantly inferior over control. Among all the treatments least root length was recorded at T_7 (sick soil) i.e., 14.33 cm followed by T_1 (16.67 cm) and were significantly different from each other. Root length in treatments T_5 (20.33 cm), T_4 (19.67 cm), T_2 (19.00 cm) are not significant with each other and significantly superior over control (Plate 4.20).

Root length in different treatments were observed in the following order:

T_8 (control) > T_6 (quizalofop ethyl + mancozeb + DM-Tr) > T_5 (mancozeb + DM-Tr) > T_4 (quizalofop ethyl + mancozeb) > T_2 (mancozeb) > T_3 (DM-Tr) > T_1 (quizalofop ethyl) > T_7 (sick plot)

Shoot length of groundnut was recorded high at T_6 (32.67 cm) i.e., significantly superior over T_7 (Sick soil) (23.33 cm) and significantly inferior over control. Among all the treatments least shoot length was recorded at T_7 (sick soil) i.e., 23.33 cm followed by T_1 (25.67 cm) and were significantly different from each other. T_4 and T_5 recorded same shoot length (31.46 cm) and are not significant with each other (Plate 4.20).

Shoot length in different treatments observed in the following order:

T_8 (control) > T_6 (quizalofopethyl + mancozeb + DM-Tr) > T_5 (mancozeb + DM-Tr) = T_4 (quizalofopethyl + mancozeb) > T_2 (mancozeb) > T_3 (DM-Tr) > T_1 (quizalofopethyl) > T_7 (sick plot)

Table 4.13. Integrated management of stem rot of groundnut in pot culture

Treatments	Seed germination (%)	Per cent reduction in germination over control	Disease incidence (%)	Per cent increase in disease incidence over control	Root length (cm)	Per cent decrease in root length over control	Shoot length (cm)	Per cent decrease in shoot length over control	Dry weight of Plants (gm)	Per cent decrease in dry weight over control
T_1	62.50 (52.21**)	37.50	17.70 (17.70)	17.70	16.67	30.54	25.67	32.44	3.03	17.21
T_2	70.83 (57.39)	29.17	0.00 (0.00)	0.00	19.00	20.83	29.67	21.92	3.06	16.39
T_3	58.33 (49.80)	41.67	8.85 (8.85)	8.85	18.33	23.62	28.33	25.44	3.00	18.03
T_4	75.00 (59.97)	25.00	0.00 (0.00)	0.00	19.67	18.04	30.67	19.28	3.26	10.92
T_5	66.67 (54.80)	33.33	0.00 (0.00)	0.00	20.33	15.29	30.67	19.28	3.10	15.30
T_6	91.67 (76.17)	8.33	0.00 (0.00)	0.00	22.33	6.90	32.67	14.02	3.26	10.92
T_7	33.33 (35.16**)	66.67	66.46 (66.44)	66.46	14.33	40.29	23.33	38.60	2.60	28.96
T_8	100.00 (90.00)		0.00 (0.00)		24.00		38.00		3.66	
C.D.	9.17		18.37		1.38		1.52		0.22	
SE(m)	3.03		6.07		0.47		0.52		0.07	
SE(d)	4.29		8.59		0.66		0.73		0.10	
C.V.	8.84		90.52		4.24		3.02		4.02	

