"EVALUATION OF BIOCONSORTIA AGAINST STEM ROT DISEASE (*Sclerotium rolfsii*) OF GROUNDNUT"

by

Mr. Nidheesh B.S.

(Reg. No. 2021/241)

A thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

In partial fulfilment of the requirements for the degree
of

MASTER OF SCIENCE (AGRICULTURE)
In

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY

POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH
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2024

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MAHARASHTRA, INDIA
2024

CANDIDATE'S DECLARATION

I hereby declared that this thesis or part
there of has not been submitted
by me or other person to any
other University or Institute
for a Degree or
Diploma

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Date : (Nidheesh B.S.)

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Maharashtra State (India)

CERTIFICATE

This is to certify that the thesis **"EVALUATION OF BIOCONSORTIA AGAINST STEM ROT DISEASE (*Sclerotium rolfsii*) OF GROUNDNUT"**, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in partial fulfilment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY**, is a record of bonafide research work carried out by **Mr. NIDHEESH B.S.,** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

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CERTIFICATE

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Place : M.P.K.V., Rahuri. (S.A. Ranpise)

Date : / / 2024

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"Gratitude is the fairest blossom which springs from the soul"

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While travelling on the path of life and education, many hands pushed me forth, learned hearts put me on the right path, unlighted by their knowledge and experience, I ever remain thankful to the mall.

Place: M.P.K.V., Rahuri. (Nidheesh B.S.)

Date : / /2024

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LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Per cent
/	:	Per
@	:	At the rate of
µg	:	Microgram
^{0}C	:	Degree Centigrade (S)
a.i	:	Active ingredient
BOD	:	Biochemical oxygen demand
CD	:	Critical Difference
cm	:	Centimetre
CRD	:	Completely Randomized Design
DAS	:	Days after sowing
et al.	:	etalli (and other)
etc.	:	For example
g	:	Gram
g/kg	:	Grams per Kilogram
ha	:	Hectar
i.e.,	:	That is
Kg	:	Kilogram
MDR	:	Mean Disease Rateing
mg	:	Milligram
ml	:	Milliliters
mm	:	Milli meter
MMT	:	Million Metric Tonne (s)
PDA	:	Potato Dextrose Agar
PDC	:	Percent Disease Control
PDI	:	Percent Disease Incidence
PDR	:	Percent Disease Reduction
pH	:	Potential of Hydrogen
SE(m)	:	Standard Error of Mean
Sp.	:	Species
viz	:	Namely

ABSTRACT

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2024

Research Guide	:	Dr. T.K. Narute
Department	:	Plant Pathology and Agril. Microbiology

Groundnut (*Arachis hypogeae*) is one of the major oil seed crop India. It contains an average of 40.1 per cent of fat and 25.3 per cent of protein and every part of groundnut has its own commercial value. In fact, it plays a pivotal role in oilseed economy of India. Several diseases like stem rot, leaf stop, bud necrosis, etc., are responsible for decreasing the quality and quantity of groundnut production. Among them stem rot of groundnut caused by soil borne pathogenic fungi is a major problem and economically important soil borne disease. This pathogen causes yield loss of 20-30 per cent and may reach up to 80 per cent during severe condition. In order to control stem rot disease of groundnut best method is biological method which is efficient and eco-friendly method.

Keeping this in mind the current work entitled on the "Evaluation of Bioconsortia Against Stem Rot Disease *(Sclerotium rolfsii)* of Groundnut" was carried out to see the cultural and morphological character of pathogen, evaluation on efficacy of bioagents on pathogen under *in vitro* condition, evaluation of bioconsortia against stem rot of groundnut under glass house condition as *Sclerotium rolfsii* is one of the most devastating pathogen which affects both quality and quantity of groundnut.

Abstract contd… **Mr. Nidheesh B.S.**

The visual observation in field revealed the symptoms such as wilting of plant, white colored mycelial growth at the base of plant, rotting of stem at base of plants were the symptoms of stem rot of groundnut. *S. rolfsii* was isolated from disease infected plant from field. The isolated and purified pathogen from diseased sample was tested for their pathogenicity.

The pathogenicity of *S. rolfsii* was tested under pot condition by soil inoculation technique (sick soil). Different seven media were selected to see the cultural and morphological properties of pathogen, among those media used Potato dextrose agar media showed maximum mean mycelial diameter of 78.5 mm and cultural characteristics of circular shaped, fluffy cottony white color with abundant sclerotial production. Study on mycelial growth of *S. rolfsii* at different pH level was carried out of which maximum mycelial growth was seen at 5.6 pH (319 mg) with abundant sclerotial production.

As a part of ecofriendly disease management strategy, efficacy of different bioagents were also studied under *in vitro* condition which showed that *T. harzianum* (74.5 %) show maximum per cent inhibition on test pathogen followed by *B. subtilis, T. viride, P. fluorescence.* Bioconsortium is a group of different strains of the same or different microorganisms the function together as a community. In a bioconsortium, the component organisms work together in a synergistic and complex way. These bio consortia help in controlling most of the soil borne diseases including stem rot in groundnut caused by *Sclerotium rolfsii*.. A study was conducted for evaluation of bioconsortium against stem rot of groundnut under glass house condition. Groundnut seed were treated with different bioconsortium of which seed treatment with MPKV's fungal and MPKV's fungal +bacterial consortium showed highest seed germination (%) (92.85 %) among bioconsortium. Maximum disease incidence in seed treated with MPKV"s fungal bioconsortium @ 5 g/kg of seed (32.07 %) with least percent disease control (52.71%) and minimum disease incidence in seed treated with MPKV's fungal + bacterial bioconsortium @10 g/kg with maximum disease control (73.8 %).

Pages 1 to

1. INTRODUCTION

Groundnut plant botanically known as *Arachis hypogeae* which belongs to the Leguminosae family. It has been reported that South America was the place, from where cultivation of Groundnut originated. Further it was spread in Brazil, Southern Bolivia and North-Western Argentina. Groundnut was introduced by the Portuguese from Brazil to West Africa and then to South-Western India in the 16^{th} century. It is native of Brazil. It is widely grown in South India, Maharashtra and Uttar Pradesh. North Gujarat is famous for peanut cultivation. The plant is a bushy or creeping annual with the peculiar habit of ripening its fruit underground. A sandy soil is best for its cultivation. The soil must be friable so that the ripening fruit can be buried and it must be well fertilized.

Groundnut is a self-pollinated, annual, herbaceous legume crop. A complete seed of groundnut is called as pod and contains one to five kernels, which develops underground in a needle like structure called as peg. After the pollination, aerial pegs grow into the soil and then convert into a pod. Groundnut has taproot system, which has many nodules, present in root and lateral roots. These nodules contain *Rhizobium* bacteria, which are symbiotic in nature and fixes atmospheric nitrogen. Outer layer of Groundnut is called shell. The shell constitutes about 25-35 per cent of the pod. The seed accounts for the remaining portion (65-75 %). The colour of the testa varies from red, brown, purple to white depending on the type and variety. The kernel and germ are normally white in colour.

Groundnut contains on an average 40.1 per cent fat, 25.3 per cent protein which is 1.3 times higher than meat, 2.5 times higher than eggs and 8 times higher than fruit and is fairly a rich source of calcium, iron and vitamin B complex like thiamine, riboflavin, niacin and vitamin A. It has multifarious usages. It is not only used as a major cooking medium for various food items but also utilized for manufacture of soap, cosmetics, shaving cream, lubricants, *etc*. In fact, it plays a pivotal role in oilseed economy of India.

Groundnut is called as the ‘King’ of oilseeds. It is one of the most important food and cash crop of country. Being a valuable source of all the nutrients, it is a low-priced commodity. Groundnut is also called as wonder nut and poor men’s cashew nut. Almost every part of groundnut has commercial value.

Groundnut is consumed as fresh, roasted, dried, boiled and in so many recipes. It is also used in the manufacturing of soaps, beauty creams, medical ointments and creams, paints, lubricants and many other industrial products. India exports groundnut kernels, in shell, HPS groundnut and oil cake. Groundnut haulms and leaves serve as a rich source of cattle feed and raw material for preparation of silage. Being a leguminous crop, groundnut also grow in crop rotation as it synthesizes atmospheric nitrogen and adds about 100-120 kg of nitrogen in the field per hectare per season. It maintains the fertility of soil and helps in reducing soil erosion. Groundnut oil cake is used as animal and poultry feed as well as an organic fertilizer. Groundnut shell is used in manufacturing industrial products like card-board boxes etc. and also for fuel purpose (Nutritive value of Indian Foods).

Globally, Groundnut covers 327 lakh hectares with the production of 539 lakh tonnes with the productivity of 1648 kg per hectare (FAOSTAT, 2021). India is one among the top three producing countries of groundnut in the world. It ranks second next to China (34 per cent contribution to the world groundnut production). Nearly 19 per cent of world groundnut production is contributed by India during 2021. Nigeria ranks third in world groundnut production with 9 per cent contribution.

Area under cultivation of groundnut in India during 2020-2021 was 6.01 Million hectare of which 5.17 Million hectare in *kharif* season and 0.8 Million hectare in *rabi* season. Production of groundnut in India during 2020-2021 was 10.24 MMT. Productivity of groundnut in India during 2020-2021 is 1703 kg/hectare (Anonymous, 2021).