** Figures in parentheses are angular transformed values

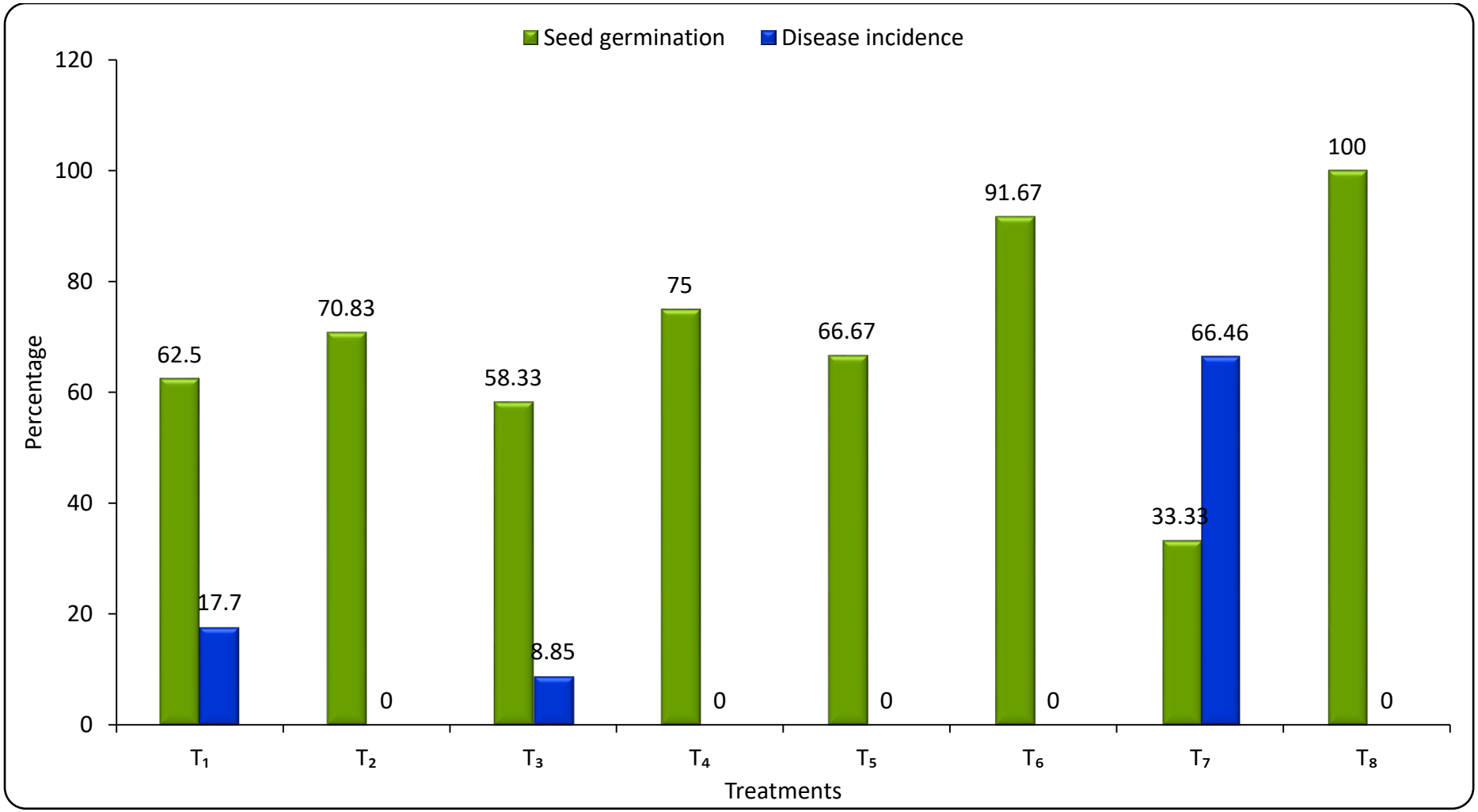


Fig. 4.14. Effect of different treatments on seed germination, disease incidence of stem rot of groundnut