Gujarat had the largest area under cultivation of groundnut in India (2.16 Million hectare) during 2020-2021, followed by Andhra Pradesh (0.87 Million hectare) and Rajasthan (0.85 Million hectare). Maharashtra had 0.3 Million hectare under groundnut cultivation during 2020-2021. Gujarat holds the first place in production of groundnut during 2020-2021 (4.1 MMT), followed by Rajasthan (1.9 MMT) and Tamil Nadu (1.02 MMT). Production of groundnut during 2020-2021 in Maharashtra was 0.4 MMT. Tamil Nadu holds first place in productivity of groundnut during 2020-2021 (2502 kg/ha) followed by West Bengal (2363 kg/ha) and Telangana (2286 kg/ha). Productivity of groundnut during 2020-2021 in Maharashtra was 875 kg/ha. (Anonymous, 2021)

Several factors are responsible for low productivity among which diseases like leaf spot, collar rot, stem rot, bud necrosis, etc., are very important. Out of all, stem rot caused by *Sclerotium rolfsii* Sacc. is a major problem and is an economically important soil borne pathogen. The pathogen *Sclerotium rolfsii* Sacc., is a soilborne pathogen that commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, stem rot, wilt and foot rot on more than 500 plant species including almost all the agricultural and horticultural crops (Aycock, 1966; Domsch *et al.,* 1980; Farr *et al.,* 1989). *Sclerotium rolfsii* was first reported by Rolfs (1892) later the pathogen was named as *Sclerotium rolfsii* by Saccardo (1911). Higgins (1927) worked in detail on physiology and parasitism of *S. rolfsii*. This was the first detailed and comprehensive study in USA. Sclerotia initially white in color, later it becomes light brown to dark brown at maturity and they are sub spherical, the surface finely wrinkled, sometimes flattened (Subramanian, 1964 and Mehan, 1995). This pathogen *Sclerotium rolfsii* forms brown sclerotia which are very well-organized compact structures, built of three layers, the rind, composed of empty melanized cells; the cortex cells, filled with vesicles and the medulla (Chet, 1975). Sclerotia may be spherical or irregular in shape and at maturity resemble the mustard seed (Taubenhaus, 1919; Barnett and Hunter, 1972). Sclerotial size was reported to be varied from 0.1 mm to 3.0 mm (Om Prakash and Singh, 1976; Ansari and Agnihotri, 2000 and Anahosur, 2001). *Sclerotium rolfsii* attacks more than 500 species, the most common hosts are legumes, crucifers and cucurbits (Punja, 1985). The disease is distributed throughout the world and prevalent particularly in warm dry climates. It was first reported by Mc Clintock (1917) in Virginia. Garren (1959) has estimated the losses in southern USA as 10 to 20 million dollars annually. Weber (1931) and Garrett (1956) reported that the fungus survived in the soil for years together by producing sclerotial bodies and causing the disease on various hosts. The loss of yield caused by the pathogen is 25 per cent, but sometimes it reaches 80-90 per cent (Grichar and Bosweel, 1987). Similarly, yield losses over 25 per cent have been reported by Mayee and Datar (1988). Stem rot causes pod yield losses of 10-25 per cent, but under severe diseased conditions yield losses may range to up 80 per cent (Rodriguez Kabana *et al*., 1975). Patil and Rane (1982) reported yield loss up to 10 to 50 per cent due to this disease. Adiver (2003) reported the yield loss of 15-70 per cent in groundnut is due to leaf spot, rust and stem rot singly or in combination.

Biological control represents both the oldest and youngest as well as ecofriendly technology for the control of plant diseases and pest. Most people agree that agriculture could not have begun without the benefits of naturally occurring biological controls. Yet modern biological control achieved with introduced micro-organisms is still in its infancy.

Combination of biocontrol agents gave better results than using them singly (Wahid 2006). Bio consortium is a group of different strains of the same or different microorganisms the function together as a community. In a bio consortium, the component organisms work together in a synergistic and complex way. These bio consortia help in controlling most of the soil born diseases including stem rot in groundnut caused by *Sclerotium rolfsii*. Keeping in mind the importance of disease, the present study was conducted with following objectives.

1. Isolation and identification of fungal pathogen from Groundnut**.**
2. To study the cultural and morphological characters of fungal pathogen.
3. *In vivo* evaluation of bioconsortium against stem rot of groundnut.

2. REVIEW OF LITRATURE

The review of literature presented here under gives an overview on stem rot of groundnut caused by *Sclerotium rolfsii* Sacc.. The detail of Occurrence of Pathogen, Distribution and Economic Importance of Pathogen, Symptomatology, Cultural and Morphological Characteristics, *In vivo* evaluation of bio consortium against stem rot of groundnut, *etc* has been given under following headings.

2.1 Occurrence of Pathogen

Singh and Pavgi (1965) studied that *Sclerotium rolfsii* usually causes collar rot, but spotted leaf rot with a single tiny sclerotium in the center has also been reported. Aycock (1966) noticed that the pathogen *S. rolfsii* is soil born pathogen that commonly occurs in the tropics, sub-tropic and other warm temperate regions of the world causing stem rot, root rot, foot rot and wilt on more than 500 plant species including almost all the agricultural and horticultural.

Tu and Kimbrough (1978) reported *Athelia rolfsii* (Curzi) as the perfect stage of *S. rolfsii* while studying *Rhizoctonia-Sclerotium* complex which is the currently accepted binomial for the sexual stages of *S. rolfsii*.

Punja (1988) described that *S. rolfsii* caused about 25 per cent seedling mortality in the groundnut cultivar JL-24 the teleomorphic state of *S. rolfsii* was discovered by confirming that the fungus was a basidiomycete. Thiribhuvanamala *et al*. (1999) distinguished this pathogen responsible for tomato crop loss of 30 per cent. Its occurrence on Crossandra has been observed about 40 to 50 per cent mortality of plants.

Narsimha Rao (2000) described this fungus *S. rolfsii* having characteristics white, radiating abundant mycelial growth on the affected portion of potato plant and tuber. Sarma *et al*. (2002) reported that *S. rolfsii* causes foot rot, collar rot, spotted rot and root rot in several plants.

2.2 Distribution and Economic Importance

Nargund (1981) carried out survey on foot rot of wheat during *rabi* 1978-79 in Malaprabha project area. He reported the maximum disease incidence of 5.20 and 10.20 per cent in irrigated and rainfed fields respectively.

Punja (1985) found pathogen attacked more than 500 species, the most common host are crucifers, cucurbits and legumes. Palakshappa (1986) reported maximum disease incidence of 30 per cent in betel vine in chikkodi taluka of belgum district. Harlapur (1988) mention incidence about 4.66 and 9.85 per cent under irrigated and rainfed condition, respectively of foot rot of wheat caused by *S. rolfsii*.

Mayee and Datar (1988) reported that stem rot occurs in all groundnut growing states. It is most severe in Gujarat, Madhya Pradesh, Karnataka, Maharashtra andhra Pradesh, Tamil Nadu and Orissa where it is estimated over 500000 ha of groundnut fields area infested with the pathogen and yield losses of over 25 per cent.

Sharma and Pathak (1990) concluded a field experiment at Sriganganagar in India during 1982-84 to investigate yield and sucrose losses in the sugar beet caused by *Sclerotium rolfsii*. They recorded the reduction of yield and sucrose by 46.50 and 62.20 per cent respectively. Mehan *et al*. (1995) worked on, the disease caused by *Sclerotium rolfsii* affected groundnut in many countries and reported reduction in yield by 10-25 per cent and pod loss of more than 50 per cent in heavily infected fields.

Shokes and Gorbet (1998) observed stem and pod rot on groundnut by *S. rolfsii* with potential death and estimated field yield losses of 10 per cent or more in the Southern-Eastern USA. Hanumanthegowda (1999) carried out survey on stem rot of groundnut during *kharif* 1998-99 and *rabi* /*summer* 1998-99 in Belgaum, Havier and Dharwad districts and reported disease incidence of 12.57 and 8.68 per cent in rainfed and irrigated fields, respectively.

Kalmesh and Gurjar (2001) reported that among the fungal diseases, root rot of chili caused by *S. rolfsii* had attained the economic importance. In recent year, this disease is causing the economic losses in chilies crop. Anand and Harikesh Bahadur (2004) stated that in peppermint, this pathogen caused about 5 to 20 per cent of crop loss was observed under field condition.

Kokub *et al.* (2007) noticed profused mycelial growth and sclerotial production contribute to the considerable crop losses associated with *S. rolfsii* and found wide host range, prolific growth and ability to produce persistent sclerotia contribute to the large economic losses associated with the pathogen.

2.3 Symptomatology

Wilson (1953) described symptoms of the stem rot as, mycelium covering the plant stem near the soil surface and produced organic acid, which where toxic to living plant tissue. This is then followed with necrosis of plant cells. The mycelium invaded the stem, gynophores and also pods and caused rotting of the tissues. The production of abundant white mycelium and small brown spherical sclerotia on the infected parts were characteristic symptoms of the disease. Beattie (1954) also observed same type of symptoms on infected plants.

Wheeler (1969) reported the symptoms that young groundnut plants are usually killed rapidly but older plants turn yellow and wilt before they die. The mycelium often appears as a white mass at stem bases of dying plants. Baruah *et al.* (1980) described the symptoms of the stem rot of groundnut caused by *S. rolfsii*. The infected seedlings were found stunted with chlorotic leaves and ultimately withered and died. Sclerotia developed on the surface of the soil and also on the affected stem.

Nyvall (1989) also narrated the symptoms as wilting of the plants due to infection of stem at base level. Further, soil level near the infected stem was covered with white mycelium. The wilted plants remained upright. The infected areas of stem shredded and covered with elongated and overcrowded brown lesions and formed reddish colour. During dry whether brown lenticular lesions occurred on stem just below the soil surface. The peg infection caused light to dark brown lesions (0.05-2.00 cm long), which resulted in tissue shredding and pod loss. Lesions on young pods of spanish peanuts were orange yellowish to light tan colour. Severely decayed karnels were shriveled and covered with mycelium. Mehrotra and Aneja (1990) distinguished cortical decay of stem base at ground level and appearance of conspicuous white mycelium which extended into the soil and on organic debries.

Kalmesh and Gurjar (2001) described the symptoms of root rot of chilli caused by *S. rolfsii.* Severe mortality of chilli plants were observed during the months of March-April in chilli growing areas. Mature plants of chilli from standing crop were collapsed and dried down suddenly.

2.4 Isolation and Identification

Bagwan (2011) studied fifty-nine isolates of *Sclerotium rofsii* isolated from stem rot of groundnut plants as well as from stem rot of groundnut infected field of major growing region of Maharashtra and Gujarat states during *Kharif* 2008. Kadam *et al*. (2011) studied that infected samples of groundnut were collected from farmers field and Oil Seed Research Station Latur. They isolated pathogen from all samples on PDA in laboratory and identified pathogen as *Sclerotium rofsii* based on morphological and colony characters.

Rakh *et al*. (2011) isolated pathogen from infected stem of groundnut plants on potato dextrose agar plates and identified it as of *Sclerotium rofsii* and stock culture of *Sclerotium rofsii* was maintained on PDA slants and stored at 4 °C for further studies. Studied biological control of *S. rolfsii* using *Pseudomonas* spp.

2.5 Pathogenicity test

Roy (1977) artificially proved pathogenicity of *S. rolfsii* was tested on Cauliflower (*Brassica oleracea* var. Capitata L.), pea (*Pisum sativum* L.) and Arun (*Colocassia spp.*). Rotting in all the cases, except *Colocassia*, infection did not reach up to core. Siddaramaiah *et al.* (1978) proved the pathogenicity by sowing 50 seeds which were artificially inoculated with 20 days old Corticium culture and the same quantity of the seeds were sown in sterilized soil as control. Out of 50 seeds, 40 germinated and remaining 10 seeds were not germinated. The fungus started infection after the third day of seed germination and all the 40 seedlings were infected within a week causing post emergence death. Based on the characteristics symptoms, fungus was identified as *Corticium rolfsii*, Curzi(1931).

Kajal Kumar and Chitreswar Sen (2000) isolated *S. rolfsii* from the roots of the affected plants. Inoculations with this isolate produced hundred per cent infection on groundnut plants, while the control plants remained healthy. Artificial inoculation of the plants with the pathogen was done by different methods. Soil inoculation by the pathogen was studied by several workers.

Awasthi *et al.* (2010) investigated the pathogenicity of different isolates of *S. rolfsii Sacc*. on groundnut. Observations revealed that all the isolates were found to be pathogenic towards groundnut but extent of their pathogenicity in respect of their disease severity differs in some isolates. Result revealed a marked variation in the virulence of the different 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 the least disease severity of 40.8 and 40.9 per cent, respectively.

2.6 Cultural and Morphological Characteristics

Grover and Chona (1960) reported that sclerotia of *Sclerotium rolfsii* were small round bodies with whitish colour in the earlier stage and buff brown, clove brown or chocolate brown in the later stage. They were formed within 6 to10 days on various culture media. Their size varied from 0.9 to 2.5 mm. A colourless drop of liquid was frequently exuded by the sclerotia.The mycelium was usually course with large cells. Clamp connections were observed only in the broader cells. The fungus Sclerotium species produced abundant white, fluffy, branched mycelium that forms numerous sclerotia.

Sulladmath *et al.* (1977) reported maximum number of sclerotia produced by isolates collected from pigeon pea followed by sunflower, wheat and groundnut. They further stated that the weight of hundred sclerotia from groundnut isolate was five to eight times higher than other isolates. Punja and Damiani (1996) studied differences in morphology (colony characteristics and sclerotial formation) and size of sclerotia in three different species of *Sclerotium*, viz. *Sclerotium coffeicola, Sclerotium rofsii and Sclerotium rofsii* from diverse geographical areas and compared for growth response to different temperature and media.

Hernandez and Ysla (1997) evaluated eight isolates of *Sclerotium rolfsii* for cultural and morphological characteristics and found variability in their mycelial density, number and diameter of sclerotia, mycelial density, presence of rhizomorphs and duration of sclerotial formation.

Ansari and Agnihotri (2000) showed existence of variation among 40 isolates of *Sclerotium rofsii* collected from different soybean growing areas in India and categorized into groups based on morphological characters of sclerotia, like sclerotial arrangement, size and colour on potato dextrose agar medium.

Sarma *et al*. (2002) studied variations in Indian isolates of *Sclerotium rolfsii* and found that, the variation was existed with respect to crop as well as regions. They observed that growth rate was more in chickpea, green gram, french bean isolates whereas, sclerotial bodies production was more in *Amorphophallus Campanulatus* isolates and green gram isolates which showed maximum sclerotial diameter. They also observed variation in same isolates from different regions and recorded that french bean isolate of Varanasi produced 576 sclerotia /Petri plate whereas the Adalpura isolate produced only 204 sclerotia/Petri plate. Among chickpea isolates the growth rate was more in Karota isolates (31 mm/ day) in comparison to Varanasi isolate (27 mm/day).

Jyothi (2006) studied variation among the isolates of *Sclerotium rolfsii* on different crops and observed considerable variation among the isolates. The colony diameter was varied from 52.00 to 89.83 mm at 72 hours of incubation. The colour of sclerotia was light to dark brown and size of sclerotia varied from 1.3 to 3.40 mm with spherical to round in shape. The test weight of 100 sclerotial bodies were recorded between 73.00 to 383.30 mg and number of sclerotia per cm^2 ranged from 1.14 to 6.55. Groundnut isolate recorded the highest dry mycelial weight (280.70 mg) while wheat isolate recorded lowest (132.70 mg) in potato dextrose broth.

Palaiah and Adriver (2006) reported that, isolates of *Sclerotium rolfsii* from groundnut, wheat, potato, guava and Bengal gram showed considerable variation in growth rate on potato dextrose agar and broth. Tripathi and Khare (2006) noticed that, the radial growth of *Sclerotium rolfsii* was maximum on potato dextrose agar at 4 and 7 days after inoculation followed by Chickpea meal agar, Rice meal agar and Richards's agar.

Darakhshanda *et al*. (2007) observed variations in *Sclerotium rolfsii* collected from different regions of Pakistan. They recorded the growth rate of 8 fungal strains of *Sclerotium rolfsii* on potato dextrose agar ranged from 0.86-1.35 mm/hour. Strains collected from Dera Ismail Khan, Chakwal and NARC (Research field) were found to be comparatively fast growing and produced highest number of sclerotia than others. All strains produced round shaped sclerotia with average diameter of 0.5-2.0 mm.

Kokub *et al*. (2007) studied the growth rate of 8 fungal strains of *Sclerotium rolfsii* on potato dextrose agar plates at 28°C were ranged from 0.86-1.35 mm $hour^{-1}$. Strains D4, D7 and D8 were found to be comparatively fast growing and produced greatest number of sclerotia than others. All strains produced round shaped sclerotia with average diameter of 0.5-2.0 mm.

Surulirajan *et al*. (2007) isolated *Sclerotium rolfsii* [*Athelia rolfsii*] the causal organism of root rot disease of lentil and studied the different morphological characters of the pathogen on two per cent potato dextrose agar medium and found to be best medium for both vegetative and reproductive growth.

Abida Akram *et al*. (2008) studied variation of chickpea isolate of *Sclerotium rolfsii* from 12 different regions and observed variation in colony morphology, mycelial growth rate, colony colour, sclerotial production, number and size of sclerotia. They recorded that out of 12 isolates, colonies of 7 isolates were fluffy, whereas 5 were compact. The isolates SRC-1, SRC-18, SRC-19 and SRC-112 were fast growing (76.7-90 mm diam.) while the isolate SRC-2, SRC-4, SRC-5 and SRC-11 were slow growing (16.0–30.6mm diam.). Most of the isolates produced more number of sclerotia (>300 sclerotia/plate), while isolates SRC-2, SRC-8 and SRC-9 produced fewer sclerotia (<300 sclerotia/plate). The average size of sclerotia for most of the isolates were >40 μm in diameter. The colour of sclerotia was found to be dark to reddish brown at maturity.

Basamma (2008) revealed that, out of 14 media used the maximum dry mycelial weight of fungus was obtained in oat meal extract broth (190.9 mg) followed by potato dextrose broth (190.4 mg), host leaf extract (188.1 mg), Richards's broth (174.8 mg) and Czapek's Dox broth (167.7 mg). Least mycelial weight was obtained in Basal broth (44.8 mg). Le *et al*. (2012) collected isolates of *Sclerotium rolfsii* from different crops like tomato, groundnut and taro, Among 103 isolates considerable variation was observed in the time to form sclerotia, their maturation time and their number and size. The number of sclerotia formed per plate after 21 days of incubation were ranged from 79 to 1080 and their size from 0.88 to 2.24 mm.

Rakholiya and Jadeja (2011) reported morphological variability among, 30 isolates of *Sclerotium rolfsii* on the basis of their mycelial growth, colony colour, mycelial dispersion and appearance, sclerotial colour, size, number of sclerotia, maturity period, weight of sclerotia and sclerotial arrangement.

2.7 *In vitro* evaluation of bioagents against stem rot of groundnut

Singh *et al*. (2003) studied collar rot disease of betel vine caused by *Sclerotium rolfsii*. Screened for their biocontrol activity against *S. rolfsii* under *in vitro* condition. Two strains *of P. fluorescens* has the ability to inhibit the mycelial growth of the pathogen significantly. Bhuiyan *et al*. (2012) described that total of 20 *T. harzianum* isolates collected from rhizosphere and rhizosplane of different crop were screened against *S. rolfsii* following dual plat culture technique. The screened isolates of *Trichoderma* significantly reduced the radial growth of *S. rolfsii*. The isolates TH-18 of *T. harzianum* showed the highest inhibition of radial growth of *S. rolfsii.*

Nawar (2013) described that the bioagent Bio-arc (*T. albium)* was the most effective against *S. rolfsii* growth responsible for 44.66 per cent inhibition. Maximum inhibition was observed in the culture filtrate of *T. harzianum* drawn from potato dextrose broth with mean reduction of 52.33 per cent.

Gomashe *et al*. (2014) reported that B. subtilis has the potential to produce bioactive compound antagonistic against plant pathogenic fungi *S. rolfsii*. Shifa *et al*. (2014) described that *B. subtilis* has G-1, *B. amyloliquefaciens* B2 and *B. subtilis* EPCO 8 were found effective biocontrol agents in inhibiting the mycelial growth of *S. rolfsii in vitro* in dual culture assay.