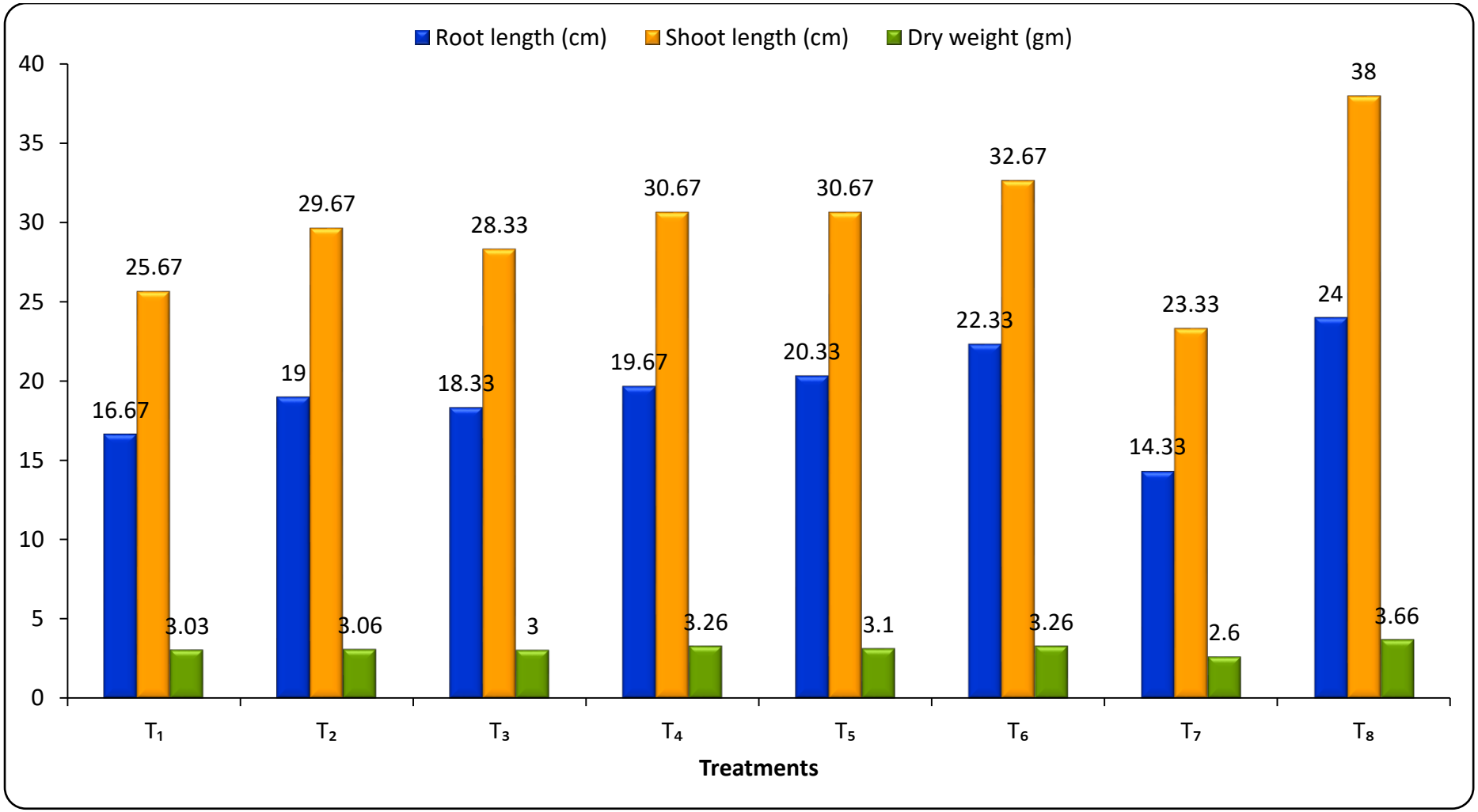


Fig. 4.15. Effect of different treatments on plant growth parameters of groundnut

Plate 4.19. Integrated management of stem rot of groundnut in pot culture

Plate 4.20. Differences in root and shoot length of groundnut plants at different treatments

Highest dry weight of the plant was recorded at T_4 and T_6 (3.26 gm) i.e., significantly superior over T_7 (Sick soil) (2.60 gm) and significantly inferior over control and not significant with each other. Among all the treatments, T_7 (Sick soil) showed least dry weight of the groundnut plant i.e., 2.60 gm followed by T_3 (3.00 gm) and the difference was significant. Least dry weight of the plant was observed in bioagent treated pot i.e., 3.00 gm and was significantly superior over sick soil.

Dry weight in different treatments observed in the following order:

T_8 (control) = T_6 (quizalofopethyl + mancozeb + DM-Tr) =
T_4 (quizalofopethyl + mancozeb) > T_5 (mancozeb + DM-Tr) >
T_2 (mancozeb) > T_1 (quizalofopethyl) > T_3 (DM-Tr) > T_7 (sick plot).

Arunasri (2003) reported that seedling root dip in thiram 0.1 per cent + seedling root dip in *Trichoderma* suspension (T_1) + seedling root dip in *Pseudomonas* spp. (B_1) reduced the *S. rolfsii* incidence in Crossandra to about 6.66 per cent compared to control (73.66%). Toorray *et al.* (2007) reported that *Trichoderma* when integrated with captan, thiram and mancozeb resulted insignificant reduction in *S. rolfsii* collor rot in chickpea. Kuldhar and Suryawanshi (2017) observed highest seed germination (98.33%) with the treatment Thiram + Carbendazim + *T. harzianum* + *P. fluorescens* + NSC + *Azadiracta indica* extract. Significantly highest reduction in pre-emergence (97.61%), post-emergence (95.77%) and average (96.69%) mortality were recorded with treatment of Thiram + Carbendazim + *T. harzianum* + *P. fluorescens* + NSC + *A. indica* extract.

Chapter ~ V

Summary & Conclusions

Chapter – V

SUMMARY AND CONCLUSIONS

The findings of the present investigation carried on 'Studies on the *Sclerotium rolfsii* Sacc, incitant of stem rot of groundnut (*Arachis hypogaea* L.)' in the P.G. laboratory at Department of Plant Pathology, S.V. Agricultural College, Tirupati, during 2017-18. The results were summarized and presented below.

The causal organism was isolated from the infected portion of the stem from the fields of S.V. Agricultural College, Tirupati. Pathogenicity of the fungus was tested by soil infestation method.

Effect of different inoculum densities of *S. rolfsii* on the development of stem rot of groundnut was assessed using soil infestation method in pot culture under green house and highest germination (45.83), root length (12.37 cm) and shoot length (15.16 cm) was observed at 1 per cent inoculum level. Germination percentage, root length and shoot length of groundnut was gradually decreased with increase of inoculum levels from 1 to 5 per cent. Lowest germination (16.66%), root length (7.50 cm), shoot length (8.33 cm) was observed in 5 per cent inoculum levels. Since the germination of groundnut seeds was reduced to more than 50 per cent at one per cent inoculums level, it was followed throughout the investigations.

Influence of soil temperature, pH and moisture was observed on the viability of sclerotia. Maximum sclerotial germination (100%) was observed at 27^0 and 30^0 C temperature. Germination of sclerotia was highly affected (76.66%) i.e., 23.34% reduction over control at lowest temperature (20^0 C) of study. Soil pH 7.2 and 7.5 was found to be best (100%) for sclerotial germination. Least sclerotial germination (76.66) occurred at soil pH 9.0 and this indicates that basic pH is less favorable for sclerotial germination.

Moisture levels of 50 per cent (control) and 40 per cent were found to be best for sclerotial germination (100%). Least germination (76.67%) was observed at 100 per cent moisture, indicating that increase in soil moisture decreases the sclerotial viability.

The antagonistic effect of three isolates Tr-DM, Tr-SNG and Tr-RGP of *Trichoderma viride* and three isolates Pf-DM, Pf-SNG and Pf-RGP of *Pseudomonas fluorescens* against *S. rolfsii* were assessed by dual culture technique *in vitro*. The isolates of fungal biocontrol agent *T. viride* were more effective in controlling the mycelial growth of *S. rolfsii* when compared with isolates of bacterial biocontrol agent *P. fluorescens.* Among the fungal biocontrol agents maximum inhibition (87.19%) of mycelial growth of *S. rolfsii* was observed with the Tr-DM isolate of *T. viride* and among bacterial isolates maximum inhibition (28.25%) of mycelial growth of *S. rolfsii* was observed with the Pf-DM isolate of *P. fluorescens* and they were significantly different from each other.

Efficacy of ten fungicides belonging to systemic and non-systemic groups were observed at various concentrations against mycelial growth of *S. rolfsii* by using poison food technique. Among different fungicides tested, fungus was most sensitive to systemic fungicides azoxystrobin, tebuconazole, hexaconazole, carboxin and non-systemic fungicide mancozeb showing cent per cent mycelial growth inhibition (100%) of *S. rolfsii* at all the concentrations tested. Carbendazim was found to be less effective among the systemic fungicides showing least inhibition even at higher concentration. Among non-systemic fungicides, copper oxychloride was less effective.