Bhatt *et al.* (2015) assess bioagents againsts *S. rolfsii* causing southern blight on bell pepper (*Capsicum annuum L*). *T. harzianum* isolates 1 and 5 and *T. viride* isolate 1and 3 inhibited mycelia growth by more than 60 per cent and completely inhibited sclerotial production in vitro. Nagmama and Nagaraja (2015) noted that *T. harzianum* (71.67 %) and *T. viride* (63.33 %) inhibited 61 per cent growth of *S. rolfsii* by dual culture technique.

2.8 *In vivo* evaluation of bio consortium against stem rot of groundnut

Ganesan and Gnanamanickam (1987) found native station of *P. fluorescens* restricted the growth of *Sclerotium rolfsii* causing stem rot of groundnut. Pushpavati and Rao (1995) found the efficacy of bio-control agent (*T. harzianum* and *T. viride*) against *Sclerotium rolfsii* groundnut. Both the *Trichoderma* species significantly reduced the growth of *S. rolfsii* in dual culture. The per cent reduction of *S rolfsii* colony diameter was significantly more with *T. harzianum* (73.37 %) isolate than that of *T. viride* (61.47 %) isolate.

Podile and Dube (1988) reported that seed coating with *P. fluoroscens* (PN-3) controlled stem rot pathogens of peanut (*S. rolfsii* and *R. solani*) in pot experiments. Bhatia *et al.* (2005) observed that *P. fluorescent* PS-I and PS–II coated seed sown in *S.*

rolfsii infected soil significantly increased seed germination by 13.1 and 8.5 per cent respectively as compared to control.

Junaid *et al.* (1991) found that in pot experiment for the control of *S. rolfsii,* seed treatment with Bavistin gave the best results followed by PCNB (quintozene) and Thiram. Seed and soil treatment with quintozene slightly reduced seedling vigor

Desai *et al*. (1999) tested forty-four isolates of *Trichoderma* belonging to 8 species groups for their ability to infect, macerate and kill the sclerotia and found that 14 isolates infected and killed sclerotia of *S. rolfsii*, 17 isolates were not effective and the remaining isolates showed an intermediate effect. Eight isolates of *T. harzianum* and only 3 isolates each *T. hamatum* and *T. koningii* could kill all the sclerotia inoculated.

Biswas *et al*. (2000) observed that, application of *T. harzianum* inoculums to soil and as seed dressing at the time of sowing in pots exhibited per cent disease reduction through seed dressing upto 33 to 50 per cent and through direct soil application it was 72 to 83 per cent.

El-Wakil and Ghonim (2000) reported that, among Topsin-M, Vitavax, Thiram, Rizolex-T and Plantgard tested against pod rot incidence caused by *S. rolfsii*. Carboxin, Thiram and Rizolex-T were found more efficient and reduced the pod rot infection.

Manjula *et al*. (2004) reported the combined application of *Pseudomonas fluorescens* and *T. viride* had an improved biocontrol activity against stem rot in groundnut. Babu and Kumar (2008) studied seven antagonists fungal mycoflora isolated from groundnut rhizosphere and found highest inhibition (83.00 %) of *S. rolfsii* (groundnut isolate) was recorded in *T. harzianum-3*. They also tested five bacterial isolates originated from groundnut rhizosphere. Of which *P. fluorescens-1(Pf-1)* completely inhibited mycelial growth and sclerotial population of *S. rolfsii*.

Kashem (2005) found that *T. harzianum* as seed treatment was effective in controlling collar rot of lentil. Singh *et al.* (2009) selected *Trichoderma* spp. and *Pseudomonas* spp. for seed and seedling treatment in tomato, to assess the synergistic effect of compatible isolates for plant growth promotion and management of *S. rolfsii.*

Varadharajan Karthikeyan *et al*. (2006) observed that, *T. viride* Tv1 was found to be the most effective isolate against *S. rolfsii* with 69.40 per cent growth inhibition followed by *P. fluorescens* resulting in 64.40 per cent inhibition. However, *T. harzianum* was observed to be the least effective bio control agent against *S. rolfsii* tested.

Arunasri *et al*. (2011) tested four fungicides, *viz*., Captan, Propiconazole, Thiophanate methyl and Thiram at five different concentrations. Among these Propiconazole showed cent per cent inhibition of pathogen at all the concentration tested followed by Captan at 1000ppm concentration (94.89 %). Belkar *et al.* (2013) described that the seed treatment with *P. fluorescens* @10g/kg of seed *Bradyrhizobium japonicum* @ 20 g/kg of seed + *P. striata* @ 20 g/kg of seed found effective with minimum stem rot incidence, i.e. 8.86, 13.33, 20.00 per cent at 20 DAS and 17.73, 33.33 and 40.00 per cent of flowering, respectively.

Shifa *et al*. (2015) tested seven bio-control agents against *S. rolfsii* Sacc, the causal agent of stem rot disease of groundnut (*Arachis hypogaea L.*) by dual culture assay. Among the various bio-control agents tested *Bacillus subtilis* strain G-1 was found most effective and inhibited the mycelia growth of *S. rolfsii* and recorded an inhibition of 28 per cent. Groundnut seeds when treated with *B. subtilis G-1* showed significant increases in root length, shoot length and seedling vigour. Similarly, their results were found that, seed treatment with the powder formulation of *B. subtilis G-1* alone effectively reduced the incidence of stem rot and increased the pod yield; but combined application through seed and soil increased the efficacy. Under greenhouse condition. Seed treatment and soil application with *Bacillus subtilis G-1* reduced the stem rot incidence from 80 per cent (with non-bacterized seeds) to 5 per cent.

Rajasekhar *et al*. (2016) evaluated the effect of consortia of *T. harzianum (TH), P. flourescens (PF), Rhizobium (Rh) and B. subtilis (BS)* in different 28 combinations against sclerotium wilt of pigeonpea under greenhouse conditions. Among all combinations, treatment TH + PF + BS + Rh significantly reduced wilt incidence by 86 per cent, followed by TH + BS (82 %) and PF+Rh (77 %) respectively.

Rani *et al*. (2017) tested nine *Trichoderma* spp. isolates against three major soil borne pathogens of groundnut such as *A. niger*, *S. rolfsii* and *M. phaseolina* against *A. niger*, GRT-3 isolate showed maximum per cent of inhibition (65.87 %) followed by TRT-2 (65.50 %). In case of, *S. rolfsii* TRT-1 isolate showed maximum per cent of inhibition (68.75 %) followed by GRT-1 (68.00 %). The highest inhibition zone was recorded in GRT-3 (0.50 cm) with 57.50 per cent inhibition followed by TRT-2 (0.43 cm) against *M. phaseolina*,TRT-2 isolate showed maximum per cent of inhibition (70.50 %) followed by TRT-1 (70.00 %), but isolate GRT-3 overgrew pathogen (3.05 cm) with sporulation, as in case of RRT-1 and RRT-2 overgrew pathogen (3.50 cm) without sporulation.

3. MATERIALS AND METHODS

The present studies were carried out at the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra. The materials and methods used and procedures followed for morphological and cultural variability among the isolates of *S. rolfsii* associated with groundnut as well as *in vivo* evaluation of bioconsortium were as follows.

3.1 Materials

The following materials were used during the present studies.

3.1.1 Laboratory Instruments

Different laboratory instruments required during the course of studies like autoclave, laminar air flow, BOD incubator, binocular research microscope, refrigerator, pH meter, electronic weighing balance, gas burner, spirit lamp, inoculating needles, forceps, blades, marker pen *etc* from Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri were used.

3.1.2 Glassware

Different Borosil make glassware, *viz*, Petri plates, test tube, glass slides, conical flasks, measuring cylinder, volumetric flasks, measuring cylinders of different capacities, pipettes etc. available at Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri were used.

3.1.3 Diseased Samples of Groundnut

The stem rotted diseased plants were collected from Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri during *kharif* 2021.

3.1.4 Chemicals

Analytical laboratory grade chemicals and reagents, required for various experiments were procured from the Department of Plant Pathology and Agricultural Microbiology, Post Graduate institute, MPKV, Rahuri, Maharashtra.

3.1.5 Cultural Media

The growth characters of *Sclerotium rolfsii* isolates were studied on seven solid media were Potato Dextrose Agar, Richards's agar, Czapek's agar, Oat meal agar, Corn meal agar, Malt extract agar, Wakeman's agar. The detailed composition of different media is given Appendix.

3.1.6 Seeds

Groundnut seeds of variety SB-11 was used during present research work was obtained from Oilseed improvement project, MPKV, Rahuri and were used for pot culture experiment to prove pathogenicity and evaluation of bioconsortium in pot under glasshouse condition.

3.1.7 Bioconsortium

Ready made MPKV's Fungal and Fungal + Bacterial bioconsortium product was used for seed treatment in this research work were obtained from Biocontrol laboratory, Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri

3.1.8 Miscellaneous Materials

These included non absorbent cotton, polypropylene and polyethylene bags, paper bags, rubber bands, blotting paper, butter paper, denatured spirit, ethyl alcohol, adhesive solutions, strain, hand instrument scissor, cutting blade, inoculating needle, forceps, pipette and micropipette of different capacities, tips of micropipettes, spirit lamp, foot scale, test tube stand, adhesive tape, adhesive labels, aluminium foil, glass markers *etc*.

3.2 Methodology

3.2.1 Isolation, Identification and Characterization of Pathogen and Its Pathogenity Test

3.2.1.1 Isolation of Pathogen

Isolation was made from the freshly infected plant samples collected during survey at seedling and vegetative stage of the crop. The roots of diseased plant showing typical symptoms were washed thoroughly with water and small pieces were surface sterilized with sodium hypochloride solution for one minute followed by three washing of sterilized distilled water aseptically to Petri plate containing sterilized PDA. Inoculated plates were incubated at 25± 2^{o}Cfor three to five days and examined at frequent intervals to see the growth of the fungus developing from different pieces. As and when fungal colony appears, they were transferred to PDA slant.

3.2.1.2 Identification of Pathogen

These isolates of *S. rolfsii* were identified by observing the colony character, colony diameter, pattern of sclerotial formation and sclerotial size measured with the help of micrometer.

3.2.1.3 Pathogenicity test of Pathogen

In vivo aggressiveness test was tested in glass house. The sick pots were prepared by using the inoculum of the pathogen @ 5% weight of soil in pot. The inoculum mass multiplication on the sand maize media isolate was added in the sterilized soil (1 % Formalin for 15 days). Two kg mixture was placed in 15 cm diameter surface sterilized plastic pots. The pots were left for 5 days for stabilization before sowing. Groundnut seeds of variety SB 11 were surface sterilized with 0.2 % Sodium hypochlorite for one minute followed by three washing with sterile water. Seed were placed two pots, one pot with inoculum and another without inoculum which act as control.

3.2.2 Characterization of *S. rolfsii*

3.2.2.1 Morphological characterization of *S. rolfsii*

The experiment was conducted in order to study the morphological characters of *S. rolfsii*. For this purpose, 15ml of potato dextrose agar was poured into Petri plates. Mycelial disc from 7 day old culture of *S. rolfsii* was placed at the center of the plate and incubated at room temperature (27±1°C) for three days and colony character like diameter, pigmentation, radial growth were recorded. *S. rolfsii* was observed under microscope after 7 days of incubation. To get matured sclerotial bodies, the culture were further incubated up to 21 days. The total number of sclerotia produced per plate and shape of sclerotia were observed and recorded.

3.2.2.2 Cultural characterization of *S. rolfsii*

a. Effect of various different growth media on growth of sclerotial production

The 20 ml each medium was poured in 90 mm petriplates. Such Petri plates were inoculated with circular disc (5mm diameter) of an actively growing fungal colony (3-4 days old) of *S. rolfsii*. The colony disc was cut with the help of cork borer and placed by facing mycelial side downwards in the center on Petri plate containing different medium under aseptic conditions and incubated at 28 ± 2^0C seven days. Each isolate was replicated thrice.

Preparation of Different Media

1. Potato dextrose agar

Potatoes were peeled off and cut into small pieces then 200 g of peeled off cut pieces of potato were boiled in 800 ml of distilled water and extract was filtered through muslin cloth. The dextrose was dissolved into the solution and agar agar was added and boiled until it was properly dissolved. Both the solutions were mixed thoroughly and the volume was made upto 1000 ml by adding distilled water. The media was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

2. Richard's agar

All the ingredients mentioned in appendix except potassium dihydrogen phosphate and agar agar were dissolved in 450 ml of distilled water. Agar-agar was melted separately in 400 ml of distilled water and was mixed with the above solution. The volume was made upto 950 ml by adding distilled water. The Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were autoclaved and subsequently mixed together.

3. Czapek's agar

Agar agar was melted in 400 ml distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one liter by adding distilled water. The media was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

4. Oat meal agar

To prepare 1000 ml of Oat meal agar medium, 72.5 g of oat meal agar was suspended in 1000 ml distilled water and boiled to dissolve it completely. The media was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

5. Corn Meal Agar

The Corn meal was boiled in 400 ml of distilled water for 20 minute and the extract was filtered through a muslin cloth. Agar agar was melted separately in 400 ml of distilled water. Both the solutions were mixed thoroughly. The volume was made upto 1000 ml with distilled water. The media was then sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

6. Malt Extract Agar

Required amount of malt extract was boiled in 500 ml distilled water and molten agar is added to this supernatant and the final volume is then made to 1L

7. Wakeman's agar

Agar agar was melted in 400 ml distilled water. The other ingredients were dissolved in 400 ml of distilled water. The two solutions were mixed thoroughly and the volume was made up to one liter by adding distilled water. The media was then sterilized by autoclaving at 15 lbs pressure (121℃) for 15 minutes.

Observation

The inoculated Petri plates were incubated at 28 ± 2°C for 7 days. The various observations on morphological and growth characteristics of isolate of *S. rolfsii* on different media were recorded after 7 days incubation at 28 ± 2°C. The following observations on morphological characters of isolate of *S. rolfsii.*

Colony diameter

The colony diameter was recorded by averaging the radial growth of the colony in two directions. The data on radial growth were analyzed statistically.

Shape and margin of colony

After incubation of 7 days the shape and margin of the colony was recorded.

Sclerotia production

After incubation of 21 days recorded the production of sclerotial bodies present in Petri plate containing *S. rolfsii* culture.

Size of Sclerotia in mm

After incubation up to thirty days, diameter of sclerotial bodies per replication of each isolate was recorded with the help of digital venier caliper and observation were statistically analyzed.

b. Effect of different pH level on growth and sclerotial production

In this aspect experiment was conducted with an object to find out suitable pH supporting good growth and sclerotial production. Influence of different pH levels on the growth of sclerotia were studied eight different pH level studies from 2.5 to 8.5 were adjusted to PD broth. This was adjusted before autoclaving with the help of HCL (0.1N) and NaOH (0.1 N) by using digital pH meter. Triplicated 250 ml flasks containing 50 ml of medium were inoculated and incubated at 30°C. Dry weight of the mycelial mat was recorded after 21 days of incubation.

3.2.3 *In vitro* evaluation of bioagents against *S. rolfsii* using dual culture technique

Two fungal antagonists *viz*., *Trichoderma viride Trichoderma harzianum* and two bacterial antagonists *Bacillus subtilis, Pseudomonas fluorescens* were evaluated under in vitro condition against *S. rolfsii* by applying dual culture technique (Rama Bhadra Raju *et al.*, 2000).

Seven days old cultures of *S. rolfsii, T. viride, T. harzianum* grown on potato dextrose agar and two old culture of *P. fluorescens, B. subtilis* grown on nutrient agar were used in this experiment. Culture disc of 5 mm of each of the fungal antagonists and target pathogens were taken with the help of sterilized cork borer and transferred to 90 mm diameter PDA culture. The 5mm disc of fungal antagonists and target pathogens was placed opposite to each other i.e. 1 cm away from the edges at equidistance. For bacterial antagonists mycelial disc of 5 mm cork borer and placed into the center of petri dish containing PDA separately. Then strain of *P. fluorescens, B. subtilis* were stricked zig-zag around the pathogen disc at center of PDA plate. The petri plate inoculated with disc of test pathogen alone served as control. The inoculated plates were then incubated at 27±1°C in BOD incubator for seven days.

Observations on linear mycelial growth of the test fungus and bioagents were recorded at interval of 24 hr and continued till untreated control plates were fully covered with mycelial growth of the test fungus. Per cent inhibition of the test fungus by the bioagents over untreated control was calculated by applying formula (Arora and Upadhyay,1978)

$$\% \text{ growth inhibition} = \frac{C - I}{C} \times 100$$

C - Colony growth in control plate

I - Colony growth in interacting plate

3.2.3.1 Experimental details

Design	:	Completely Randomized Design
Number of replication	:	Five
Number of treatment	:	Five
Method	:	Dual culture technique

3.2.3.2 Treatment details

T_1 : *Trichoderma viride*

T_2 : *Trichoderma harzianum*

T_3 : *Bacillus subtilis*

T_4 : *Pseudomonas fluorescens*

T_5 : Control

3.2.4 *In vivo* evaluation of bio consortium against stem rot of groundnut under Glass House condition

The evaluation of bioconsortium in pot under greenhouse conditions by soil sick method against *S. rolfsii* was studied. Before using this bioconsortium, soil is sterilized (Autoclaved for two consecutive days at 1.1 kg/cm^3 pressure for two hours) and mixed with inoculum of *S. rolfsii* @ 5 % weight of soil in pot. These inoculated soils were put in pots and moistened with water. After that SB-11 variety seeds were treated with different concentration of MPKV's fungal, fungal + bacterial bioconsortium, Thiram. These seeds were then sown in pot containing sick soil. Pots containing sick soil without any bioconsortium and fungicides were maintained as untreated control. For each treatment four pots were maintained as replication, kept in glass house and watered regularly.

3.2.4.1 Experimental details

Design of experiment : Completely Randomized Design (CRD)

Number of replications : Four

Number of treatments : Six

Variety : SB-11

Application method : Seed treatment

Inoculum mass multiplication : Sick soil method

3.2.4.2 Treatment details

T_1 : Seed treatment with MPKV's fungal bio consortium @ 5 g/ kg of seed

T_2 : Seed treatment with MPKV's fungal bio consortium @ 10 g/ kg of seed

T_3 : Seed treatment with MPKV's fungal + bacterial bio consortium @ 5 g/kg of seed

T_4 : Seed treatment with MPKV's fungal + bacterial bio consortium @ 10 g/kg of seed

T_5 : Seed treatment with Thiram 75% WP @ 2 to 3 g a.i/Kg of seed

T_6 : Control

3.2.4.3 Observations recorded

1. Seed Germination % - Number of seed germinated was recorded after 7 days after sowing.

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

2. $$\text{Per cent Disease incidence (PDI)} = \frac{\text{Number of plants infected in the pot}}{\text{Total number of plants in the pot}} \times 100$$

3. $$\text{Per cent Disease control (PDC)} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

3.3 Statistical Analysis

To compare the different numerical observations, the data was statistically analyzed by using the Completely Randomized Design.

4. RESULT AND DISCUSSION

Groundnut (*Arachis hypogeae*) is one of the major oil seed crop India. It contains an average of 40.1 per cent of fat and 25.3 per cent of protein and every part of groundnut has its own commercial value. In fact, it plays a pivotal role in oilseed economy of India. Several diseases like stem rot, leaf stop, bud necrosis, *etc*., are responsible for decreasing the quality and quantity of groundnut production. Among them stem rot of groundnut caused by soilborne pathogenic fungi *Sclerotium rolfsii* is a major problem and economically important soilborne disease. This pathogen causes yield loss of 20-30 per cent and may reach up to 80 per cent during severe condition. In order to control stem rot disease of groundnut best method is biological method which is efficient and eco-friendly method. Among biological method use of bioconsortium give better results than using bioagents singly. In bioconsortium, the components organisms work together in synergistic and complex way.