Efficacy of different herbicides viz., pendimethalin, quizalofop ethyl, imazethapyr were observed at various concentrations against mycelial growth of *S. rolfsii* by using poison food technique. Among different herbicides tested, quizalofop ethyl showed maximum inhibition (100%) of pathogen at higher concentration (1000 ppm) and imazethapyr was found to

be least effective among the tested herbicides. Hence, quizalofop ethyl was used for the future course of study on integrated management of stem rot of groundnut.

Studies conducted to identify the effective dosage of *T. viride* isolate (Tr-DM) and observed that at higher concentration (1×10^8) maximum sclerotial inhibition was observed i.e., 66.66% inhibition over control and least sclerotial inhibition (43.33%) was observed at 1×10^8 concentration, this indicates that increase in spore concentration increases sclerotial inhibition. Similarly, sclerotial germination was tested against effective *P. fluorescens* isolate (Pf-DM) suspension at the concentrations from 10^{-4} to 10^{-8} CFU/ml and the inhibition of sclerotial germination was 66.66 per cent at 10^{-4} and 10^{-5} concentrations, 46.66 per cent at 10^{-6} and 10^{-7} concentrations. Lower sclerotial germination (36.66%) was observed at 10^{-8} concentration (lower concentration). There was significant increase in per cent inhibition of sclerotial germination with increase in concentration of the bacterial suspension. Hence the effective isolate of *T. viride* Tr-DM was used for future course of study on integrated management of stem rot of groundnut.

All the above mentioned fungicides were tested at recommended dose against the sclerotial germination in soil. Among different fungicides tested, highest inhibition (100%) was observed with the fungicides mancozeb, tebuconazole, hexaconazole and carboxin and the least inhibition (16.66%) was observed with validamycin. Similarly, different herbicides were tested and quizalofop ethyl was highly effective in inhibiting the germination of sclerotia recording 36.66 per cent inhibition.

Compatibility of effective fungicides, herbicides and their combinations on the effective *T. viride* isolate (Tr-DM) has revealed that among different fungicides, herbicides and their combinations, mancozeb showed its compatibility with effective *T. viride* isolate (Tr-DM) and did not affect the mycelial growth of *T. viride.* Herbicide quizalofop ethyl is found to be

compatable to some extent. Tebuconazole completely inhibited the mycelial growth of Tr-DM isolate and was not compatable. As mancozeb showed its compatability towards Tr-DM, this fungicide was used for pot culture study.

Efficacy of effective herbicide, fungicide and bioagent was tested against *S. rolfsii* through pot culture under greenhouse conditions. Among different treatments, T_6 (*T. viride* @ 10 g kg^{-1} soil+ mancozeb @ 0.3% a.i kg^{-1} soil + quizalofop ethyl @ 0.3% ml a.i. kg^{-1} soil) did not show disease incidence upto 30 DAS with highest groundnut seed germination (91.67%), root length (22.33 cm) and shoot length (32.67 cm). In addition disease incidence was not observed with T_8 (control), T_6 (*T. viride* @ 10 g kg^{-1} soil+ mancozeb @ 0.3% a.i kg^{-1} soil + quizalofop ethyl @ 0.3% ml a.i. kg^{-1} soil), T_4 (mancozeb @ 0.3% a.i kg^{-1} soil + quizalofop ethyl @ 0.3% ml a.i. kg^{-1} soil), T_3 (mancozeb @ 0.3% a.i. kg^{-1} soil) and T_1 (quizalofop ethyl @ 0.3% ml a.i. kg^{-1} soil). Highest dry weight of the plant was recorded at T_8 (control) (3.66 gm) followed by T_4 (mancozeb @ 0.3% a.i kg^{-1} soil + quizalofop ethyl @ 0.3% ml a.i. kg^{-1} soil), and T_6 (*T. viride* @ 10 g kg^{-1} soil+ mancozeb @ 0.3% a.i kg^{-1} soil + quizalofop ethyl @ 0.3% ml a.i. kg^{-1} soil) (3.26 gm). From the study it was clear that integrated treatment T_6 (*T. viride* @ 10 g kg^{-1} soil + mancozeb @ 0.3% a.i. kg^{-1} soil + quizalofop ethyl @ 0.3% ml a.i. kg^{-1} soil) is highly effective with high seed germination, root length, shoot length, dry weight of the plant and least disease incidence.

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