Keeping in mind the present research work entitled "Evaluation of Bioconsortia against stem rot *(Sclerotium rolfsii)* disease of groundnut" was carried out during *kharif* 2022 at the Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, MPKV, Rahuri. A study was undertaken on various aspects likely isolation and identification of fungal pathogen, pathogenicity, cultural and morphological studies, *in vitro* efficacy of bioagents and *in vivo* evaluation of bioconsortia under glass house condition. The results of all research aspects are described and explained in this chapter.

4.1 Isolation and Identification of fungal pathogen

The disease infected plant samples showing typical symptoms of stem rot of groundnut were collected from fields of groundnut. The symptoms includes dark brown lesions on the stem just below soil surface followed by drooping and wilting of entire plant, white colored mycelia of fungus was observed at the base of groundnut plant. The pathogen was isolated by using standard procedures. By using single hyphal tip isolation technique pure culture and slant culture was obtained on PDA media as shown in (Plate1). Colony obtained on PDA media was circular, cottony, fluffy, white in color and produced mustard like brown colored spherical sclerotia after long time. Pure culture of pathogen thus obtained is preserved in the form of slant culture at 4°C for further studies.

Wilson (1953) described symptoms of the stem rot of groundnut as, mycelium covering the plant stem at the base near the soil. This was then followed with necrosis of plant cells. The mycelium invaded the stem, gynophores and also pods and caused rotting of the tissues. The production of abundant white mycelium and small brown spherical sclerotia on the infected parts were characteristic symptoms of stem rot disease of groundnut. Beattie (1954) also observed same type of symptoms on infected plants.

Maddu and Ravuri (2015) isolated and cultured *S. rolfsii* strain on PDA from the plants showing stem rot symptoms of groundnut collected from the area of Sadhanavaripalem, Chittoor district of Andhra Pradesh in *kharif* season and the pure culture of the fungus was obtained using single hyphal tip isolation technique. Colony thus obtained was circular, cottony white color.

The current studies related to isolation, identification and symptomology of pathogen are in accordance with pervious works of Baruah *et al.* (1980), Nyvall (1989), Kalmesh and Gurjar (2001), Bagwan (2011), Kadam *et al*. (2011) who also isolated and identified *S. rolfsii* from different host plant and observed similar symptomology. Hence the fungal pathogen thus obtained in this present study was identified and confirmed as *Sclerotium rolfsii*.

4.2 Pathogenicity Test

Pathogenicity of *S. rolfsii* was confirmed by proving Koch's postulates. The pathogenicity test was conducted by using soil inoculation technique in pot under glass house condition. The pathogen under study was mass multiplied on sand maize meal media at 25° C for 15 days for soil inoculum. Mass multiplied *S. rolfsii* inoculum was mixed with sterilized soil in one pot and in another pot only sterilized soil was filled which is used as control. Healthy and sterilized groundnut seeds of variety SB-11 were sown in both the pots. After growth of plants typical symptoms of stem rot were observed after 3 weeks of incubation in glass house. There was wilting of plants with white colored mycelia at the base of groundnut plant, rotting of stem lead to wilting of plant finally lead to death of seedling in pot which contain sick soil, whereas healthy plant was observed in pot which do not contain sick soil as showed in (Plate 2).

The test pathogen was reisolated from artificially diseased plant of SB-11 groundnut variety and cultural characteristics were observed on PDA media after a week of incubation. The results showed similar characters of pathogen which were isolated and incubated earlier from naturally infected disease sample obtained from field. Thus, the characteristic symptoms of the test pathogen were similar in both naturally and artificially infected plants of groundnut which confirms the pathogenicity for test pathogen *S. rolfsii* and hence Koch's postulates were proved.

Doley and Jite (2013) studied that isolate of *S. rolfsii* caused stem rot in the tested groundnut plants in pot under green house. When groundnut seeds were sown in soil and inoculated with *S . rolfsii* symptoms like damping-off , death of seedling and stem rot occurred. Some of the plants were upright in position which showed symptoms of stem rot. The disease was more severe in plants where mycorrhiza was not inoculated *S. rolfsii* were re-isolated from the seedling of groundnut and the PDA cultures were identical to original isolate.

The current study on pathogenicity of *S. rolfsii* showed similar results to that of earlier studies of Siddaramaiah *et al.* (1978), Haware and Nene (1978), Awasthi *et al.* (2010). Hence pathogenicity test for *S. rolfsii* was proved during this study.

4.3 Cultural and Morphological Studies of *S. rolfsii*

The mycelium of fungi under study was septate and hyaline with conspicuous branching at acute angle. The young growing mycelial mass of *S. rolfsii*, *in vivo* and *in vitro*, was white, cottony, fluffy, with silky lustre. Sclerotia produced by *S. rolfsii* were initially white in colour with droplets around them, which eventually turns brown in color. Theses sclerotia as they get older resembles mustard seed, measuring 1-2 mm in diameter as shown in (Plate 3).

4.3.1 Cultural characteristics of *S. rolfsii* on different solid media

Studying the cultural characteristics of pathogen on different cultural media in laboratory condition helps one to know the nature, growth pattern and other characters of fungus which is need in classification, research and its management. Keeping this in mind current study was carried out to know the cultural and morphological characteristics of *S. rolfsii* using seven different solid media.

Seven different solid media used in this current study were Potato Dextrose Agar, Richard's agar, Czapek's agar, Oat meal agar, Corn meal agar, Malt extract agar and

Wakeman's agar. During this study different observation were recorded like mean colony diameter, shape of the colony, color of the colony and sclerotial production.

The results of current study present in the Table 1 shows that the highest mean colony diameter was observed in Potato Dextrose Agar (PDA) that is 78.5 mm, having circular shaped , fluffy cottony white colored colony, followed by Czapek's agar media with mean colony diameter of 74.1 mm, followed by Malt extract agar media with mean colony diameter of 70.1 mm, followed by Wakeman's agar with mean colony diameter of 69.4 mm, followed by Oat meal agar with mean colony diameter of 68.5 mm, followed by Corn meal agar with mean colony diameter of 65.2 mm and least colony mean diameter was observed in Richards's agar that is 52.2 mm.

Profused growth of *S. rolfsii* was observed in Potato Dextrose Agar, Czapek's agar and Malt extract agar media. Moderate growth of *S. rolfsii* was observed in Wakeman's agar, Corn meal agar and Oat meal agar media. Least growth of *S. rolfsii* was observed Richards's agar. Shape and Color of the colony of *S. rolfsii* remains same in all different solid media that is circular, fluffy cottony white color.

When it comes to sclerotial production Potato Dextrose Agar, Oat meal agar media, Corn meal agar, Wakeman's agar media showed abundant sclerotial production. Malt extract agar and Czapek's agar media showed moderate sclerotial production and no sclerotial production was observed in richards's agar media.

Abeygunawardena and Wood (1957) studied the mycelial growth and sclerotial production of *S. rolfsii* on nine different media, which includes both synthetic and nonsynthetic media. Among liquid media, the growth and sclerotial production was maximum in carrot extract and Weindling's medium, whereas the growth was scanty on maize and Brown's media. Among solid media, the growth and sclerotial production was maximum on potato extract medium.

Sivakumar *et al* (2016) studied the cultural characteristics of *S. rolfsii* on six different solid media and found that Maximum radial growth (90.0 mm) was recorded on PDA medium followed by Czapek's agar which recorded 75.30 mm radial growth. Least radial growth (40.66 mm) of the test fungus was recorded on Coon's agar medium. Good sclerotia production was observed on PDA, Czapek's Dox agar and Richard's agar supported poor sclerotia formation.

Results of current studies on cultural characteristics of *S. rolfsii* and different solid media are similar to earlier studies of Abeygunawardena and Wood (1957), Sivakumar *et al.* (2016), Sulladmath *et al*. (1977), Tripathi and Khare (2006).

Table 1. Effect of different growth media on radial growth and sclerotial production of *S. rolfsii*

Sr. No.	Growth Medium	Mean Colony diameter in mm	Shape of colony	Color of the colony	Sclerotia Production
1.	Potato Dextrose Agar	78.5	Circular	Fluffy Cottony white	Abundant
2.	Richard's agar	52.2	Circular	Fluffy Cottony white	No sclerotia
3.	Czapek's agar	74.1	Circular	Fluffy Cottony white	Moderate
4.	Oat meal agar	68.5	Circular	Fluffy Cottony white	Abundant
5.	Corn meal agar	65.2	Circular	Fluffy Cottony white	Abundant
6.	Malt extract agar	70.1	Circular	Fluffy Cottony white	Moderate
7.	Wakeman's agar	69.4	Circular	Fluffy Cottony white	Abundant

4.3.2 Effect of different pH level on growth and sclerotial production of *S. rolfsii*

Present study was carried out to know the influence of pH level on growth and sclerotial production on Potato dextrose broth. Various pH levels were taken which varies from 2.5 pH – 8.5pH. During this study observations on average dry weight of mycelial mat and sclerotial production of *S. rolfsii* was recorded.

The results of present study interpreted in the Table 2 shows that the average dry weight of mycelial mat varies from 83 mg to 319 mg. Highest average mycelial mat weight of 319 mg was observed at 5.6 pH, followed by 6.2 pH with average mycelial mat weight of 315 mg ,followed by 5.1 pH with average mycelial mat weight of 306 mg, followed by 4.6 pH with average mycelial mat weight of 240,followed by 7.2 pH with average mycelial mat weight of 186 mg , followed by 3.5 pH with average mycelial mat weight of 174 mg, followed by 2.5 pH with average mycelial mat weight of 128 mg and least average mycelial mat weight of *S .rolfsii* at is 83 mg was observed at 8.5 pH level.

For sclerotial production of *S. rolfsii* at different pH level, abundant sclerotial production was observed at 5.1 pH, 5.6 pH and 6.2 pH level. Moderate sclerotial production was observed at 4.6 pH and 7.2 pH. Poor sclerotial production was seen at 2.5 pH and 3.5 pH. Very less and scanty sclerotial production was observed at 8.5 pH.

The present study shows that the growth of *S. rolfsii* was maximum at wide range of pH level that is between 5.1 pH to 6.2 pH. Growth of *S. rolfsii* was minimum at 8.5 pH with very less sclerotial production.

The results of current research are in agreement with those of Kumar *et al.* (2008) and Zape *et al.* (2013), reported that optimal mycelial growth of *S. rolfsii* was at pH 6-7 and those of Sarker *et al.* (2013) who defined that optimum pH level for *S. rolfsii* growth was at pH range of 5-6. Moreover, the restriction of *S. rolfsii* growth at higher pH values (pH 8.5) confirmed previous studies reporting that *S. rolfsii* mycelial growth was markedly less above pH 8 (Aycock, 1966; Sharma and Kaushal, 1979; Punja, 1985).

Table 2. Effect of different of pH level on mycelial growth and sclerotial production of *S. rolfsii*

Sr. No.	pH level	Average dry weight of mycelial mat in mg	Sclerotial production
1.	2.5	128	Poor sclerotial production
2.	3.5	174	Poor sclerotial production
3.	4.6	240	Moderate sclerotial production
4.	5.1	306	Abundant sclerotial production
5.	5.6	319	Abundant sclerotial production
6.	6.2	315	Abundant sclerotial production
7.	7.2	186	Moderate sclerotial production
8.	8.5	83	Very scanty sclerotial production

4.4 *In vitro* evaluation of biocontrol agents against the *S. rolfsii*

In this research studies two fungal antagonists *viz*, *Trichoderma viride, Trichoderma harzianum* and two bacterial antagonists *viz, Bacillus subtilis, Pseudomonas fluorescens* were tested to find out their antagonistic potential and type of colony interaction against test pathogen *Sclerotium rolfsii*.

In this current studies mean colony diameter of *S. rolfsii* against different bioagents in dual culture varies from 22.8 to 90 mm and growth inhibition per cent varies from 0 to 74.50 per cent. The data that is interpreted in the Table 3 revealed that, least mean colony diameter of *S. rolfsii* that is 22.8 mm was observed against *T. harzianum* in dual culture plate with maximum growth inhibition (%) of 74.5 per cent over control, followed by *B. subtilis* with growth inhibition (%) of 72.92 per cent, followed by *T. viride* with growth inhibition (%) of 70.12 per cent and least growth inhibition (%) was recorded by *P. fluorescens* among different bioagents.

Rajalakshmi (2002) evaluated *T. harzianuman* , *T. viride* evaluated for their antagonistic activity against *S. rolfsii* by dual culture technique and observed *that T. harzianum* showed maximum (41. 3 %) growth inhibition followed by *Trichoderma viride* (37. 5 %). Also observed significant reduction in the sclerotia production.

Manjula *et al*. (2004) isolate 57 bacterial and 13 isolates of *Trichoderma spp* . and evaluated for their antagonistic activity against *S. rolfsii* for management of stem rot of groundnut. They observed that four isolates of *P. fluorescens*, viz., GB 4, GB 8, G B 10, GB 27 and *T. viride pq-1* were potent antagonists of 23 *S. rolfsii* . *T . viride pq-1* produced extracellular chitinase and parasitized the mycelium of *S. rolfsii*.

Babu and Kumar (2008) also evaluated seven antagonists fungal mycoflora by dual culture technique isolated from groundnut rhizosphere and observed that *T . harzianum-3*. (Th-3) inhibited 83.00 per cent mycelial growth of *S . rolfsii*. Also evaluated five bacterial isolates for their antagonistic effect originated from groundnut rhizosphere and found that *P. fluorescens-1* (Pf-1) completely inhibited the mycelial growth and sclerotial formation of *S. rolfsii*.

Bhuiyan *et al*. (2012) screened total of 20 *T. harzianum* isolates collected from rhizosphere of different crop were screened against *S. rolfsii* by using dual culture technique. The screened isolates of *Trichoderma* species significantly reduced the radial growth of *S. rolfsii*. The isolates TH-18 of *T. harzianum* showed the highest inhibition of radial growth of *S. rolfsii.*

The results of laboratory studies were similar to that of results of earlier works of Rajalakshmi (2002), Manjula *et al* .(2004), Nawar (2013), Gomashe *et al*. (2014), Bhatt *et al.* (2015), Babu and Kumar (2008), Bhuiyan *et al*. (2012) where maximum growth inhibition was observed by *T. harzianum*.

Table 3. *In vitro* evaluation of biocontrol agents against the *S. rolfsii*

Tr. No.	Treatment	Mean colony Diameter of *S. rolfsii* (mm)	Growth Inhibition (%)
T_1	*Trichoderma viride*	26.3*	70.12
T_2	*Trichoderma harzianum*	22.8*	74.50
T_3	*Bacillus subtilis*	24.3*	72.92
T_4	*Pseudomonas fluorescens*	27.6*	69.70
T_5	Control	90.0*	0
	S.Em. $\pm$	0.291	-
	CD at 1%	1.172	-

*Mean of four replications

4.5 *In vitro* evaluation of bioconsortium against stem rot of groundnut

Now a days farmers are using different chemical fungicides for managing stem rot of groundnut caused by *S. rolfsii* which not eco-friendly. So best method to control stem rot of groundnut is biological method which economical and eco-friendly. In biological method different bioagents are used to control stem rot disease of groundnut. Use of different bioconsortium at different concentration that contains more than one compatible bioagents to control stem rot disease of groundnut might be used instead of using bioagents singly. In order to see effect of bioconsortium on seed germination and stem rot disease incidence in groundnut this the current study was conducted by seed treatment of SB-11 variety of groundnut using MPKV's fungal bioconsortium and MPKV's fungal + bacterial bioconsortium at different concentration along with thiram in pot culture under glass house condition.

4.5.1 Effect of bioconsortium on seed germination of groundnut

In this studies results on effect of bioconsortium on seed germination of groundnut as shown in the Table 4 reveals that maximum seed germination was observed in pots containing seeds which were treated with thiram that is 96.4 per cent but among different bioconsortia maximum seed germination was observed in seeds which are treated with MPKV's fungal + bacterial bioconsortium @10 g/kg of seed and MPKV's fungal bioconsortium @10 g/kg of seed with seed germination (%) of 92.85 per cent, followed by seed treated with MPKV's fungal+bacterial bioconsortium @ 5 g/kg of seed with seed germination (%) of 89.27 per cent and least seed germination 85.7 per cent was observed in seed which were treated with MPKV's fungal bioconsortium @ 5 g/kg of seed.

Table 4. Effect of bioconsortium on seed germination of groundnut (SB-11)

Tr. No.	Treatment	Mean seed germination (%)	Increase in seed germination over control (%)
T_1	MPKV's Fungal bioconsortium @ 5g/ kg of seed	85.70* (67.78)	14.3
T_2	MPKV's Fungal bioconsortium @ 10g/ kg of seed	92.85* (74.49)	23.8
T_3	MPKV's Fungal + Bacterial bioconsortium @ 5g/kg of seed	89.27* (70.87)	19.06
T_4	MPKV's Fungal + Bacterial bioconsortium @ 10g/kg of seed	92.85* (74.49)	23.8
T_5	Seed treatment of Thiram 75% WP @ 2 to 3 g a.i/kg of seed	96.42* (79.09)	28.61
T_6	Control	74.97* (59.98)	0
	S.Em. $\pm$	1.903	-
	CD @ 5%	5.654	

Figures in parenthesis are arc sine transformed values.

There was increase in seed germination of groundnut over seeds which were not treated with any bioconsortium and thiram. Maximum seed germination over control 23.3 per cent was observed in seed treated with MPKV's fungal + bacterial bioconsortium @10 g/kg of seed and MPKV's fungal bioconsortium @10 g/kg, followed with seed treated with MPKV's fungal + bacterial bioconsortium @5 g/kg that is 19.06 per cent increase over control and least increase in seed germination over control 14.3 per cent was observed in seed treated with MPKV's fungal bioconsortium @ 5 g/kg of seed.

Mishra *et al*. (2013) reported that *Trichoderma* isolate (PBAT-43) and *Pseudomonas* (PBAP-27) emerged as most compatible and efficient combination so it is used in development of mixed formulations. Mixed formulations exhibited increase in seed germination ranging from 25.5 -72.11 per cent and disease control 47.68 -76.00 per cent in mustard plants.

4.5.2 Effect of bioconsortium on stem rot disease incidence on groundnut

During the present work, the results on effect of bioconsortium on stem rot disease incidence on groundnut SB- 11 variety under glass house condition in pot culture presented in Table 5 reveals that maximum disease incidence (32.07 %) was recorded in pot containing seeds which were treated with MPKV's fungal bioconsortium @ 5 g/kg of seed which is maximum among treatments and among bioconsortium, followed by seeds treated with MPKV's fungal+bacterial bioconsortium @ 5 g/kg of seed had per cent disease incidence of 21.35 per cent, followed by seeds treated with MPKV's fungal bioconsortium @10 g/kg of seed had per cent disease incidence of 19.28 per cent, followed by seeds treated with MPKV's fungal + bacterial bioconsortium @10 g/kg of seed had per cent disease incidence of 17.77 per cent which least among different bioconsortium and least disease incidence 7.1 per cent was recorded in seeds that were treated with thiram @ 2-3 g/kg of seed.

A significant per cent disease control was observed in seed which were treated with different bioconsortium at different concentration of bioconsortium. Though the maximum disease control upto 89.53 per cent was observed in seeds which were treated with thiram @ 2-3 g/kg of seed, Maximum ecofriendly disease control of 73.8 per cent was observed in seeds which were treated with MPKV's fungal+bacterial bioconsortium @ 10 g/kg of seed and this was highest among different bioconsortium at different concentration, followed by seed which were treated with MPKV's fungal bioconsortium @ 10 g/kg of seed with disease control of 71.57 per cent, followed by seed which were treated with MPKV's fungal + bacterial bioconsortium @ 5 g/kg of seed with disease control of 68.5 per cent and least disease control 52.71 per cent was observed in seed which were treated with MPKV's fungal bioconsortium @ 5 g/kg of seed, this was the least per cent disease incidence among different bioconsortium.

Rajasekhar *et al*. (2016) studied the effect of consortia of *T. harzianum* (TH), *P. flourescens* (PF), *Rhizobium* (Rh) and *B. subtilis* (BS) in different 28 combinations against sclerotium wilt of pigeon pea under greenhouse conditions. Among all combinations, treatment TH + PF + BS + Rh showed maximum disease control by reducing wilt incidence by 86 per cent, followed by TH + BS (82 %) and PF + Rh (77 %) respectively.

Belkar *et al.* (2013) reported that the seed treatment with bioagents were found effective in reducing the collar rot disease in soybean caused by. Among different treatments tested, the most effective was seed treatment with *P. fluorescens* @ 10g/Kg of seed, followed by *Bradyrhizobium japonicum* @ 20 g/ kg of seed, *Pseudomonas striata* @ 20 g/kg of seed with minimum disease incidence *i.e*., 8.86, 13.33, 20.00 per cent at 20 days after sowing and 17.73, 33.33 and 40.00 per cent at flowering, respectively.

Manjula *et al.* (2004) reported that combined application of *P. fluorescens* GB 10 with *T. viride pq1 for* protecting groundnut seedlings from stem rot infection caused by *S. rolfsii* had shown higher per centage of pathogen mortality (78 %) when compared to application of either *T. viride pq1* (70 %) or *P. fluorescens* GB 10 (58.0) alone.

Singh *et al*. (2013) used both *Trichoderma spp*. and *Pseudomonas spp*. as seed and seedling treatment in tomato to assess the synergistic effect of compatible isolates for plant disease management of *S. rolfsii* and observed that the lowest mean disease rating (MDR) 1.96 and maximum per cent disease reduction (PDR) 53.23 per cent was observed in consortium treatment.

Patibanda *et al*. (2002) studied the feasibility of using *T. harzianum* for the management of sclerotium wilt in groundnut. *T. harzianum* application either to soil as wheat bran saw dust (WBSD) preparation or on the groundnut seeds as spore coat proved effective against sclerotium wilt caused by *Sclerotium rolfsii*. Synergistic and positive effects on disease control were reported when *T. harzianum*-WBSD preparation was applied to soil in integration with Vitavax [carboxin] or Vitavax-200. Integration of Thiram (seed coating) and soil application of antagonist was found compatible and synergistic. However, seed treatment with both antagonist and Thiram was found incompatible and hence may not be practically feasible for disease reduction.

The results of current studies on *in vitro* evaluation of ecofriendly bioconsortium against stem rot disease of groundnut was similar to results of previous studies of Rajasekhar *et al*. (2016), Belkar *et al.* (2013), Manjula *et al*. (2004), Singh *et al*. (2013) and Patibanda *et al*. (2002).

Table 5. Efficacy of bioconsortia against stem rot of groundnut (SB – 11)

Tr. No.	Treatment	Per cent Disease incidence	Per cent Disease control
T_1	MPKV's Fungal bioconsortium @ 5g/ kg of seed	32.07* (34.48)	52.71
T_2	MPKV's Fungal bioconsortium @ 10g/ kg of seed	19.28* (26.04)	71.57
T_3	MPKV's Fungal + Bacterial bioconsortium @ 5g/kg of seed	21.35* (27.52)	68.5
T_4	MPKV's Fungal + Bacterial bioconsortium @ 10g/kg of seed	17.77* (24.93)	73.8
T_5	Seed treatment of Thiram 75% WP @ 2 to 3 g a.i/kg of seed	7.10* (15.45)	89.53
T_6	Control	67.82* (55.45)	0
	S.Em. $\pm$	1.699	-
	CD @ 5 %	5.050	

Figures in parenthesis are arc sine transformed values.

5. SUMMARY AND CONCLUSION

Groundnut (Arachis *hypogeae*) which belongs to family Leguminosae is one of the important and major oil seed crop growth in India. This is also called as 'King of oilseeds'. There are several reasons for low productivity and low quality of groundnut. Disease in groundnut caused by different pathogens is one of the major reasons for low productivity. Several diseases like leaf spot, bud necrosis, stem rot etc., deteriorate the value of groundnut. Among them stem rot disease of groundnut caused by *Sclerotium rolfsii* is the major disease which reduces the yield upto 25-30 per cent. By knowing this current study entitled with "Evaluation of bioconsortia against stem rot disease *(Sclerotium rolfsii)* of groundnut" was carried out during 2022 during *kharif* season with the objectives of isolation and identification of fungal pathogen from groundnut, to study cultural and morphological characters of fungal pathogen and *in vivo* evaluation of eco-friendly bioconsortium against stem rot of groundnut.

During this study fungal pathogen was isolated from diseased plant sample collected from groundnut field. The isolated pathogen was purified using single hyphal tip isolation technique and pure culture was preserved in slant culture at 4°C for all studies. Pathogen *S. rolfsii* was tested for its pathogenicity in pot culture under glass house condition using sick soil method and Koch's postulates were proved.

Study was conducted to know the cultural and morphological studies of *S. rolfsii* on seven different solid media. Among different solid media mean colony diameter of *S. rolfsii* varies from 52.2 to 78.5 mm. Maximum mean colony diameter 78.5 mm of *S. rolfsii* was recorded on Potato dextrose agar media with circular, fluffy, cottony white colony was observed. Also effect of different pH level on growth if *S. rolfsii* on PD broth was studied with 8 different pH level varying from 2.5 – 8.5 pH. Average dry weight of mycelial mat of *S. rolfsii* varied from 83 mg to 319 mg during this study. Maximum average dry weight of mycelial mat 319 mg was recorded at 5.6 pH with abundant sclerotial production.

In *in vitro* evaluation of ecofriendly bioagents against *S. rolfsii* maximum growth inhibition was observed in *T. harzianum* dual culture plate with mean colony diameter 22.8 mm of *S. rolfsii* was recorded and least growth inhibition was observed in *P. fluorescens* dual culture plate.

In *in vivo* evaluation of bioconsortium against stem rot of groundnut study seed of groundnut variety SB-11 were treated with two different MPKV's bioconsortium at different levels of concentration along with earlier recommended thiram and sown in pots containing sick soil under glass house condition. During this study maximum seed germination of 92.85 per cent was observed in seeds which were treated with MPKV's fungal + bacterial bioconsortium @10 g/kg of seed and MPKV's fungal bioconsortium @10 g/kg of seed. Minimum disease incidences of 17.7 per cent and maximum disease control upto 73.8 per cent was recorded in seeds which were treated with MPKV's fungal + bacterial bioconsortium @ 10 g/kg of seed among bioconsortium, maximum disease incidence of 32.07 per cent was observed in seeds which were treated with MPKV's fungal bioconsortium @ 5 g/kg of seed.

Conclusions

1. The soil borne fungal pathogen *S. rolfsii* causing stem rot of groundnut was isolated, identified and grown on Potato dextrose agar media. White cottony and compact mycelia was found on petri plate containing PDA.
2. Sclerotial production was found which were white during early stage of growth but turned brown at a later stage. Sclerotia were mustard like round, spherical, with a smooth shiny surface and measured 0.5–1.1 mm in diameter.
3. Cultural studies of *S. rolfsii* on 7 different solid media revealed that Potato dextrose agar (PDA) media was superior and best culture media for in vitro growth with abundant sclerotial production. Mycelial growth and sclerotial production of *S. rolfsii* was maximum at pH between 5.1 to 6.2. Least mycelial growth and very scanty sclerotial production was observed at 8.3 pH.
4. Among biocontrol agents *T. harzianum* was more effective among different biocontrol agents and least effective was *P. fluorescens* against *S. rolfsii* under *in vitro* condition.
5. In *in vivo* evaluation of bioconsortium against *S. rolfsii* groundnut seeds treated with thiram showed maximum seed germination and maximum disease control. But among ecofriendly means of bioconsortium maximum seed germination was recorded by seeds treated with MPKV's fungal + bacterial bioconsortium @10 g/kg of seed and MPKV's fungal bioconsortium @10 g/kg of seed and maximum disease control was observed in seed treated with MPKV's fungal + bacterial bioconsortium @10 g/kg of seed.

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7. APPENDIX

Composition of laboratory medias

Following medias were prepared for the *in vitro* studies of the present research work. The composition of all medias are enlisted here,

1. Potato Dextrose Agar (PDA) medium

1. Potato - 200 g
2. Dextrose - 20 g
3. Agar - 20 g
4. Distilled water - 1000ml

2. Richard's agar

1. KNO_3 - 10 g
2. KH_2PO_4 - 5 g
3. $MgSO_4.7H_2O$ - 2.50 g
4. $FeCl_3.\ 6H_2O$ - 0.01 g
5. Sucrose - 50 g
6. Agar - 20 g
7. Distilled Water - 1000 ml

3. Czapek's agar

1. $NaNO_3$ - 2 g
2. K_2HPO_4 - 1 g
3. KCL - 0.50 g
4. $FeSO_4.7H_2O$ - 0.01 g
5. $MgSO_4.7H_2O$ - 0.50 g
6. Agar - 20 g
7. Distilled water - 1000 ml

4. Oat meal agar

1. Oat meal - 60 g
2. Agar - 12.5 g
3. Distilled water - 1000 ml

5. Corn Meal Agar

1. Corn meal - 50.0g
2. Agar agar - 15g
3. Distilled water - 1000ml

6. Malt Extract Agar

1. Malt extract - 25.00g
2. Agar-agar - 12.50g
3. Distilled water - 1000ml

7. Wakeman's agar

1. Glucose - 10 g
2. Peptone - 5 g
3. $MgSO_4$ - 0.50 g
4. KH_2PO_4 - 1.00 g
5. Agar - 20 g
6. Distilled water - 1000 ml

8. VITAE

Mr. NIDHEESH B. S.

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

In

PLANT PATHOLOGY

2024

Title of thesis		**:**	"Evaluation of Bioconsortia Against Stem Rot Disease *(Sclerotium Rolfsii)* of Groundnut"
